Intake capacity of the Icelandic dairy cow
Effect of animal factors, concentrate ratio and feeding method

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Clarification of contribution

I hereby declare that the writing of the following thesis and all data analysis presented is my work under the supervision and assistance of my advisor Dr. Jóhannes Sveinbjörnsson.

Experimental design of both Study 1 and Study 2 was in the hands of my supervisors Dr. Jóhannes Sveinbjörnsson and Grétar Hrafn Harðarson, as well as my own.

I handled the daily management of Study 1 during weekdays along with Halla Kjartansdóttir, an employee at the farmers’ alliance in south Iceland (Búnaðarsamband Suðurlands). Daily management of Study 2 was carried out by Baldur Sveinsson, apart from one week when I came to the farm and handled it. During both studies the farmers at Stóra-Ármót took care of feeding during weekends, collected milk samples and helped us with problems that came up.

Tryggvi Eiríksson prepared all feed samples for analysis. The feed samples were analysed partly at the Agricultural University of Iceland under the supervision of Tryggvi Eiríksson, while other samples were sent to Eurofins, Norway, for analysis.

________________________________________
Lilja Dögg Guðnadóttir
Abstract
Accurate prediction of dry matter intake (DMI) is crucial to formulate a feed ration that maximizes feed efficiency. The model predicting the DMI of the Icelandic dairy cow in the co-Nordic feed evaluation system NorFor needed an update to be able to predict the DMI more accurately. The aims of this study were to collect new data on the DMI of the Icelandic dairy cow in late lactation, and under feeding situations that are common today, to facilitate improvements on the prediction equation of the Icelandic dairy cow. Two studies were conducted, one observational study with the objective to obtain new data on the DMI in late lactation (Study 1) and one experimental study to compare the DMI related to different feeding strategies (Study 2). Study 2 included three treatments, a total mixed ration with a 55% concentrate ratio (TMR55) and a 45% concentrate ratio (TMR45), and separate feeding aiming at a 45% concentrate ratio (SF45). Primiparous cows fed TMR had significantly higher DMI than those fed SF45 (p<0.05), while there was no significant difference in the DMI between treatments for multiparous cows (p>0.1). Both primiparous and multiparous cows in the TMR treatments had significantly higher yield of ECM than cows fed SF45 (p<0.001). New coefficients were fitted to the Norfor equation predicting intake capacity (IC) using the data obtained in Study 1 and Study 2. The new coefficients decreased the prediction error (RMSPE) of the DMI by 0.15 and 0.24 kg DM/d for primiparous and multiparous cows respectively and increased the $R^2$ compared to the current coefficients. The suggested coefficients for the multiparous cows increase the accuracy of the DMI prediction compared to the current coefficients. The suggested coefficients for primiparous cows, however, increased the line bias substantially which suggests that the model structure is inadequate. Data from these studies and earlier studies of the DMI of the Icelandic dairy cow should be used together to fit new coefficients to the IC prediction equation.

Keywords: Dry matter intake, intake capacity, fill value, concentrate ratio, roughage, total mixed ration, separate feeding.
Ágrip
Grundvallaratriði hagkvæmrar fóðuráætlanegaðar er að geta áætlað át á tilteknu fóðri eða fóðurblöndu. Þörf var á að afla aukinna gagna um átgetu íslensku mjólkurkýrinnar til að geta endurbætt spálfíkingar fyrir át sem eru notaðar í fóðuráætlanegaði í samnorraena fóðurmatakerfinu NorFor. Markmið þessa verkefnis var annars vegar að safna gögnum á seinni hluta mjaltaskeiðs (rannsókn 1) og hins vegar að afla gagna um át við mismunandi fóðrunaraðstæður sem algengar eru í dag á íslenskum kúabúum (rannsókn 2). Rannsókn 2 byggði á þrem fóðrunarmeðferðum, heilfóðrun með annars vegar varðagur 55% og hins vegar 45% kjarnfóðurhlutfalli (TMR55 og TMR45) og aðskildri fóðrun sem miðaði að 45% kjarnfóðurhlutfalli (SF45). Heildarát fyrsta kálfs kvígna á heilfóðri var marktækt meira en þeirra sem voru á aðskildri fóðrun (p<0,05) en ekki var marktækur munur í át á milli meðferða hjá eldri kúm (p>0,1). Nyt kúla sem fengu heilfóður var marktækt meiri en kúla sem voru á aðskildri fóðrun (p<0,001). Þetta átti við um bæði fyrsta kálfs kýr og eldri kýr. Fundnir voru nýir stuðlar í spálfíkingar um átgetu annars vegar fyrir fyrsta kálfs kvígur og hins vegar eldri kýr, út frá þeim gögnum sem safnað var í báðum rannsóknum. Í báðum tilfellum lætkaði skekkja í mati á átgetu og fylgni milli spágilda og mældra gilda hækkaði þegar nýju stuðlarnir voru notaðir í samanburði við þá sem notaðir eru í dag. Allt bendir til þess að nýju stuðlarnir fyrir eldri kýr auki á öryggi spálfíkingarinnar í samanburði við stuðlana sem notaðir eru í dag. Hjá fyrsta kálfs kvígum jökst aftur á móti línulegt frávik töluvert þegar nýju stuðlarnir voru notaðir. Það bendir til þess að form líkingarinnar sé ófullnægjandi. Næst skref er að sameina þau gögn sem hér var aflað og gögn úr eldri rannsóknum og þróa úr því nýja stuðla í spálfíkingu fyrir átgetu íslensku mjólkurkýrinnar.
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<th>Definition</th>
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<tr>
<td>BCS</td>
<td>Body condition score</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>cDMI</td>
<td>Concentrate dry matter intake (barley and compound feed)</td>
</tr>
<tr>
<td>CF</td>
<td>Compound feed</td>
</tr>
<tr>
<td>CP</td>
<td>Crude protein</td>
</tr>
<tr>
<td>CR</td>
<td>Concentrate ratio</td>
</tr>
<tr>
<td>DIM</td>
<td>Days in milk</td>
</tr>
<tr>
<td>DM</td>
<td>Dry matter</td>
</tr>
<tr>
<td>DMI</td>
<td>Dry matter intake</td>
</tr>
<tr>
<td>ECM</td>
<td>Energy corrected milk</td>
</tr>
<tr>
<td>FP</td>
<td>Fermentation products (lactic acid, acetic acid, butyric acid, formic acid, propionic acid, ethanol and ammonia)</td>
</tr>
<tr>
<td>FV</td>
<td>Fill value</td>
</tr>
<tr>
<td>IC</td>
<td>Intake capacity</td>
</tr>
<tr>
<td>MSPE</td>
<td>Mean squared prediction error</td>
</tr>
<tr>
<td>MY</td>
<td>Milk yield</td>
</tr>
<tr>
<td>NDF</td>
<td>Neutral detergent fibre</td>
</tr>
<tr>
<td>OMD</td>
<td>Organic matter digestibility</td>
</tr>
<tr>
<td>rDMI</td>
<td>Roughage dry matter intake</td>
</tr>
<tr>
<td>RMSPE</td>
<td>Root mean square prediction error</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SF45</td>
<td>Separate feeding with 45% concentrate ratio</td>
</tr>
<tr>
<td>TA</td>
<td>Total acid</td>
</tr>
<tr>
<td>TMR</td>
<td>Total mixed ration</td>
</tr>
<tr>
<td>TMR45</td>
<td>Total mixed ration with 45% concentrate ratio</td>
</tr>
<tr>
<td>TMR55</td>
<td>Total mixed ration with 55% concentrate ratio</td>
</tr>
<tr>
<td>VDMI</td>
<td>Voluntary dry matter intake</td>
</tr>
<tr>
<td>WFC</td>
<td>Weeks from calving</td>
</tr>
<tr>
<td>WSC</td>
<td>Water soluble carbohydrates</td>
</tr>
</tbody>
</table>
1 Introduction

A dairy cow’s production is highly influenced by the level of its intake and the quality of the feed it is offered. Voluntary dry matter intake (VDMI) is the dry matter intake when cows are fed *ad lib.*, that is how much the animal is willing to eat when there are no restrictions. Since dairy cows are usually fed *ad lib.* the intake will, hereafter, be referred to as dry matter intake (DMI).

If energy content of the feed offered to a dairy cow is too low compared to its energy requirements, the DMI will not be sufficient to maintain the production. Similarly, the feed’s energy content can exceed the requirements of the animal, which can lead to excessive DMI, resulting in excessive fat deposition (Forbes, 2007).

To avoid both too low and too excessive DMI it is important to be able to formulate a feed ration that maximizes feed efficiency and results in an appropriate DMI that is in accordance with the desired production of the animal. In order to formulate such a ration it is important to know and understand the factors that affect the DMI, for an accurate prediction of the intake. For this sake, *i.e.* to be able to predict the DMI as accurately as possible, researchers have developed a great interest in those factors and their effects on the DMI (Ingvartsen & Andersen, 2000). During the last decades, numerous predicting models have been developed, for different breeds, under different feeding and management circumstances and including different factors (Halachmi, Edan, Moallem & Maltz, 2004; Huhtanen, Rinne, Mantysaari & Nousiainen, 2011; Jarrige *et al.*, 1986; Mertens, 1987; NRC, 2001; Volden, 2011c).

In Iceland the only cattle breed used for milk production is the Icelandic dairy cow. The Nordic feed evaluation system – NorFor (Volden, 2011c), has been adapted to this breed. The prediction of DMI of the Icelandic dairy cow in NorFor is based on the work of Baldursdóttir (2010). However, the practice of using NorFor in Iceland for the past years has revealed the need to review the model predicting the DMI of the Icelandic dairy cow. The present thesis is based on two feeding studies of Icelandic dairy cows. The main aims of the studies were to collect data on the DMI of the Icelandic dairy cow in late lactation and to investigate how different composition of total mixed ration (TMR) and separate feeding with a high concentrate ratio affects the DMI.
1.1 Regulation of feed intake

The feed intake of cows is regulated by the hypothalamus in the central nervous system. The hypothalamus receives signals from the sense organs and from numerous mechanic- and chemoreceptors in the rumen, intestines and liver. Those signals, as well as nutrients, metabolites and hormones, largely regulate the eating behaviour and food intake of the cow (Ingvartsen & Andersen, 2000). The regulation of feed intake is often categorized into three mechanisms, based on the origin of the signals of satiety/hunger. Those three categories are physical, metabolical and psychological regulation (Ingvartsen & Kristensen, 2003).

The act of physical regulation on feed intake, regulated by the capacity of the reticulo-rumen, has been substantiated in many experiments. The presence of chemo- and mechanic receptors in the rumen wall, the relationship between addition or removal of material from the rumen and the intake as well as the positive relationship between digestibility and digestion rate and the DMI, all support the presence of physical regulation (Faverdin, Baumont & Ingvartsen, 1995). Feed with low digestibility has an increased retention time in the reticulo-rumen and therefore a higher filling effect compared to feed with high digestibility. Physical regulation is therefore the main regulation when digestibility of the feed is low, but the metabolical regulation takes over with highly digestible feed when there are less physical restraints. When the regulation is mainly metabolical, physiological factors such as metabolic weight and production of the animal become more important in controlling the intake (Conrad, Pratt & Hibbs, 1964). There is a positive relationship between the intake and the production as increased production stimulates hunger and motivates the cow to eat more, while a decreased production stimulates satiety and reduces intake (Ingvartsen & Kristensen, 2003). The theory that that ruminants eat the amount they need to fulfil their requirements (metabolical regulation), as long as the intake is not physically restricted (physical regulation), is known as “the two-phase regulation” and is widely accepted as the main control of intake (Forbes, 2007).

In addition to the physical and the metabolical regulation, which are the most important regulation factors, the feed intake can also be regulated psychologically. This involves interaction between individuals, such as social hierarchy, social facilitation and stress factors (Forbes, 2007) and environmental factors, such as feed accessibility, (Albright, 1993; Forbes, 2007) and space allowance (Ingvartsen & Andersen, 1993).
1.2 Factors affecting voluntary feed intake

DMI is mostly affected by factors related to either the animal itself or the feed. The animal factors are related to the physiological state of the animal and the body reserves, and can, therefore, be referred to as long-term regulation factors. The feed characteristics, on the other hand, are more connected to the short-term regulation of intake, such as signals from the gastrointestinal tract, and induced satiety or hunger (Faverdin et al., 1995). However, the animal and feed factors interact, which is important to keep in mind. Other factors connected to the management and the environment can also affect the feed intake. Some of the animal, feed and environmental factors are listed in Table 1, but the list is not complete.

Table 1. Factors that affect the DMI (Modified by Ingvartsen (1994)).

<table>
<thead>
<tr>
<th>Animal factors</th>
<th>Feed factors</th>
<th>Management, housing and environmental factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td>Digestibility and energy concentration</td>
<td>Time of access to feed</td>
</tr>
<tr>
<td>Sex</td>
<td>Degradation and passage rate</td>
<td>Frequency of feeding</td>
</tr>
<tr>
<td>Weight and body condition</td>
<td>Chemical composition</td>
<td>Feeding method</td>
</tr>
<tr>
<td>Growth</td>
<td>Plant species</td>
<td>(separate vs. TMR)</td>
</tr>
<tr>
<td>Age/parity</td>
<td>Physical form/Particle size</td>
<td>Anabolic agents and food additives</td>
</tr>
<tr>
<td>Milk yield</td>
<td>Dry matter</td>
<td>Mineral salts and alkaline agents</td>
</tr>
<tr>
<td>Stage of lactation</td>
<td>Fermentation quality</td>
<td>Tie stalls vs. loose housing</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Palatability</td>
<td>Space allowance</td>
</tr>
<tr>
<td>Previous feeding</td>
<td></td>
<td>Photoperiod</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temperature</td>
</tr>
</tbody>
</table>

1.2.1 Animal factors

1.2.1.1 Breeds

The DMI varies largely with breeds. Most research of the DMI of dairy cows is done for a single breed, not comparing various breeds. Models predicting DMI are, therefore, generally set up only for a certain breed (Ingvartsen, 1994). NorFor, however, takes breed into account, considering three categories; large Nordic breeds (Danish Holstein, Swedish Red, Swedish Holstein and Norwegian Red), Jersey and the Icelandic Breed (Volden et al., 2011). Those breeds vary largely in body weight (BW) and size (Volden et al., 2011), which is most likely a reason for part of the difference on the DMI, but it doesn’t explain all the difference. For example, the DMI per 100 kg live weight of the Jersey cow was predicted to be 26% higher than that of the large breeds Danish Friesian and the Danish red, in the old Danish fill system of prediction of intake (Ingvartsen, 1994).
1.2.1.2 Weight and body condition

BW plays an important role in regulating the DMI and is included in most models predicting the DMI. How it is included in the models varies widely, but the most common forms are plain BW, metabolic weight (BW\(^{0.75}\)) or BW raised to other powers than 0.75 (Ingvartsen, 1994). Roseler, Fox, Chase, Pell and Stone (1997) reported that the BW accounted for about 17% of the variation in the DMI of Holstein cows.

The volume of the reticulo-rumen increases linearly with increased size and the BW of a cow (Ingvartsen & Kristensen, 2003). However, the DMI is not linearly correlated with the BW. When increased BW is caused by excessive fat, the DMI stabilizes and no longer increases with increased BW (Forbes, 2007; McDonald et al., 2011). There is a negative correlation between the DMI and the body reserves (Ingvartsen & Andersen, 2000). Therefore, the correlation between the BW and the DMI is found to be higher for primiparous cows (0.37-0.45) than multiparous cows (– 0.05-0.16) (Roseler, Fox, Chase et al., 1997). This is because the BW of a primiparous cow reflects the growth and, therefore, an increased volume of the reticulo-rumen, while an increased BW of a multiparous cow rather reflects an increase in body reserves. The BW changes after calving correlate with the potential a cow has to increase its DMI. The more BW a cow loses after calving, the less ability it has to increase its DMI postpartum (Maltz, Devir, Metz & Hogeveen, 1997).

Faverdin et al. (1995) concluded that cows have a higher motivation to eat if they have undergone a phase of undernutrition. It has also been shown that thin cows eat more than fat cows when regulation of intake is more metabolical than physical (Bines & Morant, 1983; Bines, Suzuki & Balch, 1969). When a cow gains fat the adipose tissue produces more leptin and the concentration of plasma leptin increases. It has been confirmed that both plasma leptin concentration and body condition score (BCS) have a negative effect on the DMI (Ingvartsen & Andersen, 2000; Roseler, Fox, Chase et al., 1997). Another reason for the negative effect of fat on the DMI is that the body fat can take space in the abdominal cavity that otherwise could be used by the rumen and the ingested feed (Ingvartsen & Kristensen, 2003). Roseler, Fox, Chase et al. (1997) reported that the BCS explained ≤6% of the variation in the DMI.

1.2.1.3 Age and parity

It is a well-known fact that primiparous heifers have less intake capacity (IC) than multiparous cows. A primiparous heifer calving at 2 years and weighing approximately 500 kg has only about 80% of the IC of a multiparous cow in the first part of lactation.
After taking BW and milk yield (MY) into account, a large part of the difference in the IC remains unexplained, and must be explained by the parity (Jarrige, 1986; Kristensen & Ingvartsen, 1986). The difference in the IC between primiparous and multiparous cows, however, decreases throughout the lactation period. This is caused both by of the growth of the heifers, which increases the volume of the reticulorumen, and because their MY and energy requirements don’t fall as fast as in multiparous cows (Kristensen & Ingvartsen, 2003). A slight difference between cows in their second lactation and older cows in early lactation has been reported, but this difference can be explained by difference in the BW and the MY and it should, therefore, not be included as a model factor according to Kristensen and Ingvartsen (2003).

**1.2.1.4 Milk yield and stage of lactation**

DMI is positively correlated with MY (Azizi, Kaufmann & Hasselmann, 2009; Søndergaard, Sørensen, Mao & Jensen, 2002). Higher yielding cows acquire higher DMI by increasing their meal size, but spend less time eating (Azizi et al., 2009; Dado & Allen, 1995). It can be argued that the MY should not be included as a factor in predicting DMI because milk production is certainly a result of intake. However, when intake regulation is metabolic rather than physical, the DMI is driven by energy requirements and the largest factor in the energy requirements of a lactating cow is the MY. Therefore, it can also be argued that DMI is a result of MY (NRC, 2001).

The DMI follows a pattern throughout the lactation stage, given that the feed fulfills the energy requirements of the cow. The pattern of DMI is quite similar to the pattern of the MY, with a strong dip around calving, that lasts for the first weeks postpartum (Figure 1). The peak in DMI is, however, reached 8-22 weeks after calving, while maximum MY is reached at only 5-7 weeks after calving (Ingvartsen & Andersen, 2000). After calving there is a quick release of compression on the rumen, due to the loss of the amniotic fluid, fetus and fetal membranes. If the DMI was regulated physically in that period the DMI would increase rapidly after calving. The increase is slow, however, which indicates that the intake regulation in that period is metabolic, rather than physical (Bines, 1976; Ingvartsen & Andersen, 2000).
Feeding cows in their early lactation low-concentrate ration evokes the physical regulation and under such restrictive circumstances the DMI keeps constant after it has reached its peak throughout the lactation, or as long as the feed is restrictive, instead of slowly decreasing until next calving as it does under non-restrictive circumstances. Under such restrictive circumstances, the limited energy intake can also act restrictive on the MY (Bines, 1976; Friggens, Emmans, Kyriazakis, Oldham & Lewis, 1998). The interval between peak in the DMI and the MY induces mobilization for the first weeks after calving. The mobilization is, nevertheless, partly genetically driven and, therefore, unavoidable, even with a high-energy concentrated feed (Friggens et al., 1998; Friggens, Ingvartsen & Emmans, 2004).

A few milk production parameters are used in models predicting DMI. Total MY, energy corrected milk (ECM) and 4% fat corrected milk are the ones that are most commonly used (Halachmi et al., 2004; Ingvartsen, 1994; NRC, 2001; Volden et al., 2011). According to Roseler, Fox, Chase et al. (1997) up to 45% of the variation in the DMI is explained by the MY parameters. Total MY and ECM have a higher correlation with the DMI (0.61-0.62 and 0.67 respectively for primiparous and multiparous cows) than fat corrected milk (0.58 and 0.65 respectively for primiparous and multiparous cows), but the total milk protein yield has the highest correlation with the DMI of these factors (0.69 for primiparous and multiparous cows) (Roseler, Fox, Chase et al., 1997).
1.2.1.5 Pregnancy
At pregnancy the amniotic fluid, fetus and fetal membranes take space in the abdominal cavity which otherwise could be used to consume feed. This, in addition to increased hormonal activity, depresses DMI during the last weeks of pregnancy. According to Ingvartsen, Andersen, and Foldager (1992) the depression in intake equals 1.5% per week during the last 14 weeks, and even more for the last 2 weeks. The reduction in intake is, however, generally only included in models for the last 6 weeks of pregnancy (Ingvartsen, 1994). The fall in intake during the last weeks of gestation is positively correlated with the birth weight of a calf, which supports the theory of physical regulation in that period (Forbes, 2007). There is, however, evidence that point out that the dip in intake is not only caused by physical limitation in rumen volume, but could also be metabolically regulated (Ingvartsen & Andersen, 2000). Ingvartsen (1994) points out the importance of taking pregnancy into account when predicting intake, since it would otherwise be presumed that intake increases in late pregnancy when the fetus grows rapidly, because of the increased BW. Prediction of the DMI of cows in late pregnancy is, however, seldom a point of interest, the focus is on the period when they are in full production.

1.2.1.6 Secretion of hormones and metabolites
Certain metabolites and hormones produced and secreted during certain physiological state of the animal are important factors regulating the DMI. Non-esterified fatty acids and ketone bodies that are mobilised from adipose tissue during late pregnancy and early lactation are assumed to have a negative effect on DMI in those periods (Ingvartsen & Andersen, 2000). Insulin and leptin are important in regulating both the BW and intake in ruminants. The sex hormones oestrogen and progesterone as well as hormones secreted during stress reactions, e.g. corticotrophin-releasing factor have also been shown to have a role in regulating intake (Ingvartsen & Andersen, 2000).

1.2.2 Feed factors
1.2.2.1 Energy concentration and digestibility
A positive relationship between the energy concentration or the digestibility of a feed and the DMI has been demonstrated repeatedly (Conrad et al., 1964; Montgomery & Baumgardt, 1965; Van Soest, 1965). It is known that an increase in the energy requirements because of low temperature, physical activity and increased MY or BW, increases the motivation for energy intake (Faverdin et al., 1995). Based on that knowledge, it has been suggested that as long as the intake is not physically limited by
volume of the rumen, ruminants eat to meet their energy requirements. According to that, increased energy concentration in the feed should decrease the DMI. This has been proven right in both cattle (Montgomery & Baumgardt, 1965) and sheep (Dinius & Baumgardt, 1970; Montgomery & Baumgardt, 1965). Simkins, Suttie and Baumgardt (1965) also concluded that the energy intake, represented by the production or the infusion of volatile fatty acids in the rumen, motivates satiety when there are no physical limitations on the intake. This supports the theory that ruminants eat the amount they need to fulfil their requirements, as long as the intake is not physically restricted (Forbes, 2007).

Supplementation of concentrates also clearly shows the effect of energy concentration on the DMI. Increased concentrate supplementation reduces roughage intake, corresponding to the amount of concentrate and the energy concentration of the roughage. This reduction of roughage intake with increased concentrate is called substitution rate (Forbes, 2007). The relationship between the energy concentration of the roughage and the substitution rate is positive, *i.e.* increasing energy concentration of the roughage up to a certain point increases the substitution rate, so that the roughage intake reduces for each added unit of concentrate (Forbes, 2007).

### 1.2.2.2 Degradation and passage rate

A feedstuff is built up of feed fractions that are either soluble, potentially degradable or totally indigestible, depending on the animal’s ability to digest the feedstuff. It is the rate of passage and digestion of the potentially degradable fraction as well as the passage rate of the indigestible fraction that determine how fast the feed goes through the reticulo-rumen, and therefore the fill effect of the feed in the rumen (Faverdin *et al.*, 1995; Jung & Allen, 1995). Increasing passage or digestion rate causes a faster disappearance of food from the rumen, which makes up more space for feed in the rumen and consequently increases the DMI.

A reduced particle size generally increases the passage rate, and therefore also the DMI (Jung & Allen, 1995; Martz & Belyea, 1986). However, it can also benefit the passage rate to have some structure in the feed, as it induces saliva production. More liquid in the rumen increases the passage rate of liquids and consequently also of small particles. An increased liquid passage rate also increases the production of microbial N in the rumen which has positive effect on the digestion rate (Kristensen, Hvelplund, Weisbjerg &
Nørregaard, 2003). A certain bulk in the feed could, therefore, be preferred for maximum passage and digestion rate.

To pass through, particles need to be dense enough to sink down to the orifice between the reticulum and the omasum and small enough to easily pass through the omasum. During rumen fermentation, the microbes that produce methane and carbon dioxide gas attach to the feed particles. These gas bubbles decrease the functional specific gravity and make the feed particles float. As the potentially degradable fraction of the feed ferments, the functional specific gravity increases. The particles sink, which brings them closer to the orifice and increases the possibility to pass through (Jung & Allen, 1995). The duration and the intensity of the contractions of the rumen are important factors in determining the passage rate since the contractions push particles through the orifice between the omasum and reticulum (Jung & Allen, 1995).

Grasses have a higher fraction of potentially degradable NDF and a lower digestion rate, and the reduction of functional specific gravity should, therefore, be slower than that of legumes. That is not the case, however, which suggests that other factors such as anatomical structure, also affect the functional specific gravity, and therefore the passage rate (Jung & Allen, 1995).

Passage rate increases the DMI, but also *vice versa*. Increased DMI decreases the retention time and increases the passage rate. This leads to less time for the feed particles in the rumen for digestion, but also a more effective microbial synthesis (Kristensen *et al*., 2003; Volden & Larsen, 2011).

1.2.2.3 Chemical composition

The negative effect of high NDF content in the feed on the DMI has long been known. Waldo (1986) reported it to be the best single chemical predictor on DMI, and Mertens (1987, 1994) developed a model predicting DMI, where the only feed characteristic was the NDF content, representing the fill value of the feed. The negative effect of NDF content in the feed on DMI is not linear (Dado & Allen, 1995; Mertens, 1994; Roseler, Fox, Chase *et al*., 1997; Van Soest, 1965). Van Soest (1965) reported a negative effect on the DMI when the NDF content in the roughage reached 55-60% of DM. Dado and Allen (1995) compared a feed ration of 25% NDF and 35% NDF, and reported that only the 35% NDF ration limited intake. Allen (2000), however, reported in his review that a NDF content over 25% in a ration would negatively affect the DMI. Still, even though the NDF
has a restrictive effect on feed intake, it must not be forgotten that a minimum concentration of NDF in the diet for a dairy cow is crucial for ruminal health. The NDF is the structural part of the feed and promotes chewing and saliva production which has a buffer activity in the rumen and prevents low ruminal pH (McDonald et al., 2011; NRC, 2001). Too low NDF concentration can, therefore, also limit the DMI through poor rumen health. The digestibility of the NDF also matters when it comes to effect on intake. Oba and Allen (1999) reported an increase in the DMI and the MY with an increased NDF digestibility. This is mostly explained by a higher rate of digestion and passage of NDF (Oba & Allen, 1999).

Dairy cows in high yield need additional readily fermented carbohydrates to fulfil their high-energy needs. This is usually achieved with supplementation of concentrates containing starch. It is essential that a part of the starch is soluble, and ferments in the rumen to provide enough energy for the microbes. Still, if excessive amount of starch ferments rapidly in the rumen, the rumen pH falls which negatively effects the microbes fermenting fibre. This leads to a decrease in the digestibility of NDF (McDonald et al., 2011) as well as intake (Allen, 2000). It is, therefore, favourable that a part of the starch is slowly fermented and can pass through the rumen unfermented, and rather digest in the small intestines. Maize is an example of such a starch source, while barley is rapidly fermented in the rumen (Volden, 2011b). According to Volden and Larsen (2011) the microbial protein efficiency peaks at 250 g rapidly fermented carbohydrates per kg DM in the feed, and diminishes after that.

High microbial efficiency in the rumen is a prerequisite for a high digestion rate and the DMI. It is, therefore, essential to feed the microbes of the rumen enough protein. Too low or too high protein concentration in a ration, however, depresses the DMI (Forbes, 2007). Amino acid combination in the feed does not seem to affect DMI significantly. This is because most of the amino acids absorbed are from microbial protein, which is quite constant in amino acid composition and not dependent on amino acid combination in the feed (Allen, 2000).

Increasing the energy concentration in a ration by supplementing fat usually decreases NDF digestibility and DMI (Allen, 2000). The effect of fat supplementation is dependent of the fat source. Unsaturated fatty acids inhibit digestion and intake more than saturated fatty acids and fatty acid derivatives show less digestion inhibition than free fatty acids do.
(Jenkins, 1993). Fat supplementation also contributes to less intake through secretion of the hormone cholecystokinin which has been shown to contribute to satiety (Allen, 2000).

A ration must contain all the essential minerals (*i.e.* Ca, Cu, I, Mg, P, Se, Na, Zn) as well as vitamins A and D, a deficiency or excess of it can lead to a decreased intake (Forbes, 2007).

### 1.2.2.4 Roughage species

Generally, legume roughage gives a higher intake than grass roughage (McDonald *et al.*, 2011; Oba & Allen, 1999). Grass roughages often have higher NDF digestibility than legumes, but despite that, the retention time in the rumen is longer than that for legumes which leads to a lower intake (Oba & Allen, 1999). This is because legumes contain less NDF than perennial grasses, and because the lignification in legumes is not as widely distributed as in perennial grasses (McDonald *et al.*, 2011). Legumes, therefore, have a greater particle fragility than grasses and both break down and pass through the rumen faster than grasses (Allen, 2000).

### 1.2.2.5 Physical form and particle size

Reduced particle size of roughage has been shown to both increase (Fischer, Buchanan-Smith, Campbell, Grieve & Allen, 1994; Mooney & Allen, 1997; Moore, 1964) and decrease (Woodford & Murphy, 1988b) the DMI. The effect of roughage particle size is highly dependent on the quality of the roughage. Increased DMI due to reduced particle size is mostly found when roughages are of poor quality (Moore, 1964; Poppi, Minson & Ternouth, 1981; Woodford & Murphy, 1988a). Low quality roughages with high NDF content have longer rumen retention time, and pelleting those increases passage rate and therefore the DMI (Martz & Belyea, 1986). The intake of feed rations based on roughages that are of high quality with low NDF content, and rich in energy is mostly regulated metabolically. Thus, chopping or pelleting those can hardly increase the DMI (Shaver, Nytes, Satter & Jorgensen, 1986; Tafaj, Zebeli, Baes, Steingass & Drochner, 2007). Similarly, the physiological status of the animal and its energy requirements also controls the effect of roughage particle size on intake. A recent research by Helander, Nørgaard, Arnesson and Nadeau (2014) showed that neither chopping silage nor mixing silage and concentrates affected the DMI of a pregnant ewe, while during lactation both of these treatments increased intake, due to increased energy requirements. In that case, the intake regulation was physical during pregnancy, but metabolical during lactation.
Chopping or grinding roughage increases the rate of fermentation which leads to a lower rumen pH (Tafaj et al., 2007). Lower rumen pH reduces the activity of cellulolytic microbes that produce acetate, and non-cellulolytic microbes that produce propionate become dominant (Moore, 1964; Tafaj et al., 2007). Chopping or pelleting roughage can therefore lead to less digestibility of NDF (Moore, 1964; Tafaj et al., 2007; Woodford & Murphy, 1988b). These effects are especially visible when roughage is pelleted, and this can result in reduced DMI (Woodford & Murphy, 1988b).

The effect of chopping or grinding on ruminal pH and fermentation patterns has not always been obvious. In their research, Shaver et al. (1986) did not notice any difference in ruminal fermentation patterns for long and chopped hay treatments. Also, Krause and Combs (2003) did not see a decreased pH value in the rumen when fed chopped hay as earlier had been verified. This was probably because the length difference between the particles in the treatment (5.3-5.6 vs. 2.7-2.8 mm) was not enough to find the difference (Krause & Combs, 2003). Tafaj, Steingass, Susenbeth, Lang and Drochner (1999) concluded that both long and chopped hay induced favourable fermentation conditions, but ground hay induced negative influences on ruminal fermentation. They based their conclusion on the pH value, concentration of bicarbonate and acetate:propionate ratio.

Long hay has a buffering activity in the rumen, by motivating chewing, rumination and saliva production. A moderate addition of long hay to feed rations containing high ratios of concentrates and high-quality roughages that ferment rapidly in the rumen can be advisable to prevent the decrease of rumen pH and NDF digestibility. This improves the rumen environment and can result in increased DMI (Woodford & Murphy, 1988a).

1.2.2.6 Total mixed ration (TMR)

The method of mixing various feed types together, fed as a uniform feed, often called total mixed ration (TMR), was described by Coppock (1977) as a new and without a doubt advantageous method compared to separate feeding. The fine chopping and mixing of ingredients in the TMR makes it harder for the animals to select a single feed ingredient from the ration. This means that the intake of each ingredient evens out throughout the day. From beginning this has been considered one of the main advantages the TMR has compared to separate feeding (Coppock, 1977). More dispersed intake of the concentrates during the day leads to a more stable rumen environment and can have positive effects on both the DMI (Aaes, 1993; Istasse, Reid, Tait & Ørskov, 1986; Sveinbjörnsson &
Harðarson, 2008) and the milk production (Aaes, 1993; Grainger, Auldist, O’Brien, Macmillan & Culley, 2009; Istasse et al., 1986). However, the effects of the TMR on the DMI and the MY compared to separate feeding with concentrates fed twice daily are not always distinct and are possibly affected by the concentrate ratio in the ration, where an increased concentrate ratio increases the positive effects the TMR feeding has (Istasse et al., 1986; Phipps, Bines, Fulford & Weller, 1984). Comparison of Icelandic studies of the DMI of dairy cows, using either separate feeding or a TMR has shown increased intake when fed a TMR (Sveinbjörnsson & Harðarson, 2008). It must, however, be taken into account that the concentrate ratio in the TMR feeding was considerably higher than that of the separate feeding, which presumably also has some positive effects on the DMI and makes the results somewhat less comparable.

Although feed selection is minimized when feeding a TMR compared to separate feeding, it is not eliminated, to some extent the animals generally select concentrates and small particles at the expense of roughage and NDF intake (DeVries, Beauchemin & von Keyserlingk, 2007; DeVries, Dohme & Beauchemin, 2008). The amount of feed selection in a TMR can be somewhat controlled with the right preparation. Long particles of roughage in a ration are easier to sort against and less palatable than smaller particles (DeVries et al., 2007; Leonardi & Armentano, 2003). Selection against long roughage particles seems to increase with an increased ratio of concentrates in a TMR, presumably because of better access of short particles and concentrates in such ration (DeVries et al., 2007). Decreased sorting can also be achieved with adding liquid to the TMR, either through adding moist or liquid feed (DeVries & Gill, 2012) or water to the TMR (Leonardi, Giannico & Armentano, 2005). The liquid increases adhesion of the ration which makes it harder for sorting. It can also increase the palatability of the ration, including the long particle and, therefore, decreases the desire for sorting against them (DeVries & Gill, 2012; Leonardi et al., 2005). Still, water addition to a TMR with a low DM may also increase sorting against large particles (Felton & DeVries, 2010).

1.2.2.7 Dry matter and fermentation quality

Dry matter and fermentation quality are important factors determining the intake of silage. The fermentation process of silage includes mostly lactic acid bacteria that under anaerobic conditions multiply and produce organic acids, mostly lactic acid but also propionic acid and acetic acid, using water soluble carbohydrates from the roughage. Other bacteria are also present, but under optimal conditions the lactic acid bacteria are predominant. The
Lactic acid bacteria are dependent on water in their environment, and an increased DM content in the silage results in less fermentation (McDonald et al., 2011).

Huhtanen et al. (2002) developed a model predicting silage DMI for dairy cows, revised by Huhtanen, Rinne and Nousiainen (2007). In the revision, they included the DM as an independent factor on silage DMI, in addition to the effect it has on the fermentation products. They detected a clear positive relationship between the DM concentration and the DMI, for DM concentration lower than 500 g/kg. The data restricted the estimation to a DM range from 175-500 g/kg (Huhtanen et al., 2007). Wilting silage can therefore be eligible to increase the DMI. Too much wilting may, however, cause troubles both in compaction of the silage and in achieving anaerobic conditions which can lead to bad fermentation (McDonald et al., 2011) and, therefore, lower DMI. Decreasing the DM of a ration by adding water in it has similar effects, Felton and DeVries (2010) reported that by decreasing the diet DM from 56.3% to 44.1% the DMI decreased significantly, although the intake of wet feed increased. It appeared that the cows meant to compensate for the lower DM concentration by consuming more total wet feed, but failed to consume more DM, partly because of rumen fill.

The effects of lactic acid on DMI is not easy to detect (Forbes, 2007; Huhtanen et al., 2002). Huhtanen et al. (2002) reported a strong, negative effect of lactic acid on the DMI, with stronger effects at high rates than low, which they reported was somewhat inconsistent with earlier research. The problem is that a low concentration of lactic acid can be caused by restricted fermentation, e.g. because of a high DM content, but it can also be a consequence of a secondary fermentation, which usually also includes higher concentrations of butyric acid and ethanol, which gives the silage low palatability, and, therefore, less DMI (Rotz & Muck, 1994). Information on the amount of lactic acid in the feed are, therefore, not enough, one needs to evaluate it with regards to concentrations of other fermentation products to assess its effect on the DMI.

The concentration of volatile fatty acids (propionic acid, acetic acid and butyric acid) also negatively affect intake (Huhtanen et al., 2002; Huhtanen et al., 2007). The best predictor of fermentation on intake is total acid concentration (Huhtanen et al., 2007). Huhtanen et al. (2002) concluded that when acid application was used to minimize silage fermentation, the extent of the fermentation was the best predictor of silage DMI, but when the silages
were untreated, treated with inoculants or low level of acid application, it was the type of fermentation that predicted silage DMI.

Ammonia-N is a result of an unsuccessful fermentation, and increased concentration negatively affects the DMI. Whether that is a direct effect of ammonia-N or indirect through correlation to other end-products of silage proteolysis is not known (Forbes, 2007; Huhtanen et al., 2002; McDonald et al., 2011).

### 1.2.2.8 Palatability
Palatability is based on the sensory characteristics of a particular feed; smell, touch and taste. The palatability of a certain feed depends on the individual, its experience of the feed or feed with similar sensory characteristics, as well as the animal’s metabolic status. Although some flavours, smells and tastes are known to be more palatable than others (e.g. sweet tastes, coriander and aniseed smell), this can change if the animal is forced by hunger to eat “unpalatable” feed and learns that it is safe (Blair & Fitzsimons, 1970; Forbes, 2007). Therefore, when animals only have a single feed option the palatability of the feed has minimal or no effects on the DMI. However, when they have two or more options of feed, they choose significantly more of the more palatable feed than the other (Forbes, 2007; Gherardi, Black & Colebrook, 1991). Some plants are naturally protected against the consumption of animals (e.g. by spines) or contaminated in some way that diminishes its palatability, in which case the experience of the animal of the food does not change the palatability. Generally, however, palatability is not considered an important factor in determining the DMI, especially not in high producing animals because their energy requirements are high, and the motivation to fulfil the requirements is increased (Faverdin et al., 1995).

### 1.2.3 Management and environmental factors

#### 1.2.3.1 Feeding management
Frequency of feeding does not seem to affect the total DMI of dairy cattle (DeVries, von Keyserlingk & Beauchemin, 2005; Nocek & Braund, 1985; Robles, González, Ferret, Manteca & Calsamiglia, 2007). However, Nocek and Braund (1985) concluded that although frequent feeding did not result in higher intake, feeding four times a day did result in higher feed efficiency compared to feeding once a day. Also, Robinson and McQueen (1994) noticed a more stable rumen pH while feeding a protein supplement five times a day rather than feeding it two times a day. This is consistent with the findings of
Østergaard and Gröhn (2000) and Gustafsson, Andersson and Emanuelson (1995) that frequent feeding is essential in prevention of metabolic diseases in early lactation. Østergaard and Gröhn (2000) also concluded that metabolic diseases such as ketosis and left displaced abomasum were associated with decreased DMI for weeks prior to clinical diagnoses.

Feeding once a day, compared to feeding every other day, results in more disturbances in the flock, demonstrated in less grooming and ruminating time while more time is spent standing. The disturbance is attributed to the anticipation of feeding when the cows are fed once a day compared to less frequent feeding (Phillips & Rind, 2001).

Erdman, Moreland and Stricklin (1989) reported that increasing feed access time from 8 hours up to 20 hours a day increased feed intake, but decreased the feed efficiency. They concluded, however, that access of feed could be limited down to 8 hours a day without affecting intake as long as the feed were offered *ad lib.* and the cows were adapted to the feeding time. Nevertheless, a prerequisite for *ad lib.* feeding is that the feed is accessible for 20 hours a day (Fredriksen *et al.*, 1979) and this should fully utilize the IC of the cow. Nevertheless, feed quality must be an important factor; the less feed the cow needs to ingest to fulfil its nutrient needs, and the less ruminating time required by the feed, the less feed access time should be required for *ad lib.* feeding. Models predicting the DMI are usually designed for *ad lib.* feeding but feed access time is not included in the models (Ingvartsen, 1994).

### 1.2.3.2 Social behaviour and housing

If there is a choice between two feed types, where one has less accessibility or is somehow constrained, the proportion of that feed is decreased in the total intake (Forbes, 2007). As herd animals, the cattle develop a dominance rank in the flock in loose housing. The individuals at the top of the rank have the easiest access to the feed they want, but the lower ranked individuals may have to be satisfied with the lower quality feed (Forbes, 2007). Reduced feed manger space generates more competition over the feed, and the difference in feed intake between high-ranked and low-ranked cows increases, as the high-ranked cows spend more time eating than the low-ranked (Albright, 1993). Still, if feed manger space does not limit feed intake, cows in groups stimulate each other to eat, called social facilitation. Therefore, cows in groups eat more than individually fed cows (Albright, 1993; Ingvartsen & Andersen, 1993). Space allowance is also of large influence
on DMI of loose-housed cattle. Increasing space allowance can both increase the DMI and the feed conversion ratio. This is considered a result of stress caused by small space allowance per individual. A certain distance between animals in groups is needed to prevent aggressive and abnormal behaviour of the animals that induce stress in the flock (Ingvartsen & Andersen, 1993).

Photoperiod can affect production and intake of ruminants, they tend to eat more during daylight than at night, and increasing hours of light up to at least 16 hours has been shown to increase the intake of heifers and dairy cows (Peters, Chapin, Emery & Tucker, 1980, 1981). The length of the photoperiod is, however, rarely corrected for in intake prediction models for cattle (Ingvartsen, 1994). The thermal neutral zone of dairy cattle ranges from 5-20°C (NRC, 2001). Temperature below the thermoneutral zone increases intake, probably due to increased energy requirements, while temperatures above the thermoneutral zone decrease intake (McDonald et al., 2011; NRC, 2001).
1.3 NorFor feed evaluation system

NorFor is a semi-mechanistic feed evaluation system developed by specialists in the Nordic countries, based on current and published knowledge. It is a common project between farmers’ advisory organizations in Denmark, Norway, Sweden and Iceland. It takes into account the interactions in digestion and nutrient metabolism. Because of this interaction, the feed value of a single feedstuff is not constant, but changes with e.g. the amount of intake, the microbial effectivity and the structure of the feed. Therefore, the feed value of a single feedstuff is not of any great importance, but rather the feed value of a certain ration given under certain production circumstances (Volden, 2011c).

The system has to predict the intake of a ration in order to be able to predict its feed value. The feed intake prediction is based on the theory that intake is regulated physically and metabolically. The prediction of DMI in NorFor is based on the Danish fill unit system (Kristensen, 1983), where each feed has a fill value (FV) and the animal has an intake capacity (IC).

To predict the DMI, the FV of the feed ration and the IC of the animal is calculated (Volden et al., 2011). The FV of roughage is calculated based on organic matter digestibility (OMD) and NDF (equation 7). Additionally, the FV of silage is corrected for the amount of fermentation acids and NH$_3$-N, based on the Finnish SDMI index developed by Huhtanen et al. (2002) (equation 8). Concentrates have a fixed FV of 0.22 FV/kg DM (Volden, 2011a). The IC of a cow is calculated based on its ECM, days in milk (DIM) and BW (equation 12) (Volden et al., 2011).

Generally, the following equation must be fulfilled while predicting the DMI:

\[ IC = FV_{intake} \]  

The FV must be corrected for metabolic regulation, to prevent an overestimation on intake at high-energy diets, relative to the animal’s requirements. It must also be corrected for the substitution rate of the concentrate, based on changes in the NDF digestion in the rumen and the effect of rapidly degradable carbohydrates on ruminal digestion (Volden et al., 2011).

The IC is corrected for exercise if the cows are on pasture or confined in a loose-house system. The system also corrects for situations where intake is not optimal and ad lib. feeding is not possible (Volden et al., 2011).
1.4 Earlier studies on DMI of the Icelandic dairy cow

The breeding history of the Icelandic dairy cow is quite short compared to other Nordic breeds. The settlers that moved to Iceland in the ninth century, mostly from Norway, brought their domestic animals with them to Iceland. This is considered the origin of the Icelandic cattle breed. The import of livestock since then has been very limited, and no cattle has been imported after the year 1900. The effect of import on the breed is, therefore, considered very small (Adalsteinsson, 1981). The population is quite small; in 2013 it counted 27,000 dairy cows (Hagstofa Íslands, n.d.) and up to the 1970s when deep frozen semen was first used, the use of artificial insemination was limited. The use of selection indexes in breeding work also started around that time (Sigurdsson & Jonmundsson, 2011). A mature Icelandic cow weighs on average 470 kg (Konráðsdóttir, 2011) and in 2015 the average MY per “full-year-cow” was just over 5,800 kg (RML, n.d.).

A few studies were carried out studying the DMI of the Icelandic cow in 1990-1995 (Ríkharðsson, 2002; Ríkharðsson & Gestsson, 1995; Ríkharðsson, Gestsson, Ólafsson & Harðarson, 1997) and again in 2002-2007 (Sveinbjörnsson & Harðarson, 2008). Only the last study had TMR feeding, while the others used separate feeding. The main difference between those studies was, however, the amount of concentrate fed (Table 2). Increased concentrate feeding showed an increase in total DMI, and ECM and a small decrease in roughage DMI (Sveinbjörnsson & Harðarson, 2008).

Table 2. Average DMI, concentrate ratio (CR) and MY in previous intake studies of the Icelandic cow.

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<td>DMI</td>
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<td>MY</td>
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</table>

2 Two studies, 2002-2004 and 2006-2007 (Sveinbjörnsson & Harðarson, 2008)
3 The concentrate ratio in the TMR was 46% the first 3 weeks and 62% in weeks 3-12
2 Aims
Baldursdóttir (2010) developed an equation predicting the IC of the Icelandic dairy cow to use in NorFor, based on four of the previous studies on intake of the Icelandic cow. According to advisors and farmers using NorFor, the model fails too often to predict the DMI of the Icelandic dairy cows with sufficient accuracy. A large part of the misprediction may be attributed to the shortage of data available when the model was developed, especially in late lactation. Also, the use of the TMR in Icelandic milk production has increased since most of the data was collected and the supplementation of concentrate has also increased, in general. There was, therefore, an urgent need to obtain more and new data on the DMI of the Icelandic dairy cow under situations more common today.

The main purpose of this study were the following:

1. To collect new data on the DMI of the Icelandic dairy cow in late lactation.
2. To see whether a TMR feeding would increase the DMI compared to separate feeding
3. To compare two levels of concentrate ratio in a TMR in terms of the DMI
3 Materials and methods
Two studies were performed at the research farm Stóra-Ármót in South-Iceland. Firstly, an observational study (Study 1) was performed over a 9 week period, from February 11\textsuperscript{th} until April 11\textsuperscript{th} 2013. Secondly, an experimental study (Study 2) took place over a 26 week period, from November 4\textsuperscript{th} 2013 until May 2\textsuperscript{nd} 2014.

3.1 Housing and animal handling
In both studies, the cows were housed in a tie-stall barn and milked in a SAC\textsuperscript{®} milking parlour twice a day. The SAC\textsuperscript{®} milking system automatically registered the MY of each cow at every milking. Milk samples were taken regularly in both studies and analysed for protein, fat and lactose at the Research Centre for the Milking Industry in Iceland (Rannsóknastofa mjólkuriðnaðarins). The milk contents were used to calculate the ECM according to equation 2 (section 3.5).

In both studies TMR feed rations were mixed daily in a Mullerup\textsuperscript{®} mixer. Cows were fed individually and \textit{ad lib.}, aiming at 10-15\% residues. Four days a week the cows were fed manually and feed intake measurements were conducted. Rations were fed automatically during weekends by the mixing wagon. Water was available at the stalls at all times.

In both studies the cows were weighed and their BCS was estimated once weekly, immediately after the afternoon milking. The BCS was estimated using a scale from 1-5 with a 0.25 unit precision. The scale used is based on the work of Wildman \textit{et al.} (1982) where emaciated cows score 1, and obese cows score 5.

3.2 Study 1
Eighteen cows in different parities and at various stages of lactation (Table 3) were used. Individual samples of morning and afternoon milk were taken once every two weeks for compositional analyses.

<table>
<thead>
<tr>
<th>Parity</th>
<th>N</th>
<th>DIM</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Min/max</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>116</td>
<td>44/137</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>75</td>
<td>53/87</td>
<td></td>
</tr>
<tr>
<td>3+</td>
<td>6</td>
<td>84</td>
<td>58/104</td>
<td></td>
</tr>
</tbody>
</table>
All cows in Study 1 were fed the same TMR during the whole study period. The TMR was compositied of 44.8% roughage, 20.4% compound feed, 33.6% barley, 1.0% herring meal and 0.2% salt on a DM basis. Table 4 shows the chemical analysis of the TMR and the feedstuffs used. An average particle size of the TMR during the study period was 9.7±1.6 mm (X ± SD).

Table 4. Chemical analysis of the TMR and the feedstuffs used in it.

<table>
<thead>
<tr>
<th>Feed type</th>
<th>pH</th>
<th>DM (%)</th>
<th>Ash (%)</th>
<th>CP</th>
<th>NDF g/kg DM</th>
<th>TA</th>
<th>WSC</th>
<th>Starch</th>
<th>OMD %</th>
<th>FEm g/kg DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMR</td>
<td>4.9</td>
<td>51.2</td>
<td>73.0</td>
<td>159</td>
<td>314</td>
<td>25.1</td>
<td>61</td>
<td>257</td>
<td>79.4</td>
<td>0.94</td>
</tr>
<tr>
<td>Roughage</td>
<td>4.8</td>
<td>40.3</td>
<td>79.1</td>
<td>151</td>
<td>439</td>
<td>26.0</td>
<td>70</td>
<td>565</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>4.1</td>
<td>68.5</td>
<td>25.6</td>
<td>111</td>
<td>143</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound feed</td>
<td>88.6</td>
<td>119.6</td>
<td>256</td>
<td>121</td>
<td>72</td>
<td>406</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The compound feed consisted of 37.6% maize, 19.4% wheat, 12.9% soy bean meal, 10.0% sugar beet meal, 6.0% molasses, 5.5% fish meal, 3.0% lucerne meal, 2.0% saturated fats, 1.6% shell calcium, 1.1% magnesium phosphate, 0.8% salt, 0.8% monocalcium phosphate and 0.3% mineral and vitamin mix (see appendix 1 for detailed mineral composition). Four days a week individual intake was registered and the DMI was calculated according to equation 4 (section 3.5).

3.3 Study 2

3.3.1 Animals and experimental design

In Study 2, 37 cows were divided into three treatment groups, TMR55, TMR45 and SF45. Two were based on TMR feeding with concentrate ratio set at 55% (TMR55) and 45% (TMR45). The third treatment (SF45) was based on feeding concentrates and roughage separately and aimed at 45% concentrate ratio. In that treatment the barley was mixed with the roughage and compound feed was fed separately. This method of feeding can also be defined as a partial mixed ration. The cows were divided in the groups according to parity (1, 2 and 3+), expected calving date and the MY in earlier parities. Each group was then assigned randomly to 1 of 3 treatments (Table 5). The cows calved between September 25, 2013 and January 18, 2014. Four cows were removed from the study, one had mastitis (from treatment SF45), one cow in her second parity died after calving (from treatment TMR55) and 2 heifers calved later than expected and where, therefore, not included in the study (from treatment TMR55). No significant difference in parities, the BW or the BCS
existed across treatment groups at the beginning of treatment (p>0.1). Milk samples from individual cows were collected at morning and afternoon milking once every week.

Table 5. Number of cows in each treatment and parity.

<table>
<thead>
<tr>
<th>Parity</th>
<th>TMR55</th>
<th>TMR45</th>
<th>SF45</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>3+</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>12</td>
<td>11</td>
<td>33</td>
</tr>
</tbody>
</table>

*TMR55: TMR with 55% concentrates; TMR45: TMR with 45% concentrates; SF45: Separate feeding aimed at 45% concentrates.

3.3.2 Diet and intake registration

All three groups were fed the same TMR the first weeks postpartum before they started treatment (Pre-treatment feed in Table 6). Adaption to treatments began in week 3-6 postpartum and measurements were initiated a week later and lasted for 12 weeks, except for three cows that had been in the study for 11 weeks when the study ended. See appendix 2 for information on the stage of lactation for each cow at the beginning and at the end of treatment. Composition of each ration is presented in Table 6. In order to obtain 45% concentrate ratio in SF45 it was estimated that first parity and older cows should have 7.3 and 8.5 kg of compound feed per day, respectively. The compound feed was divided in three portions per day, fed at 08:00 (after morning milking), 13:00 and 18:00 (after evening milking). The same compound feed was used as in Study 1 and the composition is described in section 3.2.

Table 7 shows the chemical analysis of the TMR in each method and the feedstuffs used in Study 2. The average particle size of the roughage used in all rations during the study period was 16.6±1.3mm.

Table 6. Composition of feed rations on a dry matter basis.

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment feed¹</th>
<th>TMR55</th>
<th>TMR45</th>
<th>SF45 ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughage%</td>
<td>58</td>
<td>45</td>
<td>55</td>
<td>90</td>
</tr>
<tr>
<td>Barley%</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Compound feed%</td>
<td>35</td>
<td>50</td>
<td>39</td>
<td>-</td>
</tr>
</tbody>
</table>

¹TMR fed to all groups the first three weeks postpartum. ²The table does not represent the final composition of the feed ingested in treatment SF45. Due to the method of separate feeding the exact ratio of compound feed of the ingested feed was not known beforehand. The composition of SF45 ration given in the table is, therefore, the ration made of roughage and barley, the compound feed was then added to that in a certain amount, aiming for a total concentrate ratio of 45%.
Table 7. Chemical analysis of the feed and feedstuff used in Study 2.

<table>
<thead>
<tr>
<th>Feed type</th>
<th>pH</th>
<th>DM %</th>
<th>Ash g/kg DM</th>
<th>CP %</th>
<th>NDF g/kg DM</th>
<th>TA g/kg DM</th>
<th>WSC g/kg DM</th>
<th>Starch %</th>
<th>NH3-N g/kg prot</th>
<th>OMD %</th>
<th>FEm /kg DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMR55</td>
<td>5.0</td>
<td>49.7</td>
<td>86.7</td>
<td>185</td>
<td>321</td>
<td>37.5</td>
<td>188</td>
<td></td>
<td>0.40</td>
<td>84.1</td>
<td>1.01</td>
</tr>
<tr>
<td>TMR45</td>
<td>4.9</td>
<td>46.9</td>
<td>84.7</td>
<td>180</td>
<td>353</td>
<td>39.9</td>
<td>158</td>
<td></td>
<td>0.38</td>
<td>84.2</td>
<td>1.01</td>
</tr>
<tr>
<td>SF45</td>
<td>4.5</td>
<td>38.2</td>
<td>81.1</td>
<td>173</td>
<td>461</td>
<td>59.1</td>
<td>38</td>
<td></td>
<td>0.34</td>
<td>79.9</td>
<td>0.94</td>
</tr>
<tr>
<td>Roughage</td>
<td>4.4</td>
<td>36.9</td>
<td>84.3</td>
<td>179</td>
<td>476</td>
<td>70.6</td>
<td>10.4</td>
<td></td>
<td>0.34</td>
<td>82.0</td>
<td>0.98</td>
</tr>
<tr>
<td>Barley</td>
<td>3.9</td>
<td>45.5</td>
<td>35.9</td>
<td>124</td>
<td>245</td>
<td>70.0</td>
<td>27.6</td>
<td>452</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF ground¹</td>
<td>89.1</td>
<td>88.9</td>
<td>187</td>
<td>129</td>
<td>72.0</td>
<td>406</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF pellets²</td>
<td>88.9</td>
<td>85.9</td>
<td>192</td>
<td>122</td>
<td>72.0</td>
<td>406</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.02³</td>
</tr>
</tbody>
</table>

¹CF ground: Ground compound feed used in TMR55 and TMR45; ²CF pellets: pelleted compound feed used in SF45; ³Energy concentration of CF according to the producer.

Four days a week individual intake was registered and the DMI was calculated. Rations were fed manually these four days (Mon-Thu) and automatically in weekends, except for the compound feed in SF45, which was fed manually at all times.

The DM of the roughage and the TMR was estimated using a microwave oven each day of feed intake registration. A sample of 20-30 g was dried in a microwave oven for 2-3 minutes, or until it reached a steady weight, without burning. The DM of the barley was estimated once a week with the same method. Each day of feeding, the weight of raw materials used in the TMR was re-evaluated based on the estimated DM of its contents.

### 3.4 Feed sample collection and analysis

The roughage and the TMR were sampled daily (four days a week), composited weekly and frozen down for later analysis in both studies (9 samples of each in Study 1 and 24 samples of each in every treatment in Study 2). A sample of residues (one from each treatment in Study 2) as well as a sample of the barley and the compound feed was collected once a week and frozen down for later analysis in both studies. Table 8 and Table 9 show the number of samples analysed in each category, and which analyses were carried out for samples in each category in Study 1 and 2 respectively.
Table 8. Sample analysis program in Study 1.

<table>
<thead>
<tr>
<th></th>
<th>TMR</th>
<th>Residues</th>
<th>Roughage</th>
<th>Barley</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>OMD</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CP</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>NDF</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Ash</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Starch</td>
<td>1(^1)</td>
<td>-</td>
<td>1(^1)</td>
<td>2</td>
<td>1(^1)</td>
</tr>
<tr>
<td>WSC</td>
<td>1(^1)</td>
<td>-</td>
<td>1(^1)</td>
<td>2</td>
<td>1(^1)</td>
</tr>
<tr>
<td>pH and FP</td>
<td>8</td>
<td>-</td>
<td>8</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^1\) Estimated starch and WSC values, not analysed.

Table 9. Sample analysis program in Study 2.

<table>
<thead>
<tr>
<th></th>
<th>TMR(^1)</th>
<th>Residues(^1)</th>
<th>Roughage</th>
<th>Barley</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>24</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td>OMD</td>
<td>24</td>
<td>6</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein</td>
<td>24</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td>NDF</td>
<td>24</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Ash</td>
<td>24</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td>Starch</td>
<td>24</td>
<td>6</td>
<td>-</td>
<td>4</td>
<td>1(^2)</td>
</tr>
<tr>
<td>WSC</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>4</td>
<td>1(^2)</td>
</tr>
<tr>
<td>FP</td>
<td>24</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^1\) Number of samples from each treatment. \(^2\) Estimated starch and WSC values, not analysed.

The DM of the fermented feed samples was determined with a single-step drying method by drying the samples in a hot-air drying cabinet at 60°C to a constant weight, for approximately 44 h, and corrected for lost volatiles according to equation 5 in section 3.5. Compound feed samples were duplicated; one sample was used for a DM analysis and the other was used for a further analysis. The DM of the compound feed was determined in a hot-air drying cabinet at 103°C as described in European Commission Regulation EC No. 152/2009.

In Study 1, the nitrogen content was analyzed using the Kjeldahl (1883) method, and in Study 2 the Dumas (1831) method was used. In both studies the crude protein (CP) was calculated as N × 6.25. The \(\text{NH}_3\)-N was determined using the MgO method (Åkerlind, Weisbjerg et al., 2011) in Study 1, but with an electrode in Study 2.

Ash was determined at 550°C according to the European Commission Regulation EC No. 152/2009 and organic matter was determined by subtracting the ash from the dry matter.
content. *In vitro* organic matter digestibility (IVOS) was determined using the method presented by Tilley and Terry (1963) in all samples except the residues in Study 1. In that case OMD was estimated with NIR technology and is based on the OMD of the TMR. The NDF was analyzed using the Ankom technique according to the method described by Van Soest, Robertson and Lewis (1991) except that sulphite and amylase were used for all samples (ISO 16472:2006 IDT).

The starch in the barley in Study 1 and in the TMR, the residues and the barley in Study 2 was analyzed using the Soxtech method with an ethanol extraction as described in the European Commission Regulation EC No. 152/2009. The water soluble carbohydrates (WSC) in the barley in Study 1 and in the roughage and the barley in Study 2 was analyzed enzymatically based on the procedure of Larsson and Bengtsson (1983). Fatty acids and ethanol were analyzed with the HPLC technique, adapted by Eurofins (Åkerlind, Weisbjerg et al., 2011).

In Study 1 the starch and the WSC were not analysed in the TMR, the roughage and the compound feed. It was estimated in the compound feed based on its ingredients and their tabulated starch and WSC contents in NorFor feed table (NorFor, 2015). The starch and the WSC content of the roughage was estimated based on the DM and the fermentation intensity using the NorFor feed table (NorFor, 2015). The starch and the WSC content of the TMR was calculated based on the analysed content of the barley and estimated content of the roughage and the compound feed. In Study 2 the starch and the WSC were not analyzed in the compound feed, but was estimated the same way as in Study 1.

In Study 1 the particle length of the TMR was estimated manually once a week with the Penn State particle separator equipped with three sieves (with pore size 1.18, 8.0 and 19.0 mm) and a bottom pan (Kononoff, Heinrichs & Buckmaster, 2003). In Study 2 the same method was used once a week to estimate the length of the roughage after chopping.

### 3.5 Calculations

ECM was calculated according to Sjaunja, Bøvre, Junkkarainen, Pedersen, and Setala (1990), see equation 2.

\[
ECM = MY \times \left( 0.01 + 0.122 \times \frac{f_{\text{milk}}}{10} + 0.077 \times \frac{p_{\text{milk}}}{10} + 0.053 \times \frac{l_{\text{milk}}}{10} \right)
\]  

Where ECM is the energy corrected milk; MY is the milk yield (kg); \( f_{\text{milk}} \), \( p_{\text{milk}} \), and \( l_{\text{milk}} \) are the contents of fat, protein and lactose in milk, respectively, g/kg.
Estimated daily ECM yield given a certain 305 days average yield of ECM was calculated by equation 3 (Åkerlind, Nielsen & Volden, 2011).

\[ ECM = a + b \times Y_{Herd} - c \times DIM + \ln(DIM) \times d \] (3)

Where \( Y_{Herd} \) is the herd’s average ECM yield per cow, kg/305d; \( DIM \) is days in milk; \( a, b, c \) and \( d \) are regression coefficients presented in the following table for primiparous and multiparous cows of the Icelandic breed.

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td>0.88</td>
<td>0.00288</td>
<td>0.04009</td>
<td>1.627</td>
</tr>
<tr>
<td>Multiparous</td>
<td>6.34</td>
<td>0.00269</td>
<td>0.06234</td>
<td>1.569</td>
</tr>
</tbody>
</table>

Four days a week in both studies the feed rations were weighed and fed manually to individual cows and the corresponding residues were weighed the morning after. A sample was taken from the TMR daily and at the end of the week they were combined in one sample. In Study 2, there were three samples each week, one for each treatment. A residue sample was taken once a week, in Study 1 it was composited of residues from all the cows included in the study, and in Study 2, there were three residue samples each week, one from each treatment. Those samples were analysed for DM.

The DMI was calculated for four days a week, and the analysed DM (corrected for lost volatiles, eq.5) of TMR and residues was used according to equation 4.

\[ DMI = \frac{TMR_{fed} \times DM_{TMR}}{100} - res \times \frac{DM_{res}}{100} \] (4)

Where \( DMI \) is the dry matter intake (kg DM/day); \( TMR_{fed} \) is the amount of ration fed to a cow (kg); \( DM_{TMR} \) is the DM of the ration fed (%); \( res \) is the amount of feed residues from the respective cow (kg) and \( DM_{res} \) is the DM of the residues %.

The DM of the fermented feed samples (TMR, roughage, barley and residues) was corrected for lost volatiles during drying according to Porter and Murray (2001), see equation 5.

\[ DM_{corr} = DM_{uncorr} + (LAF \times 0.45 - 0.09 \times pH) + ACF \times (1.5 - 0.223 \times pH) + PRF \times (1.4 - 0.182 \times pH) + BUF \times (1.9 - 0.272 \times pH) + ALF + NH_3N \times 0.6 \] (5)

Where \( DM_{corr} \) is the corrected and final dry matter, g/kg; \( DM_{uncorr} \) is the uncorrected DM; \( LAF \) is the amount of lactic acid in the feed, correction for loss of \( LAF \) is only included when \( pH \) of the feed is lower than 5.0. \( ACF \) is the amount of acetic acid in the feed; \( PRF \) is
the amount of propionic acid in feed; BUF is the amount of butyric acid in the feed; ALF is the amount of lower alcohols in the feed and NH₃N is the amount of ammonia nitrogen in the feed. All amounts are in g/kg uncorrected DM.

Energy concentration in FEm/kg DM was calculated for the roughage, TMR and residues according to Van Es (1975) and MAFF (1975), see equation 6.

\[ \text{FEm} = (0.6 \times (1 + 0.004 \times (q - 57))) \times 0.9752 \times \text{GE} / 6900 \]  

(6)

Where \( q \) is the metabolizable energy (ME) to gross energy (GE) ratio,

\[ \text{ME} = 15 \times \text{DOMD (g/kgDM)} \]  

and \( \text{DOMD (g/kgDM)} = (0.98 \times \text{OMD} - 4.8) \times 10 \)

3.5.1 Fill value calculations

In NorFor each feed has a fill value (FV) expressed in fill units. Concentrate feedstuffs have a fixed FV of 0.22 FV/kg DM and FV of roughages is calculated according to the following equation (Volden, 2011a):

\[ FV = \frac{0.86 - \text{OMD} - 0.005}{0.94 + 0.56 \times \exp(-0.000029 \times (\frac{\text{NDF}}{10})^{2.9})} \]  

(7)

Where \( FV \) is the roughage fill value, kg DM⁻¹; \( \text{OMD} \) is organic matter digestibility, %; and \( \text{NDF} \) is the feed NDF content, g/kg DM.

The fermentation quality affects the intake of silage. The FV of silage is corrected for silage fermentation products according to equation 8 (Volden, 2011a).

\[ FV_{\text{corr}} = FV \times (1 - \left(-\frac{0.000531 \times (\text{TAF})^2 - 6400}{100} + \frac{-4.765 \times (\ln(\text{NH}_3N) - \ln(50))}{100}\right)) \]  

(8)

Where \( FV_{\text{corr}} \) is the silage fill value corrected for silage fermentation products, kg DM⁻¹, \( FV \) is the roughage fill value, kg DM⁻¹ (equation 7); \( \text{TAF} \) is the content of total fermentation acids in the ensiled feed, g/kg DM and \( \text{NH}_3N \) is the content of ammonia N in the ensiled feed, g/kg N.

The FV intake is calculated based on the FV of the individual feedstuffs, but the concentrate substitution rate and a metabolic regulation factor must also be taken into account. The FV intake is calculated according to equations 9-11 (NorFor, 2014; Volden et al., 2011).

\[ FV_{\text{intake}} = \sum_i \text{DMI}_i \times FV_i + \sum_j FV_j \times FV_{\text{SubR}} + FV_{\text{MR}} \]  

(9)

Where \( FV_{\text{intake}} \) is the feed intake expressed in fill units; \( FV_i \) is the fill value of the i’th concentrate feed, kg DM⁻¹; \( FV_j \) is the basis fill value of the j’th roughage (eq. 7 and 8);
$F_{V,\text{SubR}}$ is the substitution rate factor, 0 to 1 (eq.10); $F_{V,\text{MR}}$ is the metabolic regulation factor (eq.11).

$$F_{V,\text{SubR}} = 0.97 + 0.562 \times \left( \frac{ST\_SU\_DM}{1000} - 0.2199 \right) \times 0.1 - 0.1932 \times \left( \frac{ST\_SU\_\text{intake}}{1000} - 5.122 \right) \times 0.05 \tag{10}$$

Where $F_{V,\text{SubR}}$ is the roughage substitution correction factor, 0 to 1; $ST\_SU\_DM$ is the proportion of starch and sugars in the diet, g/g DM and $ST\_SU\_\text{intake}$ is the starch and sugar intake, g/d

$$F_{V,\text{MR}} = 1.453 - \left( \frac{2530}{1 + \exp\left( (0.466 - F_{V,r})/0.065 \right) } \right) \tag{11}$$

Where $F_{V,\text{MR}}$ is the roughage metabolic correction factor; $F_{V,r}$ is the mean basis roughage fill value in the diet, kg DM$^{-1}$ (eq.7 and 8).

The individual intake capacity is expressed in the FV and calculated according to equation 12 (NorFor, 2014).

$$IC_{\text{cow}} = a \times DIM^b \times \exp^{c \times DIM} - DIM^{-d} + e \times \left( ECM + \left( \frac{(N{E}_\text{mob} + N{E}_\text{dep}) \times h}{3.14} \right) \right) + (BW - f) \times g \tag{12}$$

Where $IC_{\text{cow}}$ is the intake capacity of a lactating cow (FV/day), factors a-h are the multiple regression coefficients (current coefficients for the Icelandic breed are given in the following table; DIM is the days in milk; ECM is the production of energy corrected milk (kg/day) described in equation 2; $N{E}_\text{mob}$ is the energy supply from body reserve mobilization (MJ/day) described in equation 14.; $N{E}_\text{dep}$ is the energy requirement for deposition (MJ/day) described in equation 13; BW is the live body weight (kg).

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td>2.51</td>
<td>0.134</td>
<td>-0.0012</td>
<td>0.025</td>
<td>0.091</td>
<td>370</td>
<td>0.006</td>
<td>0.5</td>
</tr>
<tr>
<td>Multiparous</td>
<td>2.77</td>
<td>0.134</td>
<td>-0.0011</td>
<td>0.003</td>
<td>0.091</td>
<td>450</td>
<td>0.006</td>
<td>0.5</td>
</tr>
</tbody>
</table>

$$N{E}_\text{dep} = BW \_\text{change}_\text{mobdep} \times 31 \tag{13}$$

$$N{E}_\text{mob} = -1 \times BW \_\text{change}_\text{mobdep} \times 24.8 \tag{14}$$
Where $BW_{change\_mobdep}$ is the body weight change during mid and late lactation depending on deposition (kg/day) described in equation 15.

$$BW_{change\_mobdep} = (BW_{mob} + b \cdot \sqrt{DIM} \cdot \ln(DIM + 2.1) + c \cdot (\ln(DIM + 2.1))^2)$$

$$- (BW_{mob} + b \cdot \sqrt{(DIM - 1)} \cdot \ln(DIM - 1) + c \cdot (\ln(DIM - 1))^2$$

(15)

Where $BW_{mob}$ is the total body weight loss due to mobilisation in early lactation (kg) calculated according to equation 16; $b$ and $c$ are factors calculated according to equation 17 and 18; $DIM$ are days in milk. This equation applies when $DIM>1$.

$$BW_{mob} = a \cdot (1 + 2 \cdot \frac{BCS_{calv} - 3.5}{3.5})$$

(16)

$$b = 0.04 + 0.05 \cdot BW_{mob} - 0.305 \cdot (BCS_{calv} - BCS_{end}) \cdot 2$$

(17)

$$c = \frac{b}{2.4207/\sqrt{3.953}} + 0.151 \cdot (- (BCS_{calv} - BCS_{end}) \cdot 2 \cdot 2.55)$$

(18)

Where $a$ is a factor representing the mobilisation (kg) in BW change in early lactation ($DIM$ 0-70) provided $BCS_{calv}$ is 3.5. For the Icelandic breed this is 15 for primiparous cows and 20 for older cows; $BCS_{calv}$ is the body condition score at calving; $BCS_{end}$ is the body condition score at drying off.

In this thesis it is assumed that $BCS_{calv}$ and $BCS_{end}$ is at average 3.5. This is because BCS was not estimated at calving or at the end of lactation, but also because the BCS scoring is a subjective estimation. It is, therefore, not reliable to use individual BCS when the IC equation is being parameterized.

### 3.5.2 Statistical evaluation calculations

The mean square prediction error (MSPE) and the root mean square prediction error (RMSPE) are calculated according to equations 19 and 20.

$$MSPE = \frac{\sum (obs - pred)^2}{n}$$

(19)

$$RMSPE = \sqrt{MSPE}$$

(20)

Where $obs$ is the observed DMI, $pred$ is the predicted DMI and $n$ is the number of pairs of values of observed and predicted DMI being compared.
MSPE can be described as the sum of mean bias, line bias and random variation, calculated according to equations 21-23 (Roseler, Fox, Pell & Chase, 1997).

\[
\begin{align*}
\text{Mean bias} &= (\text{obs} - \text{pred})^2 \\
\text{Line bias} &= S_p^2 \times (1 - b)^2 \\
\text{Random variation} &= S_A^2 \times (1 - r^2)
\end{align*}
\]

Where \( S_p^2 \) and \( S_A^2 \) are the variances of the predicted and observed DMI, respectively; \( b \) is the slope of the regression of observed on predicted and \( r \) is the correlation coefficient of observed and predicted.

### 3.6 Statistical analysis

All statistics were analyzed using SAS Enterprise Guide 6.1© (SAS Institute, 2013). Graphs were plotted in SAS Enterprise Guide 6.1© and Microsoft® Office Excel.

In Study 1 the arithmetic means of the DMI, ECM, BW and BCS were calculated using the PROC MEANS in SAS. Difference between parities was analyzed using the PROC TTEST in SAS Enterprise Guide.

In Study 2, the effect of treatment on intake parameters, ECM and milk contents were analyzed using the PROC GLM in SAS. The models for the DMI, roughage dry matter intake (rDMI), concentrate DMI (cDMI) and FEm intake/day included the main effects of the BCS, BW, treatment and intercept. The stage of lactation was excluded from the model because it was not found significant, most likely because the cows were all in similar stage of lactation during the study period. The model analyzing the ECM included the main effects of the BCS, BW, Weeks from calving (WFC), treatment and intercept. The model analyzing the proportional intake of starch and sugars (ST+SU%), and NDF (NDF%) as well as the milk contents (fat, protein and lactose) included the main effects of the treatment and intercept. The treatment means were separated using the PDIFF statement in SAS. The results are presented as least squared means. The significancy of the difference is marked as follows, \( p<0.001: ***; p<0.01: **; p<0.05: *; p<0.1: \dagger; p>0.1: \text{NS} \).
The effects of the stage of lactation, BW, BCS and ECM on DMI was tested separately for primiparous and multiparous cows using the PROC GLM. The following model was used:

\[ Y = \mu + WFC_i + (WFC_i)^2 + BCS_j + BW_k + ECM_l + e_{ijkl} \]

Where

- \( Y_{ijkl} \) is the DMI
- \( \mu \) is the overall mean
- \( WFC_i \) is the effect of stage of lactation measured in weeks from calving (i=5 to 18)
- \( BCS_j \) is the effect of body condition score (j=1 to 5)
- \( BW_k \) is the effect of body weight
- \( ECM_l \) is the effect of energy corrected milk yield
- \( e_{ijkl} \) is the error

Data from both studies were used to estimate new regression coefficients for equation 12 (section 3.5.1) for the Icelandic breed. Separate coefficients were estimated for primiparous and multiparous cows. To parameterize the equation and fit the coefficients to the model the Solver tool (Fylstra, Lasdon, Watson & Waren, 1998) in Microsoft® Excel was used, which employs a generalized reduced gradient non-linear optimization code (Lasdon, Waren, Jain & Ratner, 1978; Sveinbjörnsson, Huhtanen & Udén, 2006). The parameters were fitted to the model by minimizing the RMSPE for animal IC.
4 Results

4.1 Study 1

At the end of Study 1 primiparous cows had reached on average 174 DIM with a maximum of 195 DIM. Multiparous cows had reached on average 139 DIM with a maximum of 162 DIM. Table 10 presents the average DMI, ECM, BW and BCS of primiparous and multiparous cows in Study 1. All cows except one increased their BW during the study period. Fourteen cows gained BCS, two were stable and two lost BCS during the period. Average BW change was 26 and 11 kg for primiparous and multiparous cows respectively and the average change in the BCS during the period was 0.19 and 0.31 for primiparous and multiparous cows respectively. The range in the BW and the BCS was quite large in both primiparous and multiparous cows (Table 10).

Table 10. DMI, ECM, BW and BCS of primiparous and multiparous cows in Study 1.

<table>
<thead>
<tr>
<th></th>
<th>Primiparous cows, n=9</th>
<th>Multiparous cows, n=9</th>
<th>p-value</th>
<th>Effect of parity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Min/max</td>
<td>Mean</td>
</tr>
<tr>
<td>DMI</td>
<td>16.1</td>
<td>0.14</td>
<td>11.7/26.3</td>
<td>20.3</td>
</tr>
<tr>
<td>ECM</td>
<td>20.4</td>
<td>0.19</td>
<td>14.3/31.7</td>
<td>32.7</td>
</tr>
<tr>
<td>BW</td>
<td>471</td>
<td>2.86</td>
<td>397/595</td>
<td>510</td>
</tr>
<tr>
<td>BCS</td>
<td>3.42</td>
<td>0.02</td>
<td>3.00/4.25</td>
<td>3.30</td>
</tr>
</tbody>
</table>

Figure 2 shows the difference between ECM and DMI through the lactation, from 40 to 200 DIM for the primiparous (a) and multiparous cows (b). The ECM of the primiparous cows peaked at around 110 DIM and decreased slowly after that throughout the lactation. The DMI, however, decreased slightly and steadily throughout the lactation. This relationship is different for the multiparous cows. The figure shows that the ECM peaks at around 70 DIM, around 7 weeks before the peak in the DMI is reached.
Figure 2. Changes in ECM and DMI of primiparous (a) and multiparous (b) cows from 40 days after calving through 200 days after calving.
4.2 Study 2

4.2.1 BW and BCS
In Study 2 the BW was 460±45 kg and 485±42 kg (X±SD) for primiparous and multiparous cows respectively. The BCS of primiparous and multiparous cows was 3.23±0.29 and 2.94±0.36 (X±SD) respectively. There was little change in the BCS of both primiparous and multiparous cows and the treatment had no significant effect on the BCS change (p=0.325 and p=0.602 for primiparous and multiparous cows respectively). Primiparous cows increased their BW more (35.5, 35.0, 35.5 kg for TMR55, TMR45 and SF45 respectively) than the multiparous cows (16.4, 17.3, and 20.9 for TMR55, TMR45 and SF45 respectively). The BW change was not affected by treatment (p=0.998 and p=0.930 for primiparous and multiparous cows respectively).

4.2.2 Intake and milk production
Primiparous cows yielded 22.5±5.3 kg ECM and multiparous cows yielded 30.4±6.3 kg ECM on average (X±SD). Primiparous and multiparous cows offered the SF45 feed consumed on average 44.7% and 46.3% concentrates of total DM intake, respectively. Among the primiparous cows, the cows offered TMR55 had the highest total DMI (Table 11) while the multiparous cows on the TMR45 diet had a tendency of a higher DMI than cows in the other two treatments (Table 12). Both primiparous and multiparous cows offered TMR55 had significantly lower DMI of roughage and higher DMI of concentrates than the cows in the other two treatments. The cows offered the TMR diets had significantly higher energy intake (FEm/day) and yield of ECM than the cows on separate feeding (p<0.001). There was, however, no significant difference between cows offered TMR55 and TMR45 in neither energy intake nor yield of ECM (p>0.1).

The primiparous cows fed TMR produced milk with significantly higher fat content than those fed concentrates separately from roughages (p<0.05). The primiparous cows offered TMR55 produced milk with significantly higher protein content than cows under the other treatments (p<0.01). Lactose content was not affected by the treatment of primiparous cows (p>0.1).

Effects of treatment on the milk content were slightly different for the multiparous cows. The milk fat content was not affected by the treatment (p>0.1), and the protein content was significantly lower for the cows fed TMR45 than the other two treatments (p<0.05). Milk
lactose content, however, was significantly lower in milk from cows fed SF45 than the other two treatments (p<0.001).

Table 11. Intake, ECM and milk contents of primiparous cows in each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TMR55</th>
<th>TMR45</th>
<th>SF45</th>
<th>p-value</th>
<th>Treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI</td>
<td>17.2a</td>
<td>16.7ab</td>
<td>16.2b</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>rDMI</td>
<td>7.8a</td>
<td>9.2b</td>
<td>9.0b</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>cDMI</td>
<td>9.4a</td>
<td>7.5b</td>
<td>7.2b</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>FEm/day</td>
<td>17.4a</td>
<td>16.9a</td>
<td>15.1b</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>NDF intake%</td>
<td>31.6a</td>
<td>35.3b</td>
<td>33.4c</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>ST+SU intake%</td>
<td>19.8a</td>
<td>16.3b</td>
<td>20.5a</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>ECM</td>
<td>24.6a</td>
<td>23.3a</td>
<td>20.7b</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>Fat%</td>
<td>3.91a</td>
<td>3.85a</td>
<td>3.65b</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Prot%</td>
<td>3.43a</td>
<td>3.23b</td>
<td>3.30b</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>Lac%</td>
<td>4.86a</td>
<td>4.79a</td>
<td>4.80a</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

1 Main effect of dietary treatment, 2 NDF intake as a proportion of total intake, 3 Starch and sugar intake as a proportion of total intake, 4 Fat, protein and lactose content of the milk.

a,b,c: Values with different superscript within a row are statistically different, p<0.05.

Table 12. Intake, ECM and milk contents of multiparous cows in each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TMR55</th>
<th>TMR45</th>
<th>SF45</th>
<th>p-value</th>
<th>Treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI</td>
<td>18.1a</td>
<td>18.6b</td>
<td>18.2a</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td>rDMI</td>
<td>8.2a</td>
<td>10.2b</td>
<td>9.8b</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>cDMI</td>
<td>10.0a</td>
<td>8.4b</td>
<td>8.4b</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>FEm/day</td>
<td>18.4a</td>
<td>18.9a</td>
<td>17.1b</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>NDF intake%</td>
<td>32.0a</td>
<td>34.4b</td>
<td>32.8a</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>ST+SU intake%</td>
<td>18.7a</td>
<td>16.5b</td>
<td>21.2c</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>ECM</td>
<td>31.0a</td>
<td>32.2a</td>
<td>27.7b</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>Fat%</td>
<td>3.92a</td>
<td>3.81a</td>
<td>3.83a</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Prot%</td>
<td>3.25a</td>
<td>3.14b</td>
<td>3.25a</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Lac%</td>
<td>4.73a</td>
<td>4.76a</td>
<td>4.63b</td>
<td></td>
<td>***</td>
</tr>
</tbody>
</table>

a,b,c: Values with different superscript within a row are statistically different, p<0.05.

4.2.3 Effects of animal factors on DMI

Regression estimates of WFC, BCS, BW and ECM on DMI in this study are given in Table 13. The intercept estimate is calculated for treatment SF45, but the intercept for the other treatments was not significantly different (p>0.1).

Effect of WFC was not significant for the primiparous cows, neither the linear effect (p=0.37) nor the quadratic effect (p=0.67). The DMI of multiparous cows increased
slightly with an increased stage of lactation, with significant quadratic effect (p=0.009),
during those 4-18 WFC that the study lasted. For the primiparous cows, a clear negative
effect of the BCS on the DMI was significant (p=0.002), while this effect was not
significant for the multiparous cows (p=0.24). The positive effect of the BW was little, but
significant for both the primiparous (p<0.0001) and the multiparous cows (p<0.0001). The
DMI increased significantly (p<0.0001) with increased ECM, and the effect was even more
distinct for the primiparous cows than for the multiparous cows.

Table 13. Effect of WFC, BCS, BW and ECM on DMI of primiparous and multiparous cows.

<table>
<thead>
<tr>
<th></th>
<th>Primiparous cows</th>
<th></th>
<th>Multiparous cows</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE</td>
<td>Estimate</td>
<td>SE</td>
</tr>
<tr>
<td>Intercept</td>
<td>4.564*</td>
<td>2.121</td>
<td>4.300*</td>
<td>1.808</td>
</tr>
<tr>
<td>WFC</td>
<td>0.191 NS</td>
<td>0.211</td>
<td>0.515**</td>
<td>0.172</td>
</tr>
<tr>
<td>WFC²</td>
<td>-0.004 NS</td>
<td>0.0094</td>
<td>-0.020**</td>
<td>0.0078</td>
</tr>
<tr>
<td>BCS</td>
<td>-2.367**</td>
<td>0.735</td>
<td>-0.354 NS</td>
<td>0.302</td>
</tr>
<tr>
<td>BW</td>
<td>0.029***</td>
<td>0.0049</td>
<td>0.019***</td>
<td>0.002</td>
</tr>
<tr>
<td>ECM</td>
<td>0.211***</td>
<td>0.038</td>
<td>0.108***</td>
<td>0.019</td>
</tr>
<tr>
<td>R²</td>
<td>0.728</td>
<td></td>
<td>0.335</td>
<td></td>
</tr>
<tr>
<td>RMSPE</td>
<td>1.295</td>
<td></td>
<td>1.456</td>
<td></td>
</tr>
</tbody>
</table>

4.3 NorFor DMI prediction model

4.3.1 Regression coefficients in IC equation

Based on data from Study 1 and Study 2 new regression coefficients for equation 12 are
suggested and presented in Table 14.

Table 14. Current and suggested regression coefficients used in equation 12 to predict animal IC.

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>2.51</td>
<td>0.134</td>
<td>-0.0012</td>
<td>0.025</td>
<td>0.091</td>
<td>370</td>
<td>0.006</td>
<td>0.5</td>
</tr>
<tr>
<td>Suggested</td>
<td>1.65</td>
<td>0.221</td>
<td>-0.0030</td>
<td>0.025</td>
<td>0.102</td>
<td>370</td>
<td>0.007</td>
<td>0.5</td>
</tr>
<tr>
<td>Multiparous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>2.77</td>
<td>0.134</td>
<td>-0.0011</td>
<td>0.003</td>
<td>0.091</td>
<td>450</td>
<td>0.006</td>
<td>0.5</td>
</tr>
<tr>
<td>Suggested</td>
<td>3.79</td>
<td>0.134</td>
<td>-0.0015</td>
<td>0.003</td>
<td>0.044</td>
<td>470</td>
<td>0.006</td>
<td>0.5</td>
</tr>
</tbody>
</table>

It is suggested that the coefficients a, b, c, e and g are changed in the equation for
primiparous cows. Raising coefficient f to 400 decreased the RMSPE slightly, but it had
negative effect on the line bias and was, therefore, not changed.
In the equation for multiparous cows it is suggested that the coefficients $a$, $c$, $e$ and $f$ are changed. The changes that made the most difference on RMSPE were raising coefficient $a$ from 2.77 to 3.79 and lowering coefficient $e$ from 0.091 to 0.044. Minimal changes were made on coefficients $c$ and $f$.

### 4.3.2 Model evaluation

The model accuracy evaluation criterions are presented in Table 15. The suggested coefficients decrease the RMSPE of 0.15 and 0.24 kg DM for primiparous and multiparous cows respectively. The suggested coefficients also result in an increased $R^2$ in both cases. The line bias, however, as a proportion of MSPE increases in the model for the primiparous cows when the suggested coefficients are used compared to the current coefficients. The suggested coefficients in the model for multiparous cows, on the other hand, greatly increase the accuracy of the model, where the line bias proportion of MSPE decreases from 27.2% to 3.2%.

<table>
<thead>
<tr>
<th></th>
<th>Primiparous cows</th>
<th>Multiparous cows</th>
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<tbody>
<tr>
<td></td>
<td>Current coefficients</td>
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<tr>
<td>Mean bias</td>
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<tr>
<td>Line bias</td>
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</tr>
<tr>
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<td>95.0%</td>
</tr>
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<td>$R^2$</td>
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</tr>
<tr>
<td>p-value</td>
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<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 15. Evaluation of DMI prediction with equation 12 using current and suggested coefficients.

Regression of predicted rDMI on observed rDMI for primiparous cows is shown in Figure 3 and for multiparous cows in Figure 4. The model predicting rDMI of primiparous cows is quite accurate in the range from 7.0 to 9.0 kg DM. The regression line for the multiparous cows, however, has a very low slope (0.28) which limits the accuracy to a very limited range of intake. When the rDMI is less than 9.0 kg, the model tends to overpredict the intake, while it underpredicts roughage intake greater than 9.0 kg DM.
Figure 3. Observed rDMI of primiparous cows compared to predicted rDMI using the suggested coefficients (p<0.001).

Figure 4. Observed rDMI of multiparous cows compared to predicted rDMI using the suggested coefficients (p<0.001).
Figures 5 and 6 plot the rDMI residuals (predicted rDMI – observed rDMI) against the observed total DMI for primiparous and multiparous cows. Both plots show a clear tendency of an overprediction of rDMI when total DMI is low (<16 kg DM and <19 kg DM for primiparous and multiparous cows respectively) and when total DMI increases the model tends to underpredict the rDMI.

**Figure 5.** Relationship between DMI residuals (predicted – observed) and the observed DMI of primiparous cows.

**Figure 6.** Relationship between DMI residuals (predicted – observed) and the observed DMI of multiparous cows.
The IC of primiparous and multiparous cows with a MY of 4,500 and 6,000 kg ECM/305 days is plotted in Figure 7. It shows that the suggested coefficients in the IC equation decrease the predicted IC of the primiparous cows compared to the coefficients currently in use. The difference increases in late lactation. The IC for multiparous cows calculated with the suggested coefficients is slightly higher than when the current coefficients are used.

Figure 7. The IC of primiparous and multiparous cows plotted as a function of DIM calculated with both current and suggested coefficients (Table 14) in equation 12.
5 Discussion

5.1 Study 1

According to Table 10, the average DMI of primiparous cows in Study 1 was 79% of the average DMI of multiparous cows. This is in accordance with earlier findings for the Icelandic dairy cow where the intake of primiparous cows has been reported to be 77%-83% of the intake of multiparous cows (parities 3 and higher) during early lactation (Ríkharðsson, 2002; Sveinbjörnsson & Harðarson, 2008). It is also known that the general IC of primiparous cows, weighing around 500 kg and calving at an age of 2 years, is around 80% of the intake of multiparous cows (Jarrige, 1986; Kristensen & Ingvartsen, 1986).

The cows in Study 1 were quite late in their lactation stage; primiparous cows were on average 174 DIM with a maximum of 195 DIM at the end of the study and multiparous cows were on average at 139 DIM, with a maximum of 162 DIM. Earlier studies on Icelandic cows have mostly been focusing on an earlier part of the lactation, i.e. only the first 84-112 DIM (Ríkharðsson, 2002; Sveinbjörnsson & Harðarson, 2008). Study 1 was meant to increase the knowledge on intake capacities of Icelandic dairy cows later in lactation, because a lack of data in late lactation was reported as one of the defects of the data used by Baldursdóttir (2010) in the development of the equation describing IC of the Icelandic cow. Due to the difference in the lactation stage, the results of Study 1 are not quite comparable with previously reported observations.

The primiparous and multiparous cows in Study 1 were on average 471 kg and 510 kg respectively, which is considerably higher BW than in earlier studies, 379-436 kg and 429-505 kg for the primiparous and multiparous cows, respectively (Ríkharðsson et al., 1997; Sveinbjörnsson & Harðarson, 2008). Higher BW can partly be explained by breeding improvements since earlier studies were conducted, but also by the stage of lactation. The primiparous cows are later in lactation stage and have grown and matured from the time of calving and are therefore considerably heavier than in early lactation. Also, this late in lactation the energy balance should be positive, and the cows have started to gain weight, after a period of mobilization during the first weeks after calving (Ingvartsen & Andersen, 2000).

In Study 1 the average DMI was 16.1 and 20.3 kg for primiparous and multiparous cows, respectively. This is considerably lower than was observed by Sveinbjörnsson and
Harðarson (2008) feeding a TMR with 62% concentrate ratio (17.1 and 22.2 kg DM/day for primiparous and multiparous cows respectively). However, the yield of ECM observed in Study 1 (20.4 and 32.7 for primiparous and multiparous cows respectively) was higher than that of the study conducted by Sveinbjörnsson and Harðarson (2008) (18.8 and 29.8 kg for primiparous and multiparous cows respectively).

A higher concentrate ratio could be the major reason for higher intake in the study by Sveinbjörnsson and Harðarson (2008). Still, it is surprising that the MY was higher in Study 1, especially because of the late stage of lactatation of the primiparous cows, since most of them have probably passed the peak of the MY at the time of the study (Figure 2). However, the low MY in the earlier study was explained at the time with a poor body condition of the cows at the time of calving, i.e. a less body mass to mobilize in the early lactation, which probably led to a lower MY. In 2002-2004 another study was conducted (Sveinbjörnsson & Harðarson, 2008), with separate feeding and the concentrate ratio was at average 47-48% during the first 12 weeks of lactation. The average DMI in that study was 14.9 and 18.0 kg, i.e. slightly lower than in Study 1. The MY in the study from 2002-2004 was also lower than in Study 1, or 19.5 and 28.7 kg for primiparous and multiparous cows, respectively. It can be assumed that both higher concentrate ratio and the method of feeding (TMR) in Study 1 results in both higher DMI and MY. As pointed out earlier, however, the difference in lactation stage in these studies must be kept in mind, and the interpretation of the results must be limited by that.

Figure 2 shows the distribution of the data of DMI and ECM obtained in Study 1, for both primiparous and multiparous cows. It must be noted that data for early lactation (<100 DIM) of primiparous cows is limited, and the results of that period may be affected by it. However, the figure shows that during 100-200 DIM, the DMI and ECM of primiparous cows slowly diminishes. The pattern for the multiparous cows is different. There is a clear peak in the ECM at around 70 DIM, and in the DMI 7 weeks later, around 120 DIM. This difference in the time of the peak in the ECM and the DMI creates a negative energy balance in early lactation which compels the cows to mobilize body reserves. This is a well-known condition in early lactation, and is in accordance with the results of Ingvartsen and Andersen (2000) who reported that the MY normally peaks at 5-7 WFC, while the DMI peaks at 8-22 WFC.
The different patterns of the DMI for primiparous and multiparous cows through stage of lactation are also well-known. The data used to develop the NRC (2001) prediction equation for the DMI show that the DMI of primiparous cows is quite stable throughout the lactation, while the DMI of multiparous cows diminishes more in late lactation. In contrast with our results, the results of Zom, André and van Vuuren (2012) even indicate that the DMI for primiparous cows should increase with an increased stage of lactation and the peak of DMI is reached at late lactation.

Because the primiparous cows continue to grow and mature after calving, the volume of their rumen and therefore their IC increases through the stage of lactation. Because of that, and because their MY doesn’t fall as much as for the multiparous cows, their energy requirements don’t fall as fast during late lactation. This leads to a slower decrease in the DMI of primiparous than multiparous cows during late lactation (Kristensen & Ingvartsen, 2003).

5.2 Study 2

5.2.1 BW and BCS

Both primiparous and multiparous cows increased their BW during the study, but the increase was greater for primiparous cows. This represents the growth of the primiparous cows. The BW and BCS of both primiparous and multiparous cows was lower in Study 1 than in Study 2. This can be explained by the difference in DIM between the two studies. In Study 1 the cows were much later in lactation than in Study 2, and the lower BW and BCS in Study 2 could, therefore, reflect the mobilization during the negative energy balance in the beginning of lactation.

5.2.2 Effect of treatment on intake

The advantage that feeding TMR has been reported to have over separate feeding in DMI (Aaes, 1993; Istasse et al., 1986; Sveinbjörnsson & Harðarson, 2008) is not distinct in these results. Primiparous cows offered TMR55 had significantly higher DMI than those on SF45 (17.2 vs. 16.2 kg) (Table 11). The DMI of multiparous cows on SF45 did not differ significantly from the cows on TMR (Table 12). One of the reasons for that could be that usually when separate feeding is practiced, the roughage is only chopped to a small extent, while the TMR feed is always chopped to a greater extent, for the feed components to mix sufficiently together and to reduce the selection of single feed components. It has been reported that chopping roughages can increase intake, at least if the feed is a limiting
factor in fulfilling the energy requirements (Helander et al., 2014; Martz & Belyea, 1986). Even though there is no reason to think that the roughage in this study didn’t fulfill the energy requirements of the cows, it should not be ruled out that chopping the roughage to the same extent for all treatments may have reduced the expected negative effect of separate feeding on the DMI.

Another reason for the little difference between feeding methods in the current study could lie in the execution of the concentrate feeding in SF45. The barley was mixed with the roughage, which is not typical for separate feeding. A feeding method that includes mixing part of the concentrates with the roughage is often referred to as a partial mixed ration, rather than separate feeding, and this may have acted in favor of the SF45 compared to the TMR feeding when it comes to the DMI. It probably also had a positive effect on the SF45 treatment that the compound feed was fed three times a day, rather than twice a day as is common in separate feeding. Mixing the barley with the roughage and a frequent feeding of the compound feed act positively on ruminal fermentation compared to a totally separate feeding, with concentrates fed only twice a day, and this has probably reduced the expected difference between the treatments. Positive effect on rumen fermentation and rumen pH is one of the main advantages of TMR (Forbes, 2007; Østergaard & Gröhn, 2000).

It was expected that a lower concentrate ratio in the TMR45 and SF45 would result in less intake than in the TMR55, as has been reported repeatedly (Allen, 2000; Dado & Allen, 1995; DeVries et al., 2007; Friggens et al., 1998). If both rations (TMR45 and TMR55) fulfill the cows’ energy demands, then increased energy concentration wouldn’t increase the DMI due to metabolic regulation (Forbes, 2007; Montgomery & Baumgardt, 1965). However, the multiparous cows offered TMR45 ingested significantly more (0.5 kg DM) than those offered TMR55. This could be traced back to the experimental design, since there was an imbalance in the number of cows in treatments and parities (Table 5), the experimental design could have failed to exclude the individual variation and consequently, caused an unexpected error in this area.

Surprisingly, higher concentrate ratio in TMR55 did not lead to increased energy concentration compared to TMR45, as was expected (Table 7). There is a possibility that the difference in concentrate ratio was too small to weigh out the high energy concentration in the roughage. However, a sampling error could also be the reason for that and should not be ruled out as a possibility.
Even though the treatment had little effect on the DMI, the energy intake was significantly higher for both TMR treatments than for SF45 (Table 11 and Table 12). This is because of the low OMD of SF45 (79.9%) compared to OMD of the TMR feeds (84.1 and 84.2% in TMR55 and TMR45 respectively) (Table 7). The low digestibility directly results in considerably lower FEm/kg DM in the SF45 ration, so low that the compound feed that is fed in addition can’t compensate for it in the total ration intake. It is surprising that the digestibility of the SF45 ration is even lower than the digestibility of the roughage, and therefore, the energy concentration as well (Table 7). It should be expected that the OMD of SF45 would be higher than that of the roughage, because the SF45 is a mix of roughage and barley. According to the NorFor feed table (NorFor, 2015) the OMD of Icelandic barley is 84-86%, but it was not measured in this study. The OMD of the roughage in this study was 82%, however, which is high according to the NorFor feed table (NorFor, 2015). Either the barley used in this study was of lower digestibility than the roughage or an error in sampling or analysis of the roughage or SF45 feed has occurred. The roughage analysis is based on an analysis on only four samples, while the SF45 analysis is based on weekly samples through the whole period so the suspicion lies with the roughage rather than SF45. The starch and sugar intake as a proportion of the total intake was significantly higher for cows fed SF45 than for cows fed TMR (Table 11 and Table 12). This was much unexpected and can hardly be true, given the higher concentrate ratio and the greater intake for the TMR treatments for primiparous cows. The reason is probably that the TMR rations were not analyzed for WSC, while both the roughage and barley were analyzed for WSC and it was estimated in the compound feed. This means that the value of WSC is missing in the TMR analysis. The starch and sugar intake is, therefore, not comparable between cows fed TMR and SF45.

5.2.3 Effect of treatment on milk production and milk content

No significant difference was noted in the yield of ECM between the TMR55 and the TMR45 treatments. The ECM of cows offered TMR was significantly greater than that of cows offered concentrates separately (Table 11 and Table 12). This can be attributed to the increased energy intake of the cows fed TMR compared to those fed concentrates separately from the roughage. This effect of TMR on the MY compared to separate feeding has been reported before (Grainger et al., 2009; Istasse et al., 1986). Rumen pH was not a factor monitored in this study, but it can be expected that the pH drop in the rumen is less for the cows offered TMR than SF45 because of a more dispersed intake of concentrates.
during the day (Østergaard & Gröhn, 2000). If that was the case, then it would also lead to a higher digestibility of the NDF and a better feed efficiency, which could contribute to a higher MY. However, since rumen pH wasn’t monitored in this study, this possible explanation can’t be verified.

The reduced milk fat content in the milk from cows fed SF45 could possibly be because this type of a feeding method can cause a rapid pH fall in the rumen. This can lead to a lower digestibility of the NDF and, therefore, less production of acetic acid in the rumen, which is the main source of milk fat production (McDonald et al., 2011). Usually the fat production also increases with an increased fiber content in the diet (McDonald et al., 2011). However, in this study there is no sign of these effects of NDF intake on the milk fat content. In fact, the highest milk fat content was among the cows that had the lowest intake of NDF as a proportion of total intake.

TMR feeding has been reported to result in a higher fat content of the milk than separate feeding (Aaes, 1993; Phipps et al., 1984), although the opposite has also been reported (Istasse et al., 1986). Friggens et al. (1998) reported a higher milk fat content feeding a high concentrate (59%) TMR compared to low concentrate (27%) TMR.

The fact that milk protein content is significantly highest in the milk from cows fed TMR55 can possibly be attributed to the high protein content of the feed (Table 7). The primiparous cows fed SF45 produced more milk protein than those fed TMR45, which was surprising since milk protein content has been reported to be positively affected by energy concentration and TMR feeding compared to separate feeding (Aaes, 1993; Istasse et al., 1986).

5.2.4 Effects of animal factors on DMI

The regression parameters of WFC, BCS, BW and ECM on the DMI of primiparous and multiparous cows are presented in Table 13. It must be kept in mind that the data behind this only applies during weeks 4-18 after calving. The ECM was not included in the model for intake parameters. This was because it was assumed that both the intake and ECM is affected by the treatment. Including the ECM would, therefore, decrease the effect of the treatment. The DMI was not included in the model for milk parameters for the same reason.

Neither the WFC nor WFC² had significant effect on the DMI of primiparous cows. This is probably because the DMI of primiparous cows is quite stable after it has reached its peak,
and does not diminish as much as it does with the multiparous cows (NRC, 2001). For multiparous cows, the DMI curve slowly increases until it reaches a peak (usually 8-22 WFC) for then to decrease after that (Ingvarsetn & Andersen, 2000). The fact that WFC² had significant effect on DMI of multiparous cows means that intake had already started to decrease at the end of the study.

The effect of BW was greater on the DMI of primiparous cows than that of the multiparous cows. This is in agreement with the results of Roseler, Fox, Chase et al. (1997) who found out that the correlation between BW and DMI is higher for the primiparous cows than for multiparous cows, that is, the change in BW has more effects on DMI for the primiparous. The effect of BCS on DMI is negative, which also is in agreement with earlier findings (Ingvarsetn & Andersen, 2000; Roseler, Fox, Chase, et al., 1997).

The effect of the ECM on the DMI turned out to be greater for the primiparous cows than for the multiparous cows. This contradicts the results of Roseler, Fox, Chase, et al. (1997), who found a slightly higher correlation for MY parameters for multiparous than primiparous cows.

5.3 NorFor DMI prediction – model evaluation
The changes suggested (Table 14) on coefficients a, b, c and d in the IC equation of primiparous cows increase the effect of DIM in early lactation and decrease it in middle and late lactation, compared to the current coefficients. This creates the gap between the IC of primiparous cows in middle and late lactation calculated with current and suggested coefficients, presented in Figure 7.

The difference between the IC of multiparous cows calculated with the current and suggested coefficients is not great, and it has similar trends throughout lactation. The suggested coefficients result in a slightly higher IC than the current coefficients do, because of the increase in coefficient a from 2.77 to 3.79. This increases the effect of DIM in early and middle lactation. In late lactation the effect of DIM decreases compared to the current coefficients.

According to the results presented in Table 14, the suggested coefficients increase the effect of ECM on the IC of primiparous cows by increasing the coefficient e up to 0.102 and decrease the effect of ECM on the IC of multiparous cows by decreasing coefficient e to 0.044. This is in contradiction with the results of Baldursdóttir (2010) where the coefficients were first fitted for the Icelandic breed in equation 12. She found more effect
of the ECM on the IC for the multiparous cows than the primiparous cows. Roseler, Fox, Chase et al. (1997) also found higher correlation between the MY and DMI for multiparous cows than primiparous cows. When NorFor predicts the IC of larger Nordic breeds and Jersey cows, coefficient $e$ is the same for primiparous cows and multiparous cows. Coefficient $g$ is also increased for the primiparous cows, leading to a greater effect of the BW on the DMI for the primiparous cows than for the multiparous cows. The effect of the BW on the DMI is the same for primiparous and multiparous cows in both larger Nordic breeds and Jersey cows in NorFor. However, Baldursdóttir (2010) found it to be higher for primiparous cows than multiparous cows.

The DMI prediction model for primiparous cows has a lower RMSPE and a higher $R^2$ than the model for the multiparous cows. This applies both when the current and the suggested coefficients are used (Table 15). Less accuracy in the DMI prediction for multiparous than primiparous cows is also reflected in Figure 3 and Figure 4. Lower MSPE values for primiparous cows than multiparous cows have earlier been reported. There, the reason suggested was less variability in the BW and the MY of primiparous cows, leading to a more stable relationship with the DMI (Roseler, Fox, Pell et al., 1997). That was not the case for the BW in the data presented here, but the standard deviation of the ECM was a little higher for the multiparous cows than the primiparous cows. Another factor that may have caused this is that the standard deviation of the DMI of multiparous cows was much lower than the standard deviation of the DMI of primiparous cows. It is easier to predict a variate as the variation is greater.

When the suggested coefficients are used, the fall in the IC of primiparous cows in late lactation is greater than the fall in the IC of multiparous cows (Figure 7). This is in contradiction with the general trend of the IC, where the intake of primiparous cows generally decreases less than the intake of multiparous cows in late lactation (Ingvartsen & Kristensen, 2003; NRC, 2001). With that in mind the curve of primiparous cows in Figure 7 using the current coefficients is more likely to represent the true IC of primiparous cows than the curve based on the suggested coefficients. Even though the suggested coefficients for the primiparous cows result in a higher $R^2$ and lower RMSPE (Table 15) than the current coefficients, the suggested coefficients of primiparous cows increase the line bias which indicates that the model structure is not adequate (Roseler, Fox, Pell et al., 1997). The suggested coefficients for the multiparous cows, on the other hand, add a great deal of accuracy to the model whether we look at RMSPE, line bias or $R^2$ (Table 15).
The suggested changes in the regression coefficients in equation 12 presented in Table 14 are based only on the data presented in this thesis. They could be combined with earlier research data in order to try to obtain an even better combination of coefficients. The evaluation presented in Table 15 is based on the same dataset as was used to fit the coefficients. It would have been preferred to use an independent dataset to evaluate the coefficients. Nevertheless, this evaluation gives an idea of how accurate they are. Before any changes are made in the coefficients, the accuracy of the model should be tested with an independent dataset to get a valid evaluation on the model.

One of the aims of these studies was to obtain data on the DMI of the Icelandic breed in late lactation, and these data certainly add to that knowledge. It would have been preferable to have more data on late lactation of multiparous cows. They reached a maximum at 162 DIM, compared to the primiparous cows that reached a maximum at 195 DIM. Nevertheless, this covers the main period of lactation which is of the most interest in predicting the DMI. For a comparison it can be noted that the data used to develop the coefficients in equation 12 for larger Nordic breeds and Jersey cows range from DIM 7-328 and 7-294 respectively with an average of 123 and 119 DIM respectively.

Separate feeding and TMR feeding call for a different approach of predicting the DMI. In separate feeding one feeds the cows a predetermined amount of concentrates and seeks to know how much roughages the cow can ingest (rDMI). The concentrate to roughages ratio is, therefore, not known beforehand. However, in TMR feeding the ratio between the feedstuffs is predetermined and the cDMI is, therefore, not known when predicting the intake and the matter of interest is the total DMI of all the feedstuff.

In this case, in order to have the same objective for both the separate feeding and the TMR, the rDMI was isolated from equation 9 in order to predict the rDMI only, for both feeding methods.

The FV equation (eq.9) takes into account both the effect of substitution rate and metabolic regulation. However, according to several published results (DeVries & von Keyserlingk, 2009; Helander et al., 2014; Martz & Belyea, 1986), the metabolic regulation is also dependent on the physical form of the feed and the feeding method. This is a factor that is hard to place in the equation and is currently not accounted for. The fact that it is not accounted for could cause an error in the DMI prediction.
When a DMI prediction is used in practice, the MY is not known at the time of prediction. Instead, a standardized lactation curve is used to predict the daily ECM yield. In order to minimize the error in the DMI prediction it is crucial that the predicted ECM yield is as close to being correct as possible.
6 Conclusions

In Study 2, feeding TMR had little effect on the DMI compared to separate feeding. The reason for that is considered to be related to the execution of the separate feeding. The roughage was chopped to the same extent as in the TMR and the concentrate was only partly fed separately since the barley was mixed with the roughage in the SF45 treatment. Increasing the concentrate ratio in the TMR from 45% to 55% did not increase the DMI either. In fact, the opposite was observed for multiparous cows. This probably reflects the metabolic regulation of the DMI; when the feed reaches a certain energy concentration, an increased energy concentration does not increase the DMI. The yield of ECM of cows fed TMR was significantly higher than that of cows on separate feeding. This was considered to be a direct consequence of increased energy intake which was observed for the cows fed TMR compared to separate feeding.

New coefficients were fitted for the IC equation (eq.12) for both primiparous and multiparous cows. The effect of DIM for primiparous cows is increased in early lactation and decreased from middle throughout late lactation compared to the current coefficients. For multiparous cows the effect of DIM is increased in early and middle lactation and decreases in late lactation compared to the current coefficients. The effect of ECM and BW on DMI is greater for primiparous cows than multiparous cows and the coefficients $e$ and $g$ are suggested to change in line with that.

The new suggested coefficients in the IC equation (eq.12) resulted in a lower RMSPE and a higher $R^2$ than the current coefficients. Nevertheless, the suggested coefficients for primiparous cows increase the line bias compared to the current coefficients, which implicates that the model structure is inadequate. Also, the suggested coefficients result in a greater fall in the IC of primiparous cows in late lactation than the current coefficients, compared to the IC of multiparous cows, which is in contradiction to the general trend of IC. The new suggested coefficients for multiparous cows look promising as an improvement in predicting IC.

These studies did certainly obtain data for late lactation, as was needed. The next step would be to parameterize new coefficients to equation 12, based on both the data obtained in the current studies, and data from the earlier research, compiled by Baldursdóttir (2010).
References


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NorFor. (2014). Equation changes since NorFor 2011 (EAAP No.130). *Unpublished.*

NorFor. (2015). *NorFor Feed Table.* Retrieved 27.01.2016, from http://www.norfor.info/


Appendix 1
Amount of minerals and vitamins in protein concentrates used in Study 1 and Study 2.

<table>
<thead>
<tr>
<th>Mineral/Vitamin</th>
<th>Amount</th>
</tr>
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<td>Cobalt (Co)</td>
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</tr>
<tr>
<td>Copper (Cu)</td>
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</tr>
<tr>
<td>Mangan (Mn)</td>
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</tr>
<tr>
<td>Selen (Se)</td>
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</tr>
<tr>
<td>Iodine (I)</td>
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<tr>
<td>Zink (Zn)</td>
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</tr>
<tr>
<td>Vitamin A</td>
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</tr>
<tr>
<td>Vitamin D</td>
<td>2.5 IU/g</td>
</tr>
<tr>
<td>Alpha-tocopherol (Vitamin E)</td>
<td>75 mg/kg</td>
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## Appendix 2
Calving dates, parity and stage of lactation of each cow in Study 1.

<table>
<thead>
<tr>
<th>Cow nr.</th>
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<td>96</td>
<td>154</td>
</tr>
<tr>
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<td>143</td>
</tr>
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