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Milk production from grass and byproducts

For improved sustainability of dairy production

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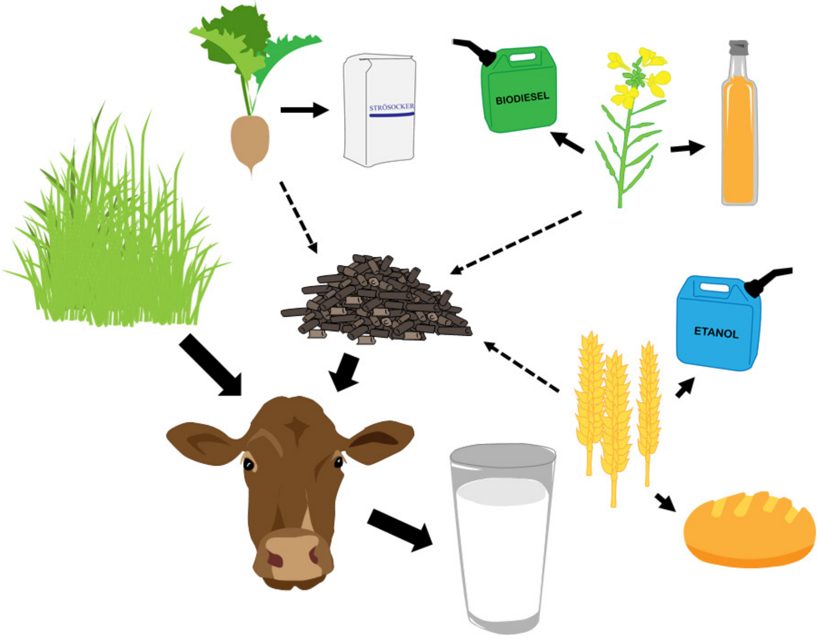

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Cover: Sources of the main feedstuffs used for dairy cows in this thesis.
(Illustration: J. Karlsson)

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Abstract

Ruminants can produce meat and milk from fibrous feed and byproducts not suitable for human consumption. However, high-yielding dairy cows are generally fed high proportions of cereal grain and pulses, which can be consumed directly by humans. This thesis investigated the effect of diets high in forage and byproducts, of low human interest, on milk production, feed efficiency, metabolic status, and fertility. Enteric methane emissions when feeding glycerol or starch were also investigated.

In a series of feeding studies, high quality grass-clover silage was included in dairy cow diets and the effects on feed intake and milk production were determined. Feeding byproduct-based concentrate to dairy cows compared with concentrate based on cereal grain and soybean meal, had no effect on feed intake and milk yield. However, feeding human-inedible byproducts increased net food production substantially.

In a study comparing methane emissions from dairy cows fed glycerol or wheat starch, the results indicated that glycerol in the feed was available to rumen microbes to a larger extent than initially assumed and therefore did not have the potential to decrease enteric methane emissions.

Dairy cows in early lactation fed low or high levels of byproduct-based concentrate showed no difference in total feed intake, milk yield, energy balance or indicators of metabolic status, although cows fed a low-concentrate diet decreased more in body weight than cows fed a high concentrate diet. When the study included only multiparous cows and extended over a whole lactation, cows offered a low-concentrate diet were found to have lower feed intake, but with no differences in milk yield, energy balance, feed efficiency or blood plasma metabolites between low- and high-concentrate diets.

Thus, high producing dairy cows can perform well on high-forage and byproduct-based diets virtually free of ingredients suitable for human consumption, without compromising milk production, feed efficiency or energy balance, thereby contributing to sustainable food production.

Keywords: silage, forage, co-products, feed efficiency, metabolism, energy balance, residual feed intake

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Mjölk på gräs och biprodukter - för en mer hållbar mjölkproduktion

Sammanfattning

Idisslare kan producera mjölk och kött av fiberrika fodermedel och biprodukter som inte lämpar sig för humankonsumtion. Högavkastande mjölkkor utfodras trots det ofta en stor andel spannmål samt ärter eller bönor som lika gärna skulle kunnat ha konsumerats direkt av människor. Syftet med denna avhandling var att undersöka effekterna av foderstater med biprodukter och en stor andel grovfoder på mjölkproduktion, fodereffektivitet, metabolisk status och fertilitet. Vi studerade även hur utfodring med glycerol eller stärkelse påverkar metanproduktionen.

Alla fyra studier som ingår i denna avhandling använde gräs-klöverensilage av hög kvalitet och utvärderade hur foderintag och mjölkproduktion påverkades. I första studien undersöktes effekten av att utfodra biproduktbaserade kraftfoder till mjölkkor jämfört med att utfodra kraftfoder baserat på spannmål och sojamjöl, och där fann vi ingen effekt på foderintag och mjölkproduktion. När korna utfodrades med produkter som inte lämpar sig för humankonsumtion så ökade nettoproduktionen av livsmedel markant. I andra studien var hypotesen att utfodring av glycerol jämfört med stärkelse skulle sänka metanutsläppen från mjölkkor. Men där tyder resultaten på att när glycerol blandas med övriga fodermedel är det tillgängligt för våmmikrober i en större utsträckning än vad som först antogs, och därför sänkte inte glycerol utsläppen av metan. I tredje studien där kor i tidig laktation utfodrades med en stor eller liten andel biproduktbaserat kraftfoder fick vi inga skillnader i totalt foderintag, mjölkavkastning, energibalans eller indikatorer av metabolisk status även om kor som utfodrades en liten andel kraftfoder minskade mer i vikt jämfört med kor som fick en stor andel kraftfoder. I den fjärde studien ingick endast kor i andra laktation eller äldre och den pågick över hela laktationen. I den studien hade korna som fick en liten kraftfodergiva ett lägre totalt foderintag men det var inga signifikanta skillnader i mjölkavkastning, energibalans, fodereffektivitet eller blodplasmametaboliter mellan stor och liten biproduktsbaserad kraftfodergiva.

Sammanfattningsvis visar denna avhandling att kor kan producera mycket mjölk utan att äventyra fodereffektivitet eller energibalans på foderstater med biprodukter och en stor andel grovfoder, som praktiskt taget inte innehåller några ingredienser som lämpar sig som livsmedel och därigenom bidra till hållbar livsmedelsproduktion.

Nyckelord: ensilage, grovfoder, fodereffektivitet, metabolism, energibalans, kor

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Dedication

Till min familj.

Knowledge is a treasure, but practice is the key to it.

Lao Tzu

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Karlsson, J.*, Spörndly, R., Lindberg, M. & Holtenius, K. (2018). Replacing human-edible feed ingredients with byproducts increases net food production efficiency in dairy cows. *Journal of Dairy Science*, 101 (8), pp. 7146-7155.

II Karlsson, J.*, Ramin, M., Kass, M., Lindberg, M. & Holtenius, K. (2019). Effects of replacing wheat starch with glycerol on methane emissions, milk production, and feed efficiency in dairy cows fed grass silage-based diets. *Journal of Dairy Science*, 102 (9), pp. 7927-7935

III Karlsson, J., Lindberg, M., Åkerlind, M. & Holtenius, K. Feed intake, milk yield, and metabolic status of early lactation Holstein and Swedish Red dairy cows of different parities fed grass-clover silage and two levels of byproduct-based concentrate. (manuscript)

IV Karlsson, J.*, Lindberg, M., Åkerlind, M. & Holtenius, K. Whole lactation feed intake, milk yield, and energy balance of Holstein and Swedish Red dairy cows fed grass-clover silage and two levels of byproduct-based concentrate. (submitted to *Journal of Dairy Science*)

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* Corresponding author.

The contribution of Johanna Karlsson to the papers included in this thesis was as follows:

I. The co-authors planned most of the study. Johanna Karlsson performed the majority of the work. She collected and prepared samples, performed most of the data analysis and statistical analysis, wrote the manuscript with regular input from the supervisors and corresponded with the journal and revised the article under supervision.

II. Johanna Karlsson was involved in planning and performing the study. She collected and prepared samples, performed most of the statistical analysis and wrote the manuscript with regular input from the co-authors and supervisors. She corresponded with the journal and revised the article under supervision.

III. Johanna Karlsson was involved in planning the study. She performed the practical preparations and performed most of the work. She collected and prepared samples, performed the data analysis and statistical analysis and wrote the manuscript with regular input from the supervisors.

IV. Johanna Karlsson performed the practical preparations for the experiment and was involved in planning the study. She performed most of the work, collected and prepared samples, performed most of the data analysis and statistical analysis and wrote the manuscript with regular input from the supervisors.

Abbreviations

AIA	acid insoluble ash
AIC	Akaike information criterion
AMR	automatic milking rotary, robot milking rotary
BCS	body condition score
BHB	β-hydroxybutyrate
BW	body weight
CG	cereal grain
CH_4	methane
CLA	commencement of luteal activity (progesterone >5ng/mL)
CO_2	carbon dioxide
CP	crude protein
DDGS	dried distiller's grain with solubles
DIM	days in milk (days from calving)
DM	dry matter
DMI	dry matter intake
EB	energy balance
ECM	energy-corrected milk
FA	fatty acid
FAME	fatty acid methyl ester
FCR	feed conversion ratio
GE	gross energy
GHG	greenhouse gas
GL	glycerol
H_2	hydrogen
HC	high concentrate
HDEAA	human-digestible essential amino acid
HeFCE	human-edible feed conversion efficiency (output/input)
HeFCR	human-edible feed conversion ratio (input/output)

IGF-1	insulin-like growth factor 1
IOFC	income over feed cost
LC	low concentrate
LCA	life cycle assessment
LUR	land-use ratio
ME	metabolisable energy
MJ	mega joule
N	nitrogen
NAD	nicotinamide adenine dinucleotides
NDF	neutral detergent fibre
NEFA	non-esterified fatty acids
NE	net energy
NEI	net energy intake
NoAA	no rumen-protected amino acids
OM	organic matter
OMD	organic matter digestibility
P	phosphorus
RFI	residual feed intake
RSM	rapeseed meal
SBM	soybean meal
SBP	sugar beet pulp
SH	Swedish Holstein (cow breed)
SR	Swedish Red (cow breed)
ST	starch
TMR	total mixed ration
VFA	volatile fatty acid
VLDL	very low-density lipoproteins
VMS	voluntary milking system, single station milking robot
WithAA	with rumen-protected amino acids

1 Introduction

The human population is now greater than ever in its history (UN, 2019a), while poverty is declining and development is increasing (UN, 2019b; Roser, 2019). The increase in material wealth and population growth are both imposing an enormous load on the planet, with at least four of the nine planetary boundaries now being transgressed (Rockström *et al.*, 2009; Steffen *et al.*, 2015). Through reduced genetic diversity, high flows of nitrogen and phosphorus, land-system change and climate change, the earth system is at risk of being destabilised to a level that may not sustain human societies. Agriculture and food production are essential for human survival, but today they are major contributing factors pushing the earth system outside its safe operating space (Campbell *et al.*, 2017). Modern agriculture has been successful in producing large amounts of food, but at a cost of environmental damage (Foley *et al.*, 2005). However, livestock such as dairy cows, are valuable in the food system and they increase food security through their ability to convert grass, and byproducts from food and biofuel systems that are non-edible for humans into nutrient-dense foods such as milk and meat (Eisler *et al.*, 2014; Van Zanten *et al.*, 2019).

1.1 Sustainability and food security

Sustainable development was first widely introduced as a concept in the Bruntland Commission report (UN, 1987), in which it was defined as “meeting the needs of the present without compromising the ability of future generations to meet their own needs”. However, the concept has many interpretations and a popular metaphor is that sustainability rest on three pillars: economic, social and environmental (Purvis *et al.*, 2019). The environment is often described as the foundation of the other two pillars, and sometimes the environment encompasses society, which in turn encompasses the economy (Figure 1).

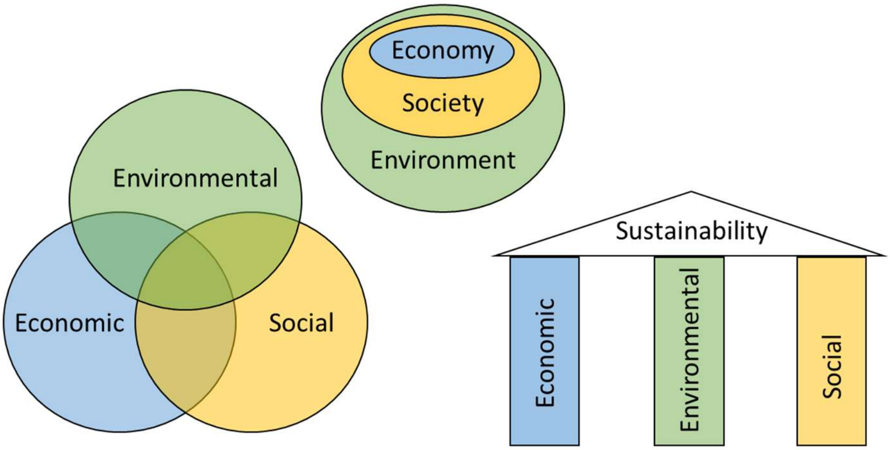

Figure 1. Metaphors of sustainability. Sustainability as three intersecting circles (left). The concentric circle approach (top) and the three pillars of sustainability (right). After Purvis *et al.* (2019).

Global policies have targeted sustainable development through UN declarations, *e.g.* Agenda 21 (UN, 1992), Millennium Declaration (UN, 2000) and Agenda 2030 (UN, 2015). The current Agenda 2030 comprises 17 inter-connected sustainable development goals (SGD) for transformation, including zero hunger, good health, well-being and climate action. Each goal contains more detailed targets on what to achieve and how to measure it. For example, the goal "zero hunger" has targets to end hunger, reach food security, improve nutrition and improve sustainable agriculture (UN, 2015).

Food security is defined by FAO (2001) as "when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life". In theory, enough food is produced on the planet today to feed the global population, even at the 10 billion level predicted for 2050 when the world population is projected to peak (Holt-Giménez *et al.*, 2012; Berners-Lee *et al.*, 2018). The proportion of undernourished people in the world decreased for many years, but in recent years has risen again (UN, 2019b). In 2017, approximately 11% of the global population, or 821 million people, were undernourished (UN, 2019b), whileat the same time approximately 2.5 billion people were overweight or obese (WHO, 2017). Due to inequality and poverty, enough food is not accessible to all (FAO, 2019). Increased food productivity, reduced post-harvest losses and reduced food waste have the potential to contribute strongly to increased food security, while at the same time mitigating climate change (Springmann *et al.*, 2018; IPCC, 2019).

1.2 Contribution of dairy production to food security and healthy diets

Milk is nutrient-dense and contributes to a healthy diet, especially for children, women, adolescents and elderly people (Dominguez-Salas *et al.*, 2019). Malnutrition in low- and middle-income countries is often caused by poor diets lacking in animal-source foods such as eggs, meat and milk products. Cow milk is a good source of fat, essential amino acids and highly digestible protein. It has a high content of calcium, is rich in vitamins A and B_2, and is an important source of vitamin B_{12}. Milk is also an important source of dietary iodine in many countries (NNR, 2012). However, milk is low in iron so it is not recommended as the main food for children below one year of age.

Milk is a food in itself, but also a raw material for products like cheese, butter and cream and fermented products like yoghurt. In the Nordic nutrition recommendations, low-fat dairy products are included as an important part of a healthy diet (NNR, 2012). The recommended intake of milk and fermented dairy products is 200-500 mL/day for adults to cover the required calcium intake, depending on the overall diet (SLV, 2015).

Dairy cows are primarily kept for milk production. However, beef is also produced in dairy production systems, because approximately half of all calves born are bulls, which are reared for beef (at least in Sweden) and because most dairy cows, culled due to not being profitable or fit for milk production any longer, are also slaughtered for beef. Depending on the dairy production system, a dairy cow is estimated to produce 46-92 kg of bone-free meat per year (calves not included) while beef production systems (including rearing of dairy calves) produce 200-350 kg bone-free meat per animal and year (Patel *et al.*, 2017). Beef meat is a good source of protein, essential amino acids and iron, which are especially important for children and women of child-bearing age (NNR, 2012; Wyness, 2016). The recommended weekly intake of prepared red meat (including beef) is maximum 500 g for adults according to WCRF (2020). However, high dietary inclusion of red meat and dairy products increases the environmental impact of the diet (Godfray *et al.*, 2018).

1.3 Land-use change

In order to decrease negative environmental impacts efficiently, it is necessary to measure these impacts. There are numerous ways to analyse environmental impacts from human activities such as livestock production (Halberg *et al.*, 2005; Kaval, 2011; Ran *et al.*, 2015). Life cycle assessment (LCA) and ecological footprint are two of the most commonly used tools (Kaval, 2011), with LCA being the current standard for evaluating potential environmental impacts of livestock systems (Ran *et al.*, 2015; Leinonen, 2019). The LCA is standardised and described in ISO 14040:2006 and ISO 14044:2006. An LCA analysis is based on an inventory of relevant inputs and selection of a functional unit, where 1 kg of energy-corrected milk (ECM) delivered at the farm gate is an example of a functional unit for milk production. Some important environmental aspects of dairy production are land-use, emissions of greenhouse gases (GHG), and emissions of eutrophying substances (Halberg *et al.*, 2005).

Of the global ice-free land area, approximately 37% is land used for grazing, 28% is not used by humans (*e.g.* pristine forests, rock and deserts), 22% is forest land managed by humans, 12% is cropland and 1% is urban structures such as roads and cities (IPCC, 2019). Worldwide, around 70% of agricultural land is used to grow animal feed (FAO, 2009), while the corresponding figure for Sweden is around 75% (Röös *et al.*, 2016). However, both these figures include grassland production, which is often carried out on land that might not be suitable for growing crops. Globally, it is estimated that only 35% of currently grazed land is suitable for conversion to cropland (Mottet *et al.*, 2017). Of total crop production (by mass) globally, 67% is eaten directly by humans, 24% is fed to animals and 9% is used for biofuels (Cassidy *et al.*, 2013). Of total crop production in Sweden, an estimated 16% is eaten directly by humans, 68% is fed to animals (16% of total crop production is fed to dairy animals) and 16% is used for biofuels (Naturskyddsföreningen, 2015). Thus although livestock worldwide are only fed about 14% human-edibles (FAO, 2017), these volumes account for a considerable amount of all crops produced.

The amount of land suitable for growing crops is limited. The best crop land is already in use, and cutting down rainforest for production of palm oil and soybeans or for grazing is harmful for the environment due to *e.g.* loss of biological diversity, release of carbon and disruption to flows of fresh water (Barlow *et al.*, 2016; Seymour & Busch, 2016). There is competition for land suitable for crop production between cultivation of food, feed and fuel (Banerjee, 2011). With the growing human population and increasing per capita income in developing economies, future increase in demand for livestock products is expected (FAO, 2011). Use and need for biofuel are also expected to increase, as fossil fuels are replaced to deal with climate change (IPCC, 2019). These increasing demands for food, feed and fuel are expected to intensify competition for cropland in the future (Banerjee, 2011).

2 Background

2.1 Byproducts for dairy cows

In the past, livestock in most parts of the world were fed products inedible to humans or of lower human interest. In today's intensive animal production systems, livestock are also fed large amounts of grain and pulses, although some rely mainly on grass, shrubs and byproducts. Since dairy cows can consume a wide variety of fibrous products, many potential byproducts can be used as feed. In this thesis, the focus was on byproducts that are readily available as animal feed in Sweden (Figure 2).

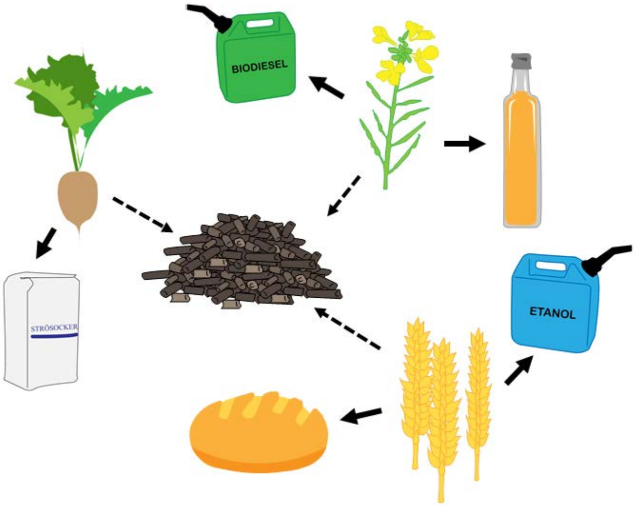

Figure 2. Byproducts tested as dairy cow concentrate in this thesis. Sugar beet pulp and molasses from sugar production (left), rapeseed meal from production of rapeseed oil and glycerol from biodiesel production (top right), wheat bran from making baking flour and distiller's grain from ethanol production (bottom right).

Sugar beet pulp is produced from the fibre fraction in sugar beet (*Beta vulgaris* L.) grown primarily for production of sugar. Sugar beet is grown in temperate climates, with the main production areas being Russia, USA, France and Germany (Heuzé *et al.*, 2019). The harvested beets are cleaned, shredded and mixed with hot water (60-70 °C), where the sugar dissolves. Sugar is obtained from the water solution by evaporation, crystallisation and centrifugation. Both molasses and sugar beet pulp are byproducts from sugar production from sugar beet. Molasses contain approximately 30% sucrose, while unmolassed sugar beet pulp contains less than 10% sugar. However, by reintroducing molasses to sugar beet pulp, the sugar content can be increased. Sugar beet pulp is usually fed pressed or dried. Pressed sugar beet pulp contains around 20% dry matter (DM), and can be fed within a few days or ensiled. Pressed sugar beet pulp can also be dried in a drum dryer and then pelled. The drying process requires much energy, which negatively affects the environmental impact of the product, with the impact depending on the energy source used.

Dried sugar beet pulp has good feeding value and is a good energy source with around 6.3 mega joule (MJ) net energy (NE)/kg DM or 12 MJ ME/kg DM (NorFor, 2020a). It contains approximately (g/kg DM) 400-500 neutral detergent fibre (NDF), 100 CP and 70-100 ash but very little starch, crude fat or lignin (NorFor, 2020a; Heuzé *et al.*, 2019). Sugar beet pulp provides ruminants with rumen-fermentable carbohydrates other than starch, such as pectin and other carbohydrate sources not included in the NDF fraction of the feed (McCready, 1966; Udén, 2007). Much of the sugar beet pulp produced is fed to horses, but it is also an excellent feed for ruminants and pigs (Heuzé *et al.*, 2019).

Rapeseed meal is the fibre and protein fraction of rapeseed (*Brassica napus* L., *Brassica rapa* L., *Brasicca juncea* L.). Rapeseed crops are mainly grown for rapeseed oil. The oil is used for human consumption, but also for biodiesel (both fatty acid methyl ester (FAME) and hydrotreated vegetable oil) production (Neste, 2020). When biodiesel (FAME) is produced from vegetable oils, triglycerides are hydrolysed to fatty acids and glycerol. The fatty acids are then esterified with methanol to FAME. The glycerol can be used as an energy-rich feed for production animals such as dairy cows, which are usually fed crude glycerol with a glycerol content of around 80-90%. Apart from water, the impurities in crude glycerol are mostly methanol and free fatty acids (Thompson & He, 2006). Methanol is toxic, although ruminants can tolerate some as rumen microbes can metabolise it to methane (CH_4) (Neumann *et al.*, 1999). Pure glycerol has many uses, but the purification process is costly.

Rapeseed is grown in temperate climates and is a common crop in Europe, China and North America (Heuzé *et al.*, 2020). The seeds are crushed and pressed to extract the oil. To increase the output of oil, heat and solvents such as hexane are used. The fibre- and protein-containing fraction of rapeseeds that is left after oil extraction is called rapeseed meal and is suitable as animal feed. Before the 1970s rapeseed contained glucosinolates and erucic acid, both toxic, but due to successful plant breeding there are now varieties available without these compounds. They are called canola, “double-low”, “double-zero” or “00”. Rapeseed meal is the second most common protein feed globally, after soybean meal (Huhtanen *et al.*, 2011a). Rapeseed meal has a high crude protein (CP) content (around 380 g/kg DM) and gives a better milk production response than soybean meal in grass silage-based diets (Huhtanen *et al.*, 2011a).

Cereal grains are seeds from graminaceous crops (*e.g.* wheat, barley, oats, rye, rice, maize) produced for human consumption, animal feed and fuel. Wheat (*Triticum* spp.) is the most commonly grown crop in the world and most of it is processed into flour (Heuzé & Tran, 2015). Refining of flour produces bran, a byproduct well suited as feed. Bran consists of the outer layer of the grain kernel and its chemical composition can vary widely depending on grain type and milling process, but it is rich in fibre (350-550 g NDF/kg DM) and moderately rich in protein (140-200 g CP/kg DM), starch (110-350 g starch/kg DM) and minerals (40-70 g ash/kg DM). The higher the fibre content of bran, the lower the starch content, and *vice versa*.

Ethanol is produced for liquor and for fuel from cereal grains with the byproduct distiller’s grain (Heuzé *et al.*, 2017). Grain is milled and bran-free flour is mixed with water and enzymes, which transform starch into dextrose. Yeast is then added and uses dextrose to produce ethanol and carbon dioxide (CO_2). However, the ethanol content in this liquid is rather low and the ethanol is separated off through distillation. The water is removed by centrifugation and the grain residues and yeast are called distiller’s grain. Wet distiller’s grain has to be fed directly while drying it increases its storage stability. As with sugar beet pulp, the drying process requires energy that increases its environmental impact. Distiller’s grain is a good feed both for pigs and ruminants. It is rich in protein (300-400 g CP/kg DM), and has a relatively high fibre content (250-500 g NDF/kg DM) (NorFor, 2020a; Heuzé *et al.*, 2017).

2.2 Forage

Forage (grass and leaves) is the major feed source in the world (FAO, 2017) and occupies approximately two-thirds of agricultural land (IPCC, 2019). It includes fresh plants directly grazed, but also conserved plants such as hay or silage. In Sweden, the most commonly used species for forage production are grasses such as timothy (*Phleum pratense* L.), perennial ryegrass (*Lolium perenne* L.), meadow fescue (*Festuca pratensis* Huds.) and tall fescue (*Festuca arundinacea* Schreber) along with legumes such as red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.) and lucerne (*Medicago sativa* L.) (Spörndly & Nilsdotter-Linde, 2011). In Sweden, all dairy cows are kept on pasture in summer, in compliance with Swedish animal welfare regulations (SFS, 2019). In Sweden, the forage that is not consumed by grazing is usually conserved as silage before feeding (Spörndly & Nilsdotter-Linde, 2011). Swedish leys are cut for silage production two to five times per growing season, with fewer cuts at higher latitudes.

Forage is an important part of dairy cow diets, as it provides fibrous material that is needed for normal rumen function (McDonald *et al.*, 2002). The cellulose-rich forage is broken down by microbes in the rumen into volatile fatty acids (VFA) that are absorbed over the rumen wall into the blood and utilised as nutrients. The nutritional value of forage depends on a number of factors, such as species, fertilisation, climate and soil. Thus, the main determinant may be plant maturity stage (*e.g.* time of harvest), where early first-cuts give the highest digestibility (Kuoppala *et al.*, 2008; Randby *et al.*, 2012). Regrowth grass has more leaves that contain less NDF but still have lower digestibility due to a higher proportion of indigestible NDF (Kuoppala *et al.*, 2010; Huhtanen *et al.*, 2006). When forage is conserved as silage, the conservation process also adds to the nutritional value, with mowing, wilting, chopping, compaction, silage additives if any, how well anaerobic conditions are met, storage time and temperature all contributing. A silage with high organic matter digestibility (OMD) and low levels of fermentation acids enables the highest silage intake (Huhtanen *et al.*, 2007). Pure forage-based diets give lower milk yields, but small amounts of concentrate are enough to give high milk production when combined with highly digestible forage (Randby *et al.*, 2012).

Grass silage has relatively low production costs and the climate conditions in Sweden are well suited for forage production. Forage is produced on approximately 37% of arable land in Sweden, while 33% of arable land is used for cereal production (SJV, 2018). The use of forage production in crop rotations contributes to a number of ecosystem services, such as improved soil quality, carbon sequestration and control of pests and weeds (Weißhuhn *et al.*, 2017). Natural and permanent grassland in particular, but to some extent also forage leys in crop rotations, contribute to increased biodiversity (Pärtel *et al.*, 2005; Albizua *et al.*, 2015). Biodiversity not only depends on the number of species grown, but also on the environment it creates for other species, such as microbes, fungi, insects, worms, reptiles, birds and wild mammals. A problem in Sweden is that semi-natural grasslands left ungrazed revert to forest, with biodiversity losses as a result (Kumm, 2003). Therefore by maintaining grasslands, grazing and grass-eating livestock are valuable in preserving biodiversity.

2.3 Feed intake and digestibility

Over time, animal breeding has increased the genetic potential for high milk production among dairy cows. However, in order to achieve their genetic potential and produce large amounts of milk, dairy cows need to consume and digest sufficient amounts of nutrients to meet the requirement for production, body maintenance and sometimes also gestation and growth. If the nutrient requirement is not met via the feed, the cow will use stored body tissues to support production and other body functions, and eventually also decrease milk production.

Dry matter intake (DMI) of dairy cows is determined by animal characteristics such as body weight (BW), milk production, stage of lactation, stage of gestation, and body condition score (BCS) (Volden *et al*., 2011), but also by health status of the cow (Bareille *et al*., 2003) and climate conditions (West, 2003). For example, fat cows with high BCS consume less feed than lean cows in early lactation (Rukkwamsuk *et al*., 1999). Other important factors influencing DMI are physical and chemical characteristics of the feed (Allen, 2000). One of the main feed characteristics determining DMI is its fill value (Jarrige *et al*., 1986; Volden *et al*., 2011) although feed intake is also regulated metabolically (Allen, 2000; Rinne *et al*., 2002). In diets with large proportions of forage, intake is normally limited by rumen fill factors (Jarrige *et al*., 1986; Mertens, 1994). Therefore, high-forage diets often result in lower total DMI than high-concentrate diets (Faverdin *et al*., 1991; Randby *et al*., 2012; Lawrence *et al.*, 2015). The fill value of forage is calculated from the OMD and NDF content, and also corrected for fermentation quality in silages in the NorFor system (Volden, 2011). Cows can consume more forage if its fill value is low, and the fill value is lower in forage with high OMD and low NDF content. Increased feed intake leads to higher passage rate of feed through the digestive tract, which in turn decreases digestibility (Tyrrell & Moe, 1975). It also takes a longer time to digest diets with a higher fibre content, so those diets reduce passage rate and intake (Dado & Allen, 1995). Maturity stage of the plants at harvest is the main determinant of forage digestibility and NDF content in forage (Buxton, 1996; Rinne *et al*., 2002). Therefore, first-cut silage that has been harvested early generally has high digestibility and low NDF content, resulting in a low fill value and enabling high forage intake.

Concentrates have a lower fill value than forage (Volden, 2011). Therefore, cows are usually able to increase total DMI if the concentrate ration is increased

(Randy *et al.*, 2012; Lawrence *et al.*, 2015). However, the concentrate substitution rate (kg DM forage/kg DM concentrate) also depend on concentrate type. Replacing starch-rich concentrates based on cereal grain with fibre-rich concentrates based on byproducts can to some extent increase forage intake (Huhtanen *et al.*, 2008).

2.4 Milk yield

Milk yield depends on a number of factors including stage of lactation, age, breed, live weight, and intake of energy and other nutrients. Feed intake is the most important nutrition-related factor influencing milk production (Huhtanen *et al.*, 2011b). However, diet composition also influences milk production, *e.g.* level and quality of protein (Colmenero & Broderick, 2006) and fat in the diet (Avila *et al.*, 2000). Milk yield and ECM yield are most often increased with higher concentrate rations along with higher energy intake (Andersen *et al.*, 2003; Kuoppala *et al.*, 2008; Randby *et al.*, 2012).

In low-protein diets (CP below 150 g/kg DM) deficiencies of some amino acids can sometimes limit milk production. Methionine and lysine are considered the first limiting amino acids for synthesis of milk in high-yielding cows fed typical North American diets, which are often are based on maize silage, alfalfa and soybean meal (Schwab *et al.*, 1992). However, it has been suggested that histidine is the first limiting amino acid for milk production in dairy cows fed grass silage-cereal based diets (Vanhatalo *et al.*, 1999; Kim *et al.*, 1999; Korhonen *et al.*, 2000). Unlike methionine and lysine, histidine is apparently not yet commercially available in rumen-protected form (Giallongo & Hristov, 2017).

Milk production increases rapidly directly after calving and most often peaks during the second month of lactation. Thereafter, it slowly decreases until the cow is dried off (Wilmink, 1987; Macciotta *et al.*, 2005; Silvestre *et al.*, 2009). The decrease in milk yield often becomes steeper at the end of lactation, because of the impact of gestation (Strandberg & Lundberg, 1991). Dry period length also influences milk yield, with 50-70 days resulting in the highest milk yield and longer or shorter periods reducing total milk yield (Andrée O'Hara *et al.*, 2020). Primiparous cows typically have a flatter lactation curve than multiparous cows (Wilmink, 1987). Moreover, primiparous cows produce around 15% less milk over the whole lactation compared with multiparous cows (Ray *et al.*, 1992; Pettersson *et al.*, 2011; A. Nyman, Växa Sverige, Uppsala, Sweden, personal communication, 2019). The parity-related increase in milk production is most pronounced between the first and second lactation, but milk production keeps increasing until the cow reaches an age of about 8 years (Akers, 2002).

The world's most widespread dairy breed is the Holstein (FAO, 2020), and in Sweden approximately 55% of dairy cows are Swedish Holstein while around 36% are of the Swedish Red breed (Växa Sverige, 2019). Holstein cows have high potential for milk production and generally produce more milk than Swedish Red cows (Växa Sverige, 2019; Andrée O'Hara *et al.*, 2020). In 2017, Swedish Holstein cows produced on average 10520 kg ECM, while Swedish Red cows produced on average 9760 kg ECM (Växa Sverige, 2019).

2.5 Milk composition

Swedish dairy cow milk contains roughly 87% water, 5% lactose, 4% fat and 3% protein along with vitamins and minerals (Lindmark-Månsson *et al.*, 2003). The composition of milk is influenced by numerous factors, such as lactation stage, genetics and nutritional factors, among many others (Jenkins & McGuire, 2006). Milk composition varies over the lactation, with fat and protein concentrations being inreversely related to milk yield (Silvestre *et al.*, 2009). Thus, when milk production is high, the fat and protein concentrations are low and *vice versa*. Holstein cows generally produce milk with a lower fat and protein concentration than Swedish Red cows, while Jersey cows produce milk with a very high fat (5.9%) and protein (4.2%) content (Växa Sverige, 2019).

Diets low in fibre and high in starch can result in low fat concentration in the milk, so-called "milk fat depression". This is believed to be due to lowering of ruminal pH, in combination with the presence of polyunsaturated fatty acids in the diet altering ruminal biohydrogenation to produce some conjugated linoleic acid isomers that inhibit *de novo* synthesis of fatty acids in the mammary gland (Bauman & Griinari, 2001; AlZahal *et al.*, 2009).

Bovine milk fat consists of approximately 98% triacylglycerol (three fatty acids esterified to one glycerol molecule, also called triglycerides), while the rest of the fat consists of phospholipids and cholesterol making up the membranes surrounding the milk fat droplets (Akers, 2002). The fatty acids in milk may principally originate from dietary lipids, adipose tissue release or mammary *de novo* synthesis (Bauman & Griinari, 2003). More than 400 different fatty acids have been identified in milk (Jensen, 2002), with the most abundant being C16:0, C18:1 cis-9, C14:0 and C18:0 (Palmquist *et al.*, 1993; Jensen, 2002). Short- and medium-chain fatty acids (≤C14) in milk and to some extent also C16, originate from *de novo* synthesis of fatty acids (Palmquist *et al.*, 1969), while long-chain fatty acids such as C18:0 and C18:1 cis-9 are pre-formed fatty acids from adipose tissue (Rukkwamsuk *et al.*, 2000). This enables milk fatty acids content to be used as an indicator of energy balance (EB) in cows (Gross *et al.*, 2011).

Diet composition can be reflected in milk fatty acid profiles (Palmquist *et al.*, 1993; Stoop *et al.*, 2009). The amount and composition of fat fed to cows is the most influential factor for milk fat composition (Palmquist *et al.*, 1993). Fat supplementation of the diet decreases short- and medium-chain fatty acids, but the changes in C16:0, C18:0 and C18:1 depend on the content of those fatty acids in the feed. Acetate and β-hydroxybutyrate (BHB) from ruminal fermentation are the major carbon sources for *de novo* fatty acid synthesis (Bauman & Griinari, 2003). Thus, high-fibre diets that increase production of acetate and butyrate in the rumen also increase milk fat concentration (Grummer, 1991). There are reports of increased concentrations of C16:0 and C18:1 cis-9 with increasing proportion of forage in the diet (Soita *et al.*, 2005; Neveu *et al.*, 2013). Regarding milk fatty acids and high-forage diets, the main research focus has previously been on increasing the concentration of polyunsaturated fatty acids beneficial to human health, such as conjugated linoleic acids, when cows are fed high-forage diets (Dewhurst *et al.*, 2003).

Milk proteins are synthesised from amino acids in the mammary epithelial cells. The amino acids are taken up from the blood and non-essential amino acids can be transaminated in the mammary epithelial cells. Milk proteins consist of approximately 80% caseins (α_s-casein, β-casein, κ-casein and γ-casein) and 20% whey proteins (mainly α-lactalbumin and β-lactoglobulin). Milk protein concentration can be manipulated by diet, but not to the same high extent as milk fat can be manipulated (Sutton, 1989). The concentration of protein in milk is positively correlated with energy intake (Sutton, 1989). The proportion of forage in the diet of dairy cows generally does not affect milk protein concentration (Kuoppala *et al.*, 2004; Randby *et al.*, 2012; Patel *et al.*, 2016), except when an increase in the proportion of forage is followed by a considerable drop in energy intake. Such diets give lower milk protein concentrations (Andersen *et al.*, 2003; Kuoppala *et al.*, 2008). Diets high in cereal grain usually increase milk protein concentration, since those diets usually increase precursors of milk synthesis such as amino acids and glucose in blood plasma (Walker *et al.*, 2004). Feeding more than 50-60g/kg DM of fat can reduce the protein concentration in milk (Walker *et al.*, 2004). However, as long as cows are not severely protein under-nourished there are no clear correlation between how much crude protein fed to cows and milk protein concentration (Beever *et al.*, 2001).

Lactose, a disaccharide comprised of one molecule of glucose and one of galactose, is the most common carbohydrate in milk. It is synthesised in the Golgi apparatus of mammary epithelial cells by the enzyme lactose synthase in the final step (Akers, 2002). Lactose is highly osmotic and the main driver for milk secretion (Sjaastad *et al.*, 2003) and its concentration in milk varies very little. In general, it is considered that lactose content cannot be changed by dietary factors. However, some studies have indicated that high proportions of forage or of lipids can reduce the lactose concentration in cow milk (Sutton, 1989).

2.6 Energy balance and metabolic status

The energy demand of dairy cows varies over time, mainly due to stage of lactation and gestation. In late pregnancy glucose and amino acids are required for foetal development (Bell *et al.*, 1995). After parturition milk production starts and lactation increases energy, protein and mineral demand drastically and rapidly (Ingvartsen, 2006). In early lactation, dairy cows are usually not able to consume enough feed to meet their energy needs and are in negative energy balance.

When they are in negative energy balance, cows use energy from body reserves to compensate for the lack of energy by intake. Energy stored in adipose tissue is broken down to non-esterified fatty acids (NEFA) and glycerol, which are released into the blood (Adewuyi *et al.*, 2005). Most tissues can oxidise NEFA as a source of energy, although most of them are metabolised by the liver (Herdt, 2000). In the liver, they can be completely oxidised to CO_2 and water or partly oxidised to ketone bodies, mainly β-hydroxybutyrate. The NEFA that are not oxidised are re-esterified to triglycerides that may be exported from the liver bound to very low-density lipoproteins (VLDL) and used elsewhere in the body for energy or synthesis of milk fat. The capacity of VLDL to export NEFA is limited and if the inflow of NEFA is substantial re-esterified triglycerides are also stored in the liver cells. A certain degree of triglyceride storage in the liver occurs in virtually all cows after parturition. However, a high level of triglyceride infiltration in the liver (“fatty liver”) is harmful since it reduces the normal function of the liver (Herdt, 2000).

Energy balance can be measured in a respiration chamber (Erdmann *et al.*, 2019). However, it is often estimated from feed intake and composition, milk yield and composition, together with animal factors such as body weight, growth and gestation, for example. Another way to estimate energy balance is by loss or gain of body tissue, assessed by body condition scoring (Edmonson *et al.*, 1989). Change in body condition score or body weight over time may reflect the cow’s energy balance status. Impaired metabolic status can be indicated by higher blood plasma concentrations of NEFA and β-hydroxybutyrate, along with lower concentrations of glucose, insulin and insulin-like growth factor 1 (IGF-1) in blood plasma (as reviewed by Adewuyi *et al.*, 2005).

In dairy cows and other adult ruminants, glucose is synthesised by liver gluconeogenesis mainly from propionate, but also from glucogenic amino acids, glycerol and lactate (Aschenbach *et al*., 2010). This because virtually all dietary carbohydrates are fermented to volatile fatty acids, mainly acetate, propionate and butyrate, in the rumen. In ruminants, glucose is shunted to tissues and organs that have limited possibilities to use other substrates for their metabolism. At onset of lactation, glucose requirements rapidly increase since glucose is required for mammary production of lactose, the main driver of milk secretion (Sjaastad *et al*., 2003).

Insulin is a peptide hormone that plays a pivotal role in regulation of the metabolism during the transition period from late gestation to early lactation (Weber *et al*., 2016). In the transition period dairy cows show reduced insulin sensitivity and responsiveness in peripheral tissues. After calving, blood plasma concentrations of insulin decrease (Holtenius *et al*., 2003). Glucose is thus directed to the mammary gland, which does not require insulin for its glucose uptake (Weber *et al*., 2016).

Studies of metabolic responses to low-concentrate diets in early lactation are inconsistent. Andersen *et al*. (2004) observed lower plasma concentrations of glucose, insulin, and IGF-1, along with higher concentrations of β-hydroxybutyrate and no effect on NEFA, while Lawrence *et al*. (2015) reported lower β-hydroxybutyrate and higher NEFA with no effect on glucose. In both those studies, cows fed a low-concentrate diet had lower energy intake than cows fed a high-concentrate diet.

2.7 Health and fertility

For dietary regimes to be sustainable in the long term, it is important to ensure that animal health and fertility are good. Poor health and fertility increase costs (Dijkhuizen *et al*., 1997) and also the environmental impact of dairy production through *e.g.* increased methane emissions (Garnsworthy, 2004). Animal health can be measured as mortality or the presence of diseases. Metabolic status can be used as an indicator of animal health, *e.g.* high concentrations of NEFA in blood plasma are related to increased risk of metabolic disease (LeBlanc, 2010) and high blood plasma concentrations of β-hydroxybutyrate are related to impaired health and reproductive performance in dairy cows (Rutherford *et al*., 2016; Benedet *et al*., 2019). Mastitis is one of the most common and costly diseases in dairy production (Hogeveen *et al*., 2011) and udder health can be indicated by the number of white blood cells in the milk, which is called somatic cell count (SCC). High somatic cell count has been related to high-concentrate diets (Barnouin *et al*., 1995). Generally, Swedish Red cows have lower somatic cell count and lower incidence of mastitis than Swedish Holsteins, and older cows have higher somatic cell count and higher incidence of mastitis than younger cows (Andrée O'Hara *et al.*, 2020).

Fertility assessed by reproductive measures such as calving interval, time to first insemination and total number of inseminations is greatly influenced by management practices and skilfulness. More objective measures such as time from calving to commencement of luteal activity (CLA), determined by analysing the progesterone (P4) profiles based on P4 levels in the milk, provide a better reflection of the fertility of individual cows (Petersson *et al.*, 2006). Specifically, CLA is determined as the first rise in P4 after calving. When cows ovulate the P4 levels are low, while pregnancy can be detected as consistently high P4 levels (Sjaastad *et al.*, 2003). The oestrus cycle in dairy cows is normally around 21 days, but some disturbances can cause delayed onset of cyclicity (low P4 for at least the first 56 days after calving), cessation of cyclicity (low P4 levels for at least 14 days) and prolonged luteal phase (high P4 levels for at least 20 days) according to Petersson *et al.* (2006). Severe negative energy balance can cause delayed onset of cyclicity (Leroy *et al.*, 2008) and fertility is generally better in dairy cows with blood plasma concentrations high in glucose, insulin and IGF-1, and low in NEFA and β-hydroxybutyrate (Butler, 2014).

Generally, Swedish Red cows have better fertility than Swedish Holsteins (Muuttoranta *et al.*, 2019; Andrée O'Hara *et al.*, 2020) and younger cows have better fertility than older cows (Andrée O'Hara *et al.*, 2020). Concentrate level does not seem to have a direct impact on fertility (Armstrong *et al.*, 1990). In addition, fertility is not improved by increasing the amount of concentrate, as high-yielding dairy cows seem rather to partition the extra energy to milk production (Butler, 2014). Overall, the available literature indicates that feeding regimes leading to deep negative energy balance in early lactation contribute to impaired fertility.

2.8 Feed efficiency

High efficiency is defined as getting high output from low input, often measured as output divided by input. Feed efficiency is generally measured as feed conversion ratio (FCR) calculated as input (kg feed) divided by output (kg meat). Comparing FCR between different production animals, it takes 6-10 kg of cereals to produce 1 kg beef, 3-5 kg of cereals to produce 1 kg pork and 2 kg of cereals to produce 1 kg chicken (Fry *et al.*, 2018). To compare dairy cows or dairy cow systems similar measures are used. However, the custom is then that the numerator and denominator are switched and the metric is calculated as energy-corrected milk output divided by dry matter intake or net energy intake (ECM/DMI or ECM/NEI). However, these comparisons fail to account for the kind of diet the animals are fed. Monogastric animals such as pigs and poultry are mainly fed diets based on cereal grains, *e.g.* such as wheat and maize, together with soybean and other pulses. Cereal grain and soybean can just as well be fed to humans directly. Ruminants, on the other hand, can produce meat and milk from forage and fibrous byproducts not suitable for human consumption. Wilkinson (2011) introduced the idea of human-edible FCR (HeFCR) calculated as the amount of human-edible products fed to animals divided by the amount of human-edible products (meat, milk or eggs) produced by the animals, expressed as a dimensionless ratio. The numerator and denominator can be switched to calculate human edible feed conversion efficiency (HeFCE). Lower values of HeFCR and higher values of HeFCE indicat higher efficiency. A similar but quantifiable measure is net food production (human-edible output minus human-edible input), which was introduced by Ertl *et al.* (2016a). In terms of HeFCR, dairy production is the most efficient animal production system (Wilkinson, 2011). Nevertheless, dairy cows in conventional high-producing dairy systems in Sweden and worldwide are fed large amounts of cereal-based concentrate (Emanuelson *et al.*, 2006; FAO *et al.*, 2014). Feeding dairy cows diets based on human-inedible byproducts and forage is much more efficient from a human-edible production point of view than feeding them cereal grain and soybean meal in large volumes (Ertl *et al.*, 2015a, 2016a; Pang *et al.*, 2018).

Net food production and HeFCR/HeFCE have been calculated for gross energy (GE) and crude protein (Wilkinson, 2011; Ertl *et al.*, 2015a, 2016a). However, humans not only require energy and protein but also, *e.g.* essential amino acids. These amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (NRC, 1989). Protein of animal origin generally has a better amino acid profile and slightly higher digestibility than protein of plant origin (FAO, 2012). Patel *et al.* (2017) proposed the concept of comparing different cattle production systems for efficiency of human-digestible essential amino acids (HDEAA) and found that cattle production generally upgrade protein quality by producing more HDEAA than consumed. Patel *et al.* (2017) also concluded that more extensive production systems where most feed comes from forage resulted in the highest net quantity of HDEAA.

Another aspect to take into consideration when it comes to efficient and sustainable food production is the use of different types of land (Wilkinson & Lee, 2018). Ruminants can produce nutrient-dense meat and milk from pasture on land not suitable for growing arable crops such as cereal grain and pulses, while other types of land would be more suitable for arable crops. In order to determine the production system that is most efficient for different types of land, Van Zanten *et al.* (2016) developed the concept of land use ratio (LUR). This ratio is calculated by dividing the maximum amount of human-digestible protein from food crops on land used to cultivate feed required to produce 1 kg of animal-source food by the amount of human-digestible protein in that 1 kg of animal-source food. A LUR value less than 1 indicates that livestock produce human-digestible protein more efficiently than crops on that type of land.

All these efficiency measures are primarily used to compare production systems against each other. However, when looking for the most efficient individuals for breeding purposes these measures might not always be applicable. For this purpose residual feed intake (RFI) was developed by Koch *et al.* (1963) and is defined as the actual feed intake minus the expected feed intake of each animal. The expected feed intake of an animal is adjusted for that animal's estimated requirements. An animal with negative RFI consumes less than expected and is more feed-efficient. The RFI metric seems to be reasonably stable for different types of diets (Potts *et al.*, 2015). However, it has been suggested that 9-31% of the variation in RFI in low-starch diets, and none of the variation in RFI in high-starch diets, is explained by digestibility (Potts *et al.*, 2017).

From a farmer's perspective, the most important efficiency measure might be one that also takes economic aspects into consideration. One of the simplest and most widely used measures for this in dairy production is income over feed cost (IOFC). It is often calculated as a mean per cow and day by taking average milk yield times the milk price and then deducting feeding costs, based on feed intake and feed cost (Buza *et al.*, 2014). However, it is important to note that IOFC fluctuates with market prices for milk and different feed ingredients, so the most profitable feeding strategy can differ over time. In addition, it can take slightly more effort to calculate the cost for home-grown feeds such as forage.

2.9 Nitrogen efficiency

Eutrophication of surface water bodies is a major environmental problem (Rockström et al., 2009), and thus minimising the nitrogen (N) and phosphorus (P) losses from different animal production systems is important. Proteins and other nitrogenous compounds are degraded by rumen microbes to amino acids, peptides and ammonia (NH_3). These can be synthesised to microbial protein when energy is available for the microbes. Most of the microbial protein together with undegraded feed protein is absorbed in the small intestine. If energy is limited for rumen microbes, excess ammonia is absorbed from the rumen and eventually excreted in urine as urea (Nocek & Russell, 1988). It is therefore important to balance the levels of protein and fermentable carbohydrate in the diet to achieve optimal rumen fermentation and to minimise nitrogen excretion from ruminants (Broderick, 2003). The urea in urine is degraded to ammonia when it reaches the animal house floor or soil. It is then emitted into the air where it contributes to acidification, climate change and eutrophication of water and soil. In this regard, the efficiency of use of nutrients as nitrogen is a valuable indicator (Gerber *et al.*, 2014). In dairy cows, nitrogen efficiency is calculated by dividing the nitrogen content in milk (milk protein yield/6.38) by nitrogen intake via the diet (CP intake/6.25). Nitrogen efficiency depends mainly on dietary protein content, with diets low in CP content being more efficient (Nadeau *et al.*, 2007). However, too low crude protein levels in the diet will decrease milk yield, with the optimum level in the diet reported to be approximately 16.5% (Colmenero & Broderick, 2006; Nadeau *et al.*, 2007).

2.10 Methane and enteric fermentation

Livestock is responsible of approximately 15% of all anthropogenic GHG emissions globally (Gerber *et al.*, 2013). The two main sources of emissions regarding livestock production are feed production and enteric fermentation from ruminants (45% and 39%, respectively). Cattle are the main contributor to GHG emissions of all livestock, with beef cattle and dairy cattle generating similar amounts. Enteric fermentation lead to emissions of methane, while the main impact from feed production comes from nitrous oxide (N_2O) related to fertilisation. In order to compare different GHGs, they are converted into CO_2-equivalents according to their global warming potential. Nitrous oxide and methane have a global warming potential around 265 and 28 times higher than carbon dioxide when measured over a 100-year period (IPCC, 2013). As agricultural systems are biological and complex, estimates of GHG emissions are associated with a wide range of uncertainty (IPCC, 2007; Greber *et al.*, 2013).

Enteric fermentation enables ruminants to utilise energy in cellulose and other structural carbohydrates through the symbiosis between rumen microbes such as protozoa, fungi, archaea and bacteria. Carbohydrates are degraded into monosaccharides, and further metabolised into pyruvate by primary fermenters. During that process, nicotinamide adenine dinucleotides (NAD) are reduced to NADH (H-acceptor), which needs to be re-oxidised to NAD. During this re-oxidation, hydrogen (H_2) is produced. Pyruvate is then used by other microbes, secondary fermenters, resulting in end-products such as the volatile fatty acids acetate, butyrate and propionate along with CO_2 and H_2 but also other molecules to a lesser extent (Van Soest, 1994). The VFAs are absorbed through the rumen wall into the blood and used for energy and as carbon sources. Formation of acetate from carbohydrates is the main H_2 source in the rumen, while butyrate formation generates smaller amounts of H_2 and propionate formation is a H_2-consuming reaction. However, the major route to eliminate H_2 is by methanogens (Archaea) that reduce CO_2 to CH_4 (Moss *et al.*, 2000).

It is possible to reduce methane emissions from rumen fermentation by altering the diet of the ruminant. It is important to measure the reduction in methane emissions per unit produced (*e.g.* per kg ECM) and not per animal. Increased productivity per animal dilutes the effect of animal maintenance (Johnson & Johnson, 1995), so it is more favourable to have fewer high-producing animals than many low-producing animals. Dietary means to reduce methane emissions from ruminants are *e.g.* to increase the amount of starch in the diet, to include up to 6-7% fat in total dietary dry matter, and to increase forage digestibility (Hristov *et al*, 2014). Increasing starch works by increasing animal productivity and by shifting the proportions of volatile fatty acids, favouring propionate (Johnson & Johnson, 1995). Fat has an overall suppressive effect on rumen microbes, and thus too high an inclusion level will reduce feed intake and fibre digestibility and thereby reduce productivity (Hristov *et al.*, 2014). Increasing forage digestibility leads to increased feed intake and reduced methane production per unit of feed consumed (Brask *et al.*, 2013). Various feed additives also have the potential to lower methane emissions, but their long-term effect has not yet been established (Hristov *et al.*, 2014).

3 Aims

The overall aim of this thesis was to investigate the effect of replacing human-edible feed in dairy cow diets with byproducts and grass, not suitable for human consumption, on milk production, metabolic status and feed efficiency. A second aim was to evaluate the effect of dietary glycerol on enteric methane emissions in lactating dairy cows.

Specific objectives of the studies reported in Papers I-IV were to:

- Examine whether feeding byproduct-based concentrate combined with grass silage to dairy cows affected feed intake, milk production and efficiency of human food production compared with a concentrate based on cereal grain and soybean meal (Paper I).

- Investigate whether enteric methane emissions were reduced or feed intake, milk production and organic matter digestibility were affected when starch was replaced with glycerol in a grass silage-based diet fed to dairy cows (Paper II).

- Compare how cows of the breeds Swedish Holstein and Swedish Red responded in feed intake, milk production, metabolic status and feed efficiency in early lactation and over the whole lactation when fed diets high or low in byproduct-based concentrate in combination with grass-clover silage (Papers III and IV).

4 Material and methods

For detailed descriptions of the methods used in the studies on which this thesis is based, see Papers I-IV. The work performed in the four studies is summarised in Table 1. The studies for Papers I, III and IV were conducted in the Lövsta dairy house at the Swedish Livestock Research Centre (Uppsala, Sweden). The study described in Paper II was conducted at Röbäcksdalens research farm (Department of Agricultural Research, SLU, Umeå, Sweden). All work was conducted between 2015 and 2018. The studies were approved by the Uppsala Ethics Committee for Animal Research (Uppsala, Sweden; diary number C98/15 and C99/16) and the Umeå Ethics Committee for Animal Research (Umeå, Sweden; diary number A72/15).

Table 1. *Overview of experimental design, animals, housing, diets and feeding in the four studies on which this thesis is based (Papers I-IV)*

Main topic	Byproducts	Glycerol	Early lactation	Whole lactation
Paper	I	II	III	IV
Herd	Lövsta	Röbäcksdalen	Lövsta	Lövsta
Time period	Nov 2015- Feb 2016	Feb-Apr 2016	Feb-Apr 2016	Feb 2017- May 2018
Experimental design	Change-over	Change-over, switch-back	Randomised	Randomised
No. of animals	24	22	26	37
Lactation stage	Mid	Mid	Early	Early, mid, late
Breeds[1]	SR and SH	SR	SR and SH	SR and SH
Parities	Primiparous and multiparous	Primiparous and multiparous	Primiparous and multiparous	Multiparous
Housing	Loose housing	Loose housing	Loose housing	Loose housing (partly out-door)
Milking system	VMS[2]	Parlour (twice/d)	AMR[3](twice/d)	VMS
Feeding strategy	Silage + concentrate	Total mixed ration (TMR)	Silage + concentrate	Silage + concentrate
Forage	Grass silage, 1st and 2nd cut	Grass-clover silage, 1st cut	Grass-clover silage,1st cut	Grass-clover silage, 1st cut
Concentrate based on	Byproducts or cereal grain + soybean meal	Byproducts and cereal grain	Byproducts	Byproducts
Concentrate ration (kg/d)	11		≤5 & ≤15	≤6 & ≤12
Treatments	Concentrates: 3 byproduct-based, 1 cereal grain + soybean meal	Wheat starch vs glycerol	High- vs low-concentrate level	High- vs low-concentrate level × WithAA vs NoAA[4]

[1]Swedish Red (SR) and Swedish Holstein (SH). [2]Single-station automatic milking with FeedFirst cow traffic system. [3]Automatic milking rotary with batch milking morning and evening. [4]With or without rumen-protected lysine and methionine amino acids.

4.1 Experimental design, animals and housing

In Paper I, the effects of three different byproduct-based concentrates and one concentrate based on cereal grain and soybean meal on milk production, feed intake and efficiency were compared. In Paper II, the effect of replacing wheat starch with dietary glycerol on enteric methane, milk production and feed intake was investigated. The experimental design in Papers I and II was change-over studies on cows in mid-lactation. In Paper I, four dietary treatments were fed during four periods of three weeks each, while in Paper II two dietary treatments were fed during three periods of three weeks each in a switch-back design with treatments sequence ABA and BAB. In both Papers I and II, the first two weeks were used for adaptation, while sampling and data collection were performed during the last week of each period. In Paper I, multiparous (n=12) and primiparous (n=12) Swedish Holstein (SH, n=8) and Swedish Red (SR, n=16) dairy cows were blocked by breed and parity, and then randomly assigned to the four treatment groups. In Paper II, multiparous (n=14) and primiparous (n=8) SR cows were blocked by parity and yield and then randomly assigned to two treatment groups.

In Papers III and IV, the effects of a high and low byproduct-based concentrate diet on feed intake, milk production and energy balance were studied. In Paper III, the cows were followed from lactation week 2 to 6, while in Paper IV they were followed over the whole lactation. In Papers III and IV, a randomised experimental design was used to compare high- and low-concentrate diets and the design was unbalanced in both cases due to a parallel genetic study on the low-concentrate cows. The study described in Paper IV had a 2 × 2 factorial design, comparing high- vs. low-concentrate diet, with or without rumen-protected amino acid-supplementation. The study described in Paper III included 100 cows in total, but only the first 26 cows entering the study were included in Paper III, since during that period every other cow entering the study was assigned to either the high- or low-concentrate diet, while during the remaining period all cows were assigned to the low-concentrate diet. Both primiparous and multiparous cows were included in the study and, from the 100 cows, the 51 cows calving first in the next lactation were also included in the study in Paper IV, which thus only included multiparous cows. Of the 51 cows entering the study in Paper IV, 37 were followed for their whole lactation, while the rest were excluded due to illness or mistakes in feeding.

All cows were kept in insulated loose houses with rubber mats and sawdust-bedded cubicles in groups of approximately 60 cows. The cows included in Paper IV also had access to a grass-covered permanent paddock for exercise and recreation at night-time during summer in compliance with Swedish animal welfare regulations. Cows in Paper I and IV were milked in a single-station automatic milking system (VMS, DeLaval International AB, Tumba, Sweden) with the FeedFirst cow traffic system resulting in approximately 2.5 milkings per day. Cows in Paper II were milked morning and evening in a 2 × 8 milking parlour (SAC, S.A. Christensen & Co. A/S, Kolding, Denmark) while the cows in Paper III were milked morning and evening in an automatic milking rotary (AMR, DeLaval International AB, Tumba, Sweden) (Table 1).

4.2 Diets and feeding

In Papers I, III and IV, the cows were offered grass or grass-clover silage *ad libitum* in roughage troughs and fixed rations of pelleted concentrate from dispensers, while those in Paper II were offered a total mixed ration (TMR) *ad libitum* from roughage troughs.

In Paper I, the forage consisted of grass silage from the first and second cut of a perennial grass sward of timothy, perennial ryegrass, tall fescue hybrid and tall fescue conserved in round bales. Three of the concentrates were byproduct based and contained dried and unmolassed sugar beet pulp as approximately 50% of fresh matter in combination with dry distiller's grain, rapeseed meal or a combination of these two. The fourth concentrate (control) was based on cereal grain (wheat, barley, oats) and soybean meal. The cows in Paper I were offered 11 kg of concentrate per day.

The two different TMR used in Paper II were based on (g/kg of DM) grass-clover silage (605), rapeseed meal (120), crimpled barley (70), and a mineral mix (5). The dietary treatments consisted of a control with wheat starch (200 g/kg DM) and a treatment diet with refined glycerol (200 g/kg DM). The silage was a first-cut of a second-year perennial sward sown with timothy and red clover.

The cows in Papers III and IV were fed first-cut grass-clover silage from perennial swards sown mainly with timothy, with inclusion of perennial ryegrass, tall fescue and red clover. The silage was of high digestibility and conserved in bunker silos. All cows in Papers III and IV received 3 kg of concentrate starting approximately two weeks before expected calving and continuing until they entered the group of lactating cows. The concentrate fed in Paper III was the same as one of the concentrates fed in Paper I and was mainly based on (g/kg fresh matter) sugar beet pulp (501), rapeseed meal (168) and distiller's grain (150). The cows on a low-concentrate diet were offered up to 5 kg of concentrate per day and those on a high-concentrate diet were offered up to 15 kg of concentrate per day. When the cows entered the group of lactating cows, the concentrate ration was increased by 0.5 kg/day until they reached the maximum allowance for their treatment.

The concentrates offered in Paper IV were mainly based on (g/kg DM) sugar beet pulp (566), wheat bran (120), wheat flour (100), rapeseed meal (70), and distiller's grain (70). Half the cows in Paper IV had their concentrate supplemented with rumen-protected lysine and methionine, while the other cows were fed a concentrate without amino acid supplementation. The cows on a low-concentrate diet were offered up to 6 kg concentrate per day while those on a high-concentrate diet were offered up to 12 kg concentrate per day. At calving the cows was offered 3 kg concentrate per day. When the cows entered the group of lactating cows, the concentrate was increased over a period of 21 days until it reached the maximum allowance for the treatment. The cows stayed on their maximum allowance until 210 days in milk (DIM), when the concentrate amount was gradually decreased to 0 kg/day over 95 days. For cows that had started dry-off before 305 DIM, the concentrate ration was decreased to 0 kg/day before drying off at 9 weeks before expected calving.

4.3 Data and sample collection

Individual daily feed intake was recorded automatically in Papers I-IV. Forage or TMR intake was recorded by roughage troughs (CRFI, BioControl Norway A/S, Rakkestad, Norway at Lövsta (Papers I, III, IV); Insentec B.V., Marknesse, the Netherlands at Röbäcksdalen (Paper II)). At Lövsta (Papers I, III, IV) concentrate intake was recorded by dispensers (FSC400, DeLaval International AB, Tumba, Sweden).

At Lövsta (Papers I, III, IV), silage was sampled five times a week (Mon-Fri), while concentrates were sampled once a week. At Röbäcksdalen (Paper II), separate feed ingredients and TMR were sampled daily during the experimental periods. All silage samples were stored frozen until analysis.

Milk yield was recorded at each milking. At Lövsta (Papers I, III, IV), milk yield was measured with optical milk meters (MM25/27, DeLaval International AB, Tumba, Sweden), while at Röbäcksdalen it was measured with gravimetric milk recorders (SAC, S.A. Christensen & Co. A/S, Kolding, Denmark). Milk sampling was carried out at all milkings for 24h in the middle of the sampling week in each experimental period in Paper I. In the glycerol study (Paper II), milk samples were collected during four consecutive milkings at the end of each experimental period. In the early lactation study (Paper III), milk sampling was carried out at two consecutive milkings in lactation weeks 2, 4 and 6. Milk samples in the study for Paper IV were collected at two consecutive milkings every second week. All milk samples were preserved with bronopol, stored at maximum 8 °C and analysed within three days. In the two studies comparing high- and low-concentrate rations (Papers III, IV), additional milk samples were taken once a day twice weekly for progesterone (P4) analysis until the cows were confirmed pregnant or left the study.

In all studies, BW and BCS were recorded. The cows were all weighed automatically, either when passing through a sorting gate when leaving the feeding area (Papers I, IV) or after each milking (Papers II, III). At Röbäcksdalen (Paper II), BCS was assessed simultaneously by two trained individuals according to Edmonson *et al.* (1989). At Lövsta (Papers I, III, IV), BCS was assessed automatically with a 3D camera (DeLaval International AB, Tumba, Sweden) as described by Hallén Sandgren & Emanuelson (2016) after each milking and presented as a daily value.

Spot samples of faeces for estimation of diet DM and organic matter (OM) digestibility were collected in all studies once a day on three consecutive days per sampling period. In the two change-over studies (Papers I, II) sampling was performed at the end of each experimental period. In the early lactation study (Paper III), faeces sampling was carried out on two different occasions per cow, three weeks apart. In the whole lactation study (Paper IV), faeces sampling was carried out in early and mid-lactation. Faeces samples were pooled per cow and sampling period and then stored frozen at -20°C until analysis.

In the two studies comparing high- and low-concentrate rations (Papers III, IV), blood samples were drawn from the coccygeal vein or artery of the tail-head in lactation weeks 2, 4 and 6, plus once in lactation weeks 19-21 in the whole lactation study (Paper IV). The blood was immediately centrifuged and plasma was transferred to Eppendorf tubes and stored at -20°C until analysis.

Enteric CH_4 emissions were measured in an open-circuit head chamber system (GreenFeed, C-Lock Inc., Rapid City, SD, United States) in the glycerol study (Paper II). The cows received several small drops of concentrate per day in the GreenFeed station to extend each visit to some minutes, so that mass flux of CH_4 in the breath of each individual cow could be measured.

4.4 Analyses and calculations

The feed analyses were performed by the laboratory at the Department of Animal Nutrition and Management (SLU, Uppsala, Sweden). For all feeds, concentrations of DM, ash, acid-insoluble ash (AIA), CP and NDF were analysed. Concentrates were analysed for crude fat and starch. Silages were analysed for water-soluble carbohydrates, ruminal fluid-digestible OM (VOS), pH and ammonium-nitrogen (NH_4-N).

Net energy content in the feed and energy intake were estimated according to the NorFor system (Volden & Nielsen, 2011). Energy balance and RFI were calculated as:

$$EB = (NE_{intake}) - (NE_{maintenance} + NE_{milk})$$

$$RFI = (NE_{intake}) - (NE_{maintenance} + NE_{milk} - NE_{mobilisation} + NE_{deposition})$$

with NE_{intake}. $NE_{maintenance}$, NE_{milk}, $NE_{mobilisation}$ and $NE_{deposition}$ calculated according to the NorFor system.

Samples of faeces were analysed for DM, ash and AIA. The total amount of faeces was calculated from the total intake of AIA (by feed intake and feed content of AIA) and the content of AIA in the faeces. Apparent DM digestibility and OM digestibility were calculated from feed intake of DM and OM, and estimated excretion of DM and OM.

Milk samples were analysed for composition of fat, the four most abundant FA (C14:0, C16:0, C18:0, C18:1 cis-9), protein, and lactose using infrared Fourier-transform spectroscopy (Combiscope FTIR 300 HP, Delta Instruments B.V., Drachten, the Netherlands). Energy-corrected milk yield was calculated based on fat, protein and lactose content. Milk samples were analysed for P4 using ELISA (enzyme-linked immunosorbent assay). The limit for luteal activity was set a milk P4 concentration >5 ng/mL.

The concentration of glucose, insulin, NEFA, BHB and IGF-1 in blood plasma were determined.

The proportion of potential human-edibles in feeds was calculated according to the classification of Wilkinson (2011). Feeds categorised as byproducts had an estimated human-edible proportion of 0.2. Human-edible feed conversion efficiency (HeFCE) for CP and GE was calculated as the human-edible content in the milk produced divided by the potential human-edible content of the feeds that the cows consumed during the study. Net food production per cow and day for CP and GE was calculated as the human-edible content in the milk produced minus the potential human-edible amount in the feed consumed by the cow during the study according to Ertl *et al.* (2016a). Amount of HDEAA in Paper I was calculated according to Patel *et al.* (2017).

For calculation of IOFC the milk price was set to 3.44 SEK/kg ECM and the silage cost to 1.90 SEK/kg DM. The concentrate feed cost used in the calculations was the price at which the feed was purchased for in the actual studies (SEK/kg DM).This was: Paper I: cereal grain and soybean meal 2.97, sugar beet pulp and distiller's grain 2.83, sugar beet pulp and rapeseed meal 2.82, sugar beet pulp, rapeseed meal and distiller's grain 2.80; Paper III: 3.22; Paper IV: with rumen-protected lysine and methionine 3.49, no rumen-protected amino acids 3.26. Forage efficiency was calculated as kg forage DMI/BW$^{0.75}$.

4.5 Statistical analysis

All data were analysed using the statistical software SAS (version 9.4, SAS Institute Inc., Cary, NC, United States). Treatment effects of feed and nutrient intake, digestibility, milk yield and composition, efficiency parameters, BCS, BW, blood plasma parameters, gases and continuous fertility data were analysed using the procedure MIXED in all papers. In the two randomised studies (Papers III, IV) lactation week was repeated autoregressively. For treatment effects of digestibility in the two randomised studies with only two measures per cow, the model was adapted in that cow was not treated as random effect and lactation week was repeated unstructured.

The model used in Paper I included the effect of block, cow within block, period and treatment. When the effect of breed and parity was investigated using data from the byproduct study (Paper I) the statistical model was adjusted to include treatment sequence, parity and breed instead of block, and by having only cow and not cow within block as a random effect. The model used in Paper II included the effect of block, period, pre-treatment, treatment sequence, treatment, cow within block and treatment × cow interaction. When the effect of parity was investigated using data from the glycerol study (Paper II) the statistical model was adjusted to include parity instead of block. The model used in Paper III included the effect of cow, parity, breed, concentrate level, lactation week, breed × lactation week interaction and concentrate level × lactation week interaction. The model used in Paper IV included the effect of cow, parity, breed, concentrate level, concentrate type, lactation week, ECM in previous lactation, concentrate level × lactation week interaction and breed × concentrate level interaction. In Paper III primiparous cows formed one parity class and cows of parity 2 and older formed another. In Paper IV, cows in their second parity formed one parity class and cows in parity 3 and older formed another parity class.

Treatment effects of changes on BCS and BW in the two randomised studies (Papers III, IV) were analysed by PROC GLM. Binomial fertility data in these two randomised studies were analysed by PROC LOGISTIC. Correlations were analysed by PROC CORR and expressed as Pearson correlation coefficient (r_{xy}).

In Papers I and II, interactions were calculated but removed from the final model if not significant. In Papers III and IV, the models with the lowest Akaike information criterion (AIC) value were used. All residuals were tested for normality and log transformation was applied to those that did not follow normal distribution. Values presented in Chapter 5 and in tables are least square means calculated using the LSMEANS/PDIFF option. Statistically significant differences were determined following Tukey’s adjustment declared at $P \leq 0.05$.

5 Results and discussion

Detailed descriptions of all results can be found in Papers I-IV.

5.1 Feed intake and digestibility

There was no difference in total DMI between different concentrates in the study comparing different byproduct-based concentrates with concentrate based on cereal grain and soybean meal (Paper I). Although the control concentrate based on cereal grain and soybean meal led to higher intake of starch and lower intake of NDF than the byproduct-based concentrates, the byproduct-based concentrates had a high content of sugar beet pulp that probably contained sufficient levels of rumen-fermentable carbohydrates other than starch to supply the cows with energy. Similarly, other comparisons of diets based on cereal grain or soybean meal with diets based on different byproducts have not demonstrated any effect on total DMI (Anderson *et al.*, 2006; Maxin *et al.*, 2013; Dann *et al.*, 2014; Ertl *et al.*, 2015a, 2016a).

In Paper I, the digestibility of NDF was lower when cows were fed the concentrate based on cereal grain and soybean meal than when they were fed the concentrates based on byproducts. This was expected, because the byproduct-based concentrates had overall lower NDF content and because sugar beet pulp has a high ruminal NDF digestion rate (Voelker & Allen, 2003). The reported effects of replacing soybean meal with rapeseed meal on CP digestibility are not consistent, with results varying from higher (Paula *et al.*, 2018) to no effect (Paula *et al.*, 2020) or lower (Waldern, 1973), as in Paper I. Others have not observed any difference in CP digestibility when comparing sugar beet pulp with maize (Bhattacharya & Sleiman, 1971) or soybean meal with rapeseed meal (Huhtanen *et al.*, 2011a; Gidlund *et al.*, 2015; Paula *et al.*, 2018). In addition, the OM digestibility in Paper I was lower in the diets with rapeseed meal, than in those with soybean meal or distiller's grain.

Based on the results obtained for cows in mid-lactation in Paper I, the effect of byproducts in combination with high-forage diets was explored in subsequent studies. In the early lactation study (Paper III) and whole lactation study (Paper IV), all cows received byproduct-based concentrate, at two levels. In Paper III, where 26 cows were followed between lactation weeks 2 and 6, there was no difference in total DMI between the high- and low-concentrate diets. In Paper IV, where 37 multiparous cows were followed over a whole lactation, the cows fed a high-concentrate diet consumed 1.0 kg more DM than those fed the low-concentrate diet over the whole lactation (Figure 3).

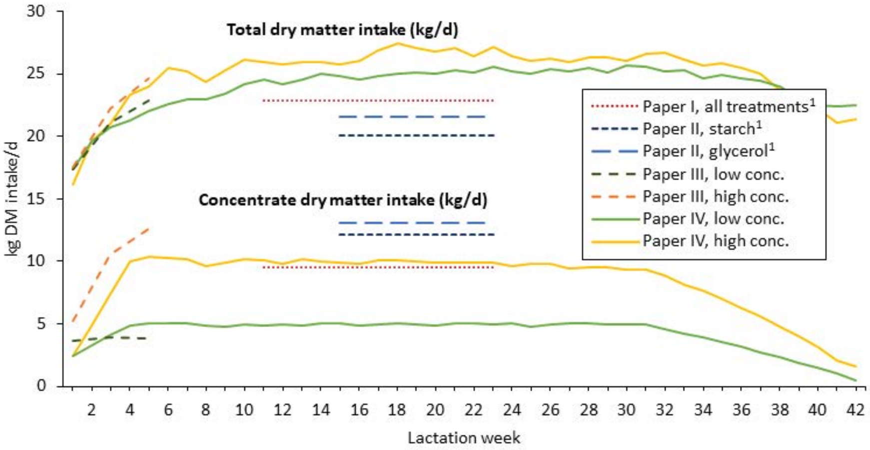

Figure 3. Total dry matter intake (kg/d, top lines) and concentrate dry matter intake (kg/d, bottom lines), as least square means per lactation week in the whole lactation study (Paper IV), per lactation week 2, 4 and 6 in the early lactation study (Paper III), and as a mean for the whole study period in the change-over studies (Papers I and II). Since there were no differences ($P \geq 0.05$) between treatments for these parameters in the byproduct study (Paper I), only one mean is presented (all treatments). [1]Mean value for the whole study period.

Others have also found that cows fed higher concentrate levels have a higher total DMI (Kuoppala *et al*., 2008; Randby *et al*., 2012; Lawrence *et al*., 2015). The explanation for this could be that forage has a higher rumen fill value than concentrates (Jarrige *et al*., 1986; Volden *et al*., 2011), and rumen fill often limits total DMI in diets with a large proportion of forage (Jarrige *et al*., 1986; Mertens, 1994). In both the early and whole lactation studies (Papers III and IV), total DMI was relatively high compared with that reported in others studies examining different proportions of forage and concentrate (Ferris *et al*. 2001, Lawrence *et al*., 2015; Patel *et al*., 2016). However, some studies have found high total DMI when using highly digestible forage (Kuoppala *et al*., 2008; Randby *et al*., 2012). The estimated *in vivo* digestibility of OM was very high for the silage in Papers III and IV, and there were no differences in OM or DM digestibility between the high- and low-concentrate diets in either study. The estimated *in vivo* digestibility of OM was (g/kg DM): 905 ± 2 in Paper I (4 samples), 864 ± 1 in Paper II (6 samples), 931 ± 9 in Paper III (12 samples) and 921 ± 2 in Paper IV (31 samples).

Cows had higher total DMI when fed a TMR with glycerol compared with wheat starch (Paper II). Since the feed was provided as a TMR in that study, intake of OM, CP, NDF and crude fat was also higher when cows were fed the glycerol diet. However, due to somewhat higher energy level estimated for starch than for glycerol (NorFor, 2020a), there was no difference in energy intake between the two diets (Paper II). The higher intake of the glycerol diet may be explained by it being more palatable since glycerol is sweet tasting.

There was no breed effect on total DMI (Table 2) or forage intake in the byproduct study when adjusting the statistical model to include the effect of breed and parity (Paper I). In the glycerol study, all cows were of the breed SR, and thus it was not possible to explore breed effects in that study. In both the early and whole lactation studies, SR cows had lower intake of forage and total DMI compared with SH cows (Paper III and IV). These results confirm previous findings that SH cows consume more feed than SR cows (Li *et al.*, 2018; Andrée O'Hara *et al.*, 2018). Some have attributed this higher intake to higher BW of SH cows compared with SR cows (Li *et al.*, 2018), as BW is related to digestion volume (Beecher *et al.*, 2014). Similarly, cows of SH breed were heavier than those of SR breed in the whole lactation study with only multiparous cows (Paper IV). However, there was no such difference in the early lactation study, where both primiparous and multiparous cows in early lactation were followed (Paper III). High BCS is associated with lower DMI (Roche *et al.*, 2008), and SR cows were both fatter and had lower DMI than SH cows in the byproduct study (Paper I). However, there were no breed difference in DMI/BW in any of the studies in this thesis.

The effect of parity in all studies (Papers I-IV) is presented in Table 3. As expected, primiparous cows in Papers I-III had lower feed intake than multiparous cows (second lactation and older) but there did not seem to be any difference in DMI between cows in second lactation and older cows based on the results from the whole lactation study (Paper IV). The most straight forward explanation is that the lower BW of primiparous cows limits their feed intake.

Table 2. *Effect of dairy cow breed (Swedish Red (SR) and Swedish Holstein (SH)) on feed intake, milk yield, energy-corrected milk yield (ECM), milk composition, body weight (BW), body condition score (BCS), blood plasma concentrations of glucose, insulin, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB) and insulin-like growth factor 1 (IGF-1), and energy balance (EB) presented as least square mean with standard error of the mean (SEM) and P-value, in Papers I, III and IV*

	Paper I				Paper III				Paper IV			
	SR	SH	SEM	P-value	SR	SH	SEM	P-value	SR	SH	SEM	P-value
Number of cows	16	8	-	-	14	12	-	-	24	13	-	-
Dry matter intake, kg/d	22.1	23.6	0.613	0.10	19.9	22.0	0.64	0.04	23.6	25.4	0.33	0.001
Milk yield, kg/d	31.0	32.3	1.08	0.43	31.2	35.1	1.15	0.03	32.2	33.2	0.96	0.46
ECM yield, kg/d	33.8	32.4	0.98	0.36	32.5	36.1	0.95	0.02	34.9	35.3	1.03	0.81
Milk fat, %	4.71	4.18	0.121	0.006	4.45	4.41	0.141	0.83	4.56	4.36	0.084	0.10
Milk protein, %	3.49	3.27	0.062	0.02	3.43	3.27	0.040	0.01	3.59	3.48	0.044	0.10
Milk lactose, %	4.52	4.58	0.030	0.18	4.54	4.51	0.032	0.49	4.69	4.77	0.030	0.07
BW, kg	630	661	12.0	0.09	631	648	18.9	0.53	722	780	12.6	0.003
BCS, scale 1-5	3.53	3.19	0.065	0.001	3.60	3.25	0.081	0.007	3.47	3.21	0.064	0.009
Glucose	-	-	-	-	3.47	3.51	0.085	0.75	2.96	3.05	0.079	0.44
Insulin[1]	-	-	-	-	0.16	0.16	-	0.91	0.10	0.17	-	0.02
NEFA[1]	-	-	-	-	0.27	0.26	-	0.85	0.33	0.24	-	0.004
BHB[1]	-	-	-	-	0.39	0.38	-	0.76	1.01	0.89	-	0.20
IGF-1[1]	-	-	-	-	92.1	70.4	-	0.03	72.0	83.1	-	0.16
EB, MJ NE/d	-4.75	6.31	4.144	0.08	-14.0	-13.0	2.98	0.82	-0.23	7.39	2.55	0.05

[1] Statistics run on log10 values, values shown are antilog.

Table 3. *Effect of the parity classes primiparous (1; first lactation) and multiparous (2+; second lactation and older) in Papers I, II and III, or second parity (2; second lactation) and multiparous (3+; third lactation and older) in Paper IV on feed intake, milk yield, energy-corrected milk yield (ECM), milk composition, body weight (BW), body condition score (BCS), blood plasma concentrations of glucose, insulin, non-esterified fatty acids (NEFA), β-hydroxybutyrat (BHB) and insulin-like growth factor 1 (IGF-1), and energy balance (EB) presented as least square mean with standard error of the mean (SEM) and P-value*

	Paper I				Paper II				Paper III				Paper IV			
	1	2+	SEM	P-value	1	2+	SEM	P-value	1	2+	SEM	P-value	2	3+	SEM	P-value
Number of cows	12	12	-	-	8	14	-	-	12	14	-	-	20	17	-	-
Dry matter intake, kg/d	21.0	24.7	0.64	<0.001	19.2	21.7	0.49	0.003	18.5	23.4	0.63	<0.001	24.5	24.4	0.53	0.92
Milk yield, kg/d	29.5	33.9	1.15	0.007	23.1	28.2	1.03	0.003	28.8	37.5	1.14	<0.001	34.8	30.7	1.55	0.15
ECM yield, kg/d	30.9	35.4	1.02	0.002	25.4	31.2	1.15	0.003	29.4	39.3	0.95	<0.001	37.9	32.3	1.64	0.07
Milk fat, %	4.45	4.44	0.127	0.95	4.73	4.83	0.126	0.58	4.32	4.54	0.141	0.28	4.49	4.43	0.14	0.82
Milk protein, %	3.39	3.38	0.065	0.84	3.69	3.70	0.063	0.88	3.32	3.38	0.040	0.26	3.50	3.56	0.07	0.61
Milk lactose, %	4.58	4.53	0.031	0.25	4.47	4.34	0.030	0.009	4.61	4.44	0.031	0.001	4.79	4.67	0.05	0.18
BW, kg	604	687	12.5	<0.001	511	630	15.3	<0.001	608	672	18.8	0.03	722	780	20.3	0.12
BCS, scale 1-5	3.48	3.24	0.068	0.01	3.04	3.18	0.055	-[3]	3.54	3.30	0.080	0.05	3.25	3.43	0.100	0.33
Glucose	-	-	-	-	-	-	-	-	3.73	3.25	0.08	0.001	2.92	3.09	0.128	0.44
Insulin[1]	-	-	-	-	-	-	-	-	0.28	0.09	-	0.001	0.13	0.12	-	0.97
NEFA[1]	-	-	-	-	-	-	-	-	0.23	0.31	-	0.05	0.36	0.22	-	0.01
BHB[1]	-	-	-	-	-	-	-	-	0.33	0.45	-	0.06	1.05	0.86	-	0.29
IGF-1[1]	-	-	-	-	-	-	-	-	105	61	-	<0.001	87.5	68.4	-	0.22
EB, MJ NE/d	-2.56	4.12	4.335	0.26	13.7	10.4	3.15	0.50	-13.7	-13.3	2.98	0.91	-2.75	9.91	4.10	0.09

[1] Statistics run on log10 values, presented values are antilog. [2] Fed a total mixed ration. [3] Not possible to estimate.

5.2 Milk yield and composition

There was no difference between dietary treatments for milk and ECM in the byproduct, early and whole lactation studies (Papers I, III and IV). The low number of animals, but perhaps also due to the 2 × 2 factorial design in the whole lactation study which reduced the degrees of freedom, could explain why these results were not significantly different although there were some numerical differences (Figures 4 and 5). As feed intake is the most important nutrition-related factor influencing milk yield, higher DMI of diets with a higher content of concentrate often results in higher milk yields (Kuoppala *et al*., 2004; 2008; Randby *et al*., 2012; Lawrence *et al*., 2015).

The cows fed concentrate based on cereal grain and soybean meal in the byproduct study had a lower milk fat concentration than cows fed concentrate based on sugar beet pulp and distiller's grain, and higher lactose concentration than those fed byproduct-based concentrates (Paper I). Diets high in starch and low in fibre can reduce milk fat content (Bauman & Griinari, 2001; AlZahal *et al*., 2009), but that is unlikely to be the explanation in Paper I, as all cows in that study consumed 58% forage on a DM basis, with at least 327 g NDF/kg DM. There were no differences in concentration of fat, protein and lactose in the milk between diets high and low in byproduct-based concentrate in the early and whole lactation studies (Papers III and IV).

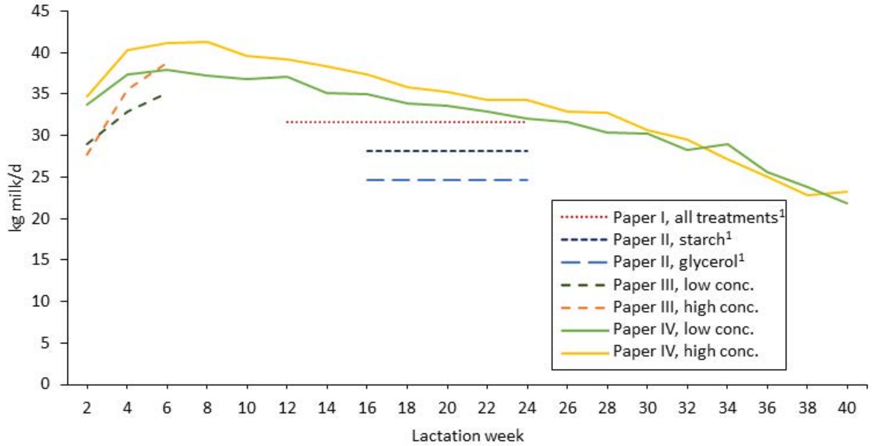

Figure 4. Milk yield presented as least square means per alternate lactation week in the whole lactation study (Paper IV), per lactation week 2, 4 and 6 in the early lactation study (Paper III), and as a mean for the whole study period in the change-over studies (Papers I and II). Since there were no difference between treatments (P>0.05) for these parameters in Paper I, only one mean is presented (all treatments). [1]Mean value for the whole study period.

The lower milk yield when cows were fed a diet with glycerol compared with starch in the glycerol study was evened out for ECM as cows fed glycerol had a higher concentration of both fat and protein (Paper II). Gaillard *et al.* (2018) found an increase in fat and protein with increasing amounts of glycerol, while others only found an increase in protein (Kass *et al.*, 2012) or no effect on either fat or protein (Donkin *et al.*, 2009). A higher fat and protein concentration with high levels of dietary glycerol might be related to increased butyrate production (Huhtanen *et al.*, 1993) from feeding glycerol as reported by others (Rémond *et al.*, 1993; Ariko *et al.*, 2015; Castagnino *et al.*, 2018).

In Sweden, Holstein cows typically have higher milk and ECM production than SR cows (Växa Sverige, 2019). In the present work however, SH cows had a higher milk and ECM production compared with SR cows only in the early lactation study (Paper III), while in the byproduct study (Paper I) and the whole lactation study (Paper IV) no breed differences in milk production were found (Table 2). Swedish Holstein cows also generally produce milk with lower concentrations of fat and protein than SR cows (Växa Sverige, 2019). That was the case in the byproduct study (Paper I) and for protein in the early lactation study (Paper III), but in the whole lactation study there were no breed differences in milk composition (Paper IV).

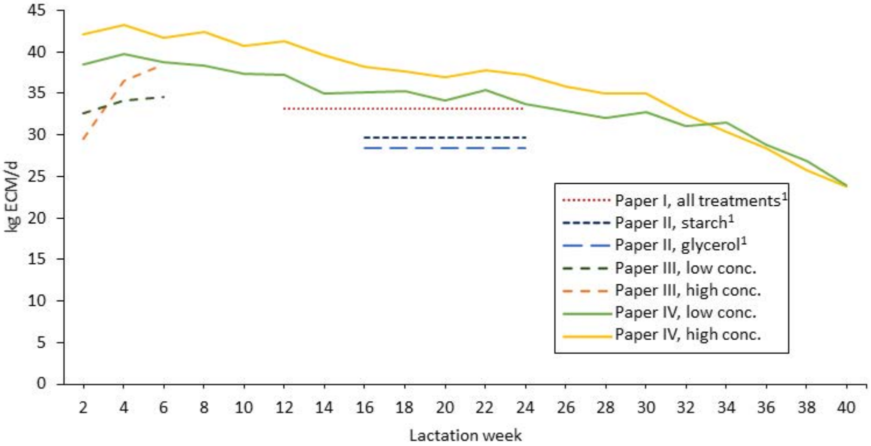

Figure 5. Energy corrected milk (ECM) yield presented as least square means per alternate lactation week in the whole lactation study (Paper IV), per lactation week 2, 4 and 6 in the early lactation study (Paper III), and as a mean for the whole study period in the change-over studies (Papers I and II). Since there were no difference (P>0.05) between treatments for these parameters in the byproduct study (Paper I) only one mean is presented (all treatments). [1]Mean value for the whole study period.

Primiparous cows yielded less than multiparous cows in Papers I-III, as also found by others (Ray *et al.*, 1992; Patel *et al.*, 2016; A. Nyman, Växa Sverige, Uppsala, Sweden, personal communication 2019). In the whole lactation study, where only multiparous cows were studied, there were no statistically significant differences in milk yield between second parity cows and older cows (Paper IV).

Short- and medium-chain FA (≤C14) and about half of the C16 FA in milk originate from *de novo* synthesis of FA (Palmquist *et al.*, 1969) while long-chain FA in milk are pre-formed from adipose tissue or originates from feed (Rukkwamsuk *et al.*, 2000). Therefore, milk FA can be used as an indicator of EB in cows (Gross *et al.*, 2011). The four most abundant milk FA were analysed in the early and whole lactation studies (Papers III-IV). In the glycerol study, there was a higher concentration of C14:0 and lower concentration of C18:1 cis-9 in milk fat when cows were fed a diet with glycerol indicating that that diet led to more positive EB than the starch diet (Paper II). The low and high byproduct-based concentrate diets in the early and whole lactation studies did not result in any differences regarding these four milk FA, suggesting a less pronounced effect on EB from any of the diets (Papers III and IV).

5.3 Energy balance and metabolic status

In all studies included in this thesis (Papers I-IV), EB was estimated based on parity, gestation, BW, feed intake and milk yield. Changes in both BCS and BW can also indicate whether cows are in positive or negative EB. Impaired metabolic status of dairy cows can be indicated by higher blood plasma concentrations of NEFA and BHB, along with lower concentrations of glucose, insulin and IGF-1 (as reviewed by Adewuyu *et al.*, 2005). In the byproduct study (results not published in Paper I), it was estimated that the cows had a more negative EB when fed the concentrate based on sugar beet pulp and distiller's grain, compared with the other three concentrates (Figure 6). However, there were no differences in BCS change or BW change. In the byproduct study (Paper I), blood was not analysed since blood metabolites are less informative in mid-lactation cows. However, others have measured NEFA, BHB, and glucose in mid-lactation and post-peak lactation dairy cows fed either cereal grain and pulse-based or byproduct-based concentrate and have found no effect of diet on blood metabolic indicators (Ertl *et al.*, 2015a; Whelan *et al.*, 2017).

When the cows were fed dietary glycerol in the glycerol study they had a more positive EB than when fed wheat starch (Paper II), which is supported by the concentrations of milk FA, but not by changes in BCS and BW. However, the cows in the gycerol study were assessed for BCS manually instead of automatically, which may have resulted in small changes in body condition not being detected in that study, as manual scoring is more subjective than the BCS camera. In addition, each measurement period was three weeks, which is a relatively short time as regards detecting body condition changes in mid-lactation cows.

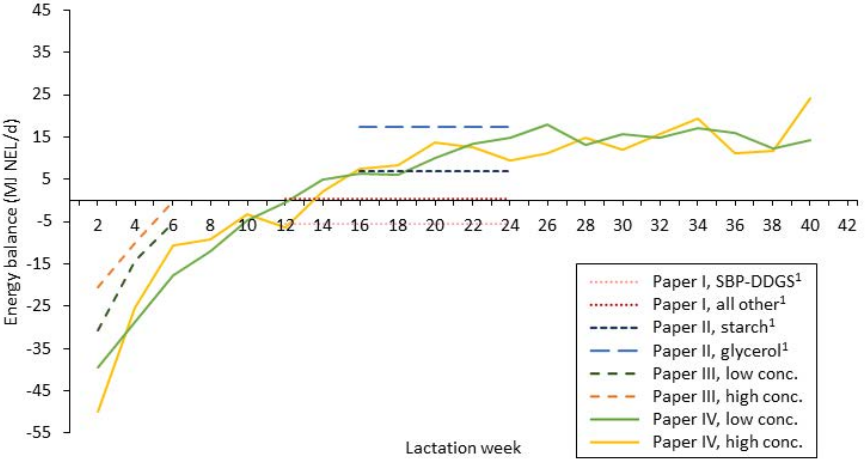

Figure 6. Energy balance (EB; MJ NE/d) presented as least square means per alternate lactation week in the whole lactation study (Paper IV), per lactation week 2, 4 and 6 in the early lactation study (Paper III), and as a mean for the whole study period in the change-over studies (Papers I and II). Since there were no differences in EB (P≥0.05) between concentrates based on cereal grain and soybean meal, and byproduct-based concentrate with rapeseed meal in the byproduct study (Paper I), only one mean is presented for those three concentrates (all other). The concentrate based on sugar beet pulp and distiller's grain (SBP-DDGS) is presented as one line, as it had lower EB values (P-value 0.041). [1]Mean value for the whole study period.

The blood parameters used as indicators of metabolic status were analysed in the early and whole lactation studies (Papers III and IV). In Paper III, where the cows were followed only in early lactation, concentrate level did not have any effect on EB, BCS change or on any of the blood parameters. However, cows fed a high-concentrate diet had a less reduced BW change compared with cows fed a low-concentrate diet. It is possible that those changes in BW partly reflect the weight of digesta.

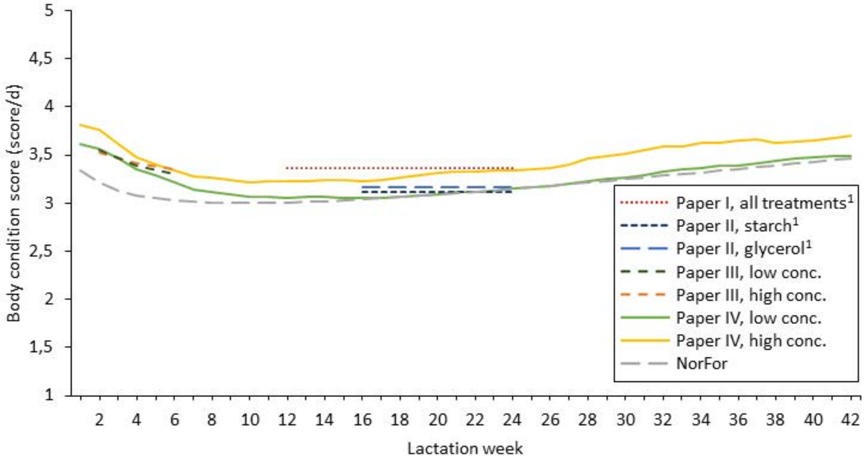

Figure 7. Body condition score (BCS) presented as least square means per lactation week in the whole lactation study (Paper IV), per lactation week 2, 4 and 6 in the early lactation study (Paper III), and as a mean for the whole study period for the change-over studies (Paper I and II). Since there was no difference ($P \geq 0.05$) between treatments in the Byproduct study (Paper I) only one mean is presented (all treatments). The NorFor BCS-curve is included for comparison (NorFor, 2020b). [1]A mean value for the whole study period.

In Paper IV, where the cows were followed over a whole lactation, there were no effects of concentrate level on EB, BCS change, or blood plasma concentration of glucose, NEFA, or BHB, as aslo seen in the early lactation study (Paper III). However, in the whole lactation study the cows fed a high concentrate diet had higher levels of insulin and IGF-1 (Paper IV), which could be explained by higher energy intake. Higher insulin concentrations could be an effect of more propionate being produced in the cows fed a high-concentrate diet (Bines & Hart, 1984). However, the high-concentrate diet did not affect propionic acid concentration or proportion of total VFA (Table 4). There was no effect on BW change in the whole lactation study (Paper IV). In the whole lactation study, cows fed a low-concentrate diet had lower BCS than cows fed a high-concentrate diet (Paper IV), but the difference in BCS is not likely to have been a treatment effect since it was present already in lactation week 1, before any treatment effect could have emerged, and the difference persisted throughout the whole lactation (Figure 7). The BW/BCS ratio calculated based on results in the whole lactation study (Paper IV) was 68 kg/unit score, which can be compared with the NorFor estimate of 60 kg BW per unit of BCS in SH and SR cows (Åkerlind *et al.*, 2011).

Table 4. *Effect of a low (LC) or high (HC) level of byproduct-based concentrate, with (WithAA) or without (NoAA) rumen-protected lysine and methionine, on rumen fluid concentration (mol/100 mol) and proportion of volatile fatty acids (VFA) in mid-lactation cows, presented as least square mean with standard error of the mean (SEM) and P-value (data from R. Danielsson on the cows included in the whole lactation study)*

	LC	HC	SEM[1]	P-value	WithAA	NoAA	SEM[1]	P-value
mol/100 mol								
Acetate (A)	66.7	69.6	2.12	0.39	64.9	71.4	2.44	0.05
Propionate (P)	17.6	19.0	0.82	0.29	17.4	19.2	0.95	0.17
Butyrate (B)	10.8	12.1	0.46	0.09	10.9	12.0	0.53	0.13
Total VFA	98.7	104.7	3.43	0.28	97.0	106.4	3.95	0.08
Proportion								
Acetate	0.68	0.67	0.005	0.16	0.67	0.67	0.006	0.95
Propionate	0.18	0.18	0.003	0.97	0.18	0.18	0.004	0.50
Butyrate	0.11	0.12	0.002	0.06	0.11	0.11	0.002	0.85
(A+B)/P	67.3	70.3	2.11	0.38	65.5	72.1	2.44	0.06

The effect of breed on EB was not significant in the byproduct study (Paper I) and the early lactation study (Paper III), but in the whole lactation study (Paper IV) SH cows had a more positive EB than SR cows (see Table 2). Regarding the blood parameters for indicating metabolic status and effect of breed, the finding of more positive EB of SH cows in the whole lactation study (Paper IV) was supported by higher insulin and lower NEFA concentrations. Concentrations of glucose and BHB were not affected by breed in Papers III and IV. This is in agreement with findings by Andrée O'Hara *et al.* (2018; 2019) of no effect of breed on EB in a study on multiparous cows. In a study on primiparous cows in early lactation, however, Ntallaris *et al.* (2017) found that SH cows had lower glucose plasma concentrations and more negative EB than SR cows. Moreover, in the whole lactation study in this thesis, SH cows had lower BHB concentrations than SR cows in lactation week 4 (P=0.03) (Paper IV). Plasma concentrations of IGF-1 were lower in SH cows than in SR cows in the early lactation study (Paper III), as also reported by Andrée O'Hara *et al.* (2019), while there were no breed differences for IFG-1 in the whole lactation study (Paper IV). There were no interactions of breed × concentrate level or breed × parity for any of the blood parameters analysed in Papers III and IV. In both those studies, the cows were sampled for blood in lactation week 2, 4 and 6. In the whole lactation study they were additionally sampled once in mid-lactation. Only one sample from mid-lactation differed between the two studies, so the blood parameter results are probably comparable to a large extent. In summary, the effect of breeds SH and SR on EB and blood plasma metabolic indicators seems to be somewhat inconsistent.

In the early lactation study, primiparous cows had higher levels of glucose, insulin and IGF-1, and lower levels of NEFA, than multiparous cows (Paper III). The blood plasma metabolic indicator data indicated that multiparous cows had higher pressure on their metabolism and used more body tissue to support their higher milk production, which is similar to findings by Wathes (2007). Multiparous cows also lost more BCS than primiparous cows in Paper III, but EB did not differ between parity classes.

There were no differences between second parity cows and multiparous (third lactation and older) cows as regards concentration of glucose, insulin and BHB in the whole lactation study (Paper IV), as also reported by Andrée O'Hara *et al.* (2019). However, second parity cows had higher concentrations of NEFA than multiparous cows in Paper IV, whereas Andrée O'Hara *et al.* (2019) found no differences. The higher concentrations of NEFA could be related to the milk yield of second parity cows in Paper IV. Andrée O'Hara *et al.* (2019) recorded higher concentrations of IGF-1 in second parity cows than in multiparous cows, which was not found in Paper IV. Higher levels of growth-promoting IGF-1 can be expected in growing cows, and cows continue to grow until at least their third lactation (Coffey *et al.*, 2006). Differences in body condition as such might also have an influence on IGF-1 levels (O'Hara *et al.*, 2016). Overall, the results in Paper III and IV indicate that the metabolism of primiparous cows differs from that of older cows, while the difference between second parity cows and older cows is smaller. Another factor that can have influenced the results between papers is that in Paper III the cows were only sampled for blood in early lactation, while in Paper IV they were also sampled once in mid-lactation. However, there were no effects of parity × lactation week or parity × concentrate level in either study (Papers III and IV).

Estimated EB and metabolic status in dairy cows based on BW, feed intake and milk production along with changes in BCS and BW, in blood plasma concentrations of glucose, insulin, NEFA, BHB and IGF-1 and in milk FA composition, were not completely consistentin Papers I-IV. The gold standard of measuring EB is in calorimetric respiration chambers, which is a much more precise but also more expensive and labour-intensive method, and was not used in the studies included in this thesis. Comparisons of EB values obtained from respiration chambers with values obtained from equations based on feed intake, BW and milk production generally show that the latter approach underestimates the negative EB postpartum (Erdmann *et al.*, 2019). However, the more parameters of EB and metabolic status measured, the more pieces of a complex puzzle can be added, hopefully giving a clearer picture of the effect of diet on EB and metabolic status. In particular, the concentration of C18:1 cis-9 in milk appears to be a promising determinant of negative EB in cows (Churakov *et al.*, unpublished).

5.4 Fertility

Progesterone was analysed in milk samples taken twice weekly between lactation week 2 and 6 in the early lactation study (Paper III) and from calving to confirmed pregnancy in the whole lactation study (Paper IV), to get an indication of the effect of high- and low-concentrate diets on fertility. There were no effects of concentrate level on CLA in either study. Most fertility traits, including CLA, only give one data point per cow and lactation. Hence, concidering the low numbers of animals included in the studies in this thesis, the number of data points was relatively low. In Paper IV, where the cows were followed for the whole lactation, it was possible to record other fertility measures apart from CLA, but no effect of concentrate level or of concentrate type was found for any of the fertility traits measured. These results agree with previous findings (Armstrong *et al.*, 1990; Pedernera *et al.*, 2008; Cutullic *et al.*, 2011; Ntallaris *et al.*, 2017).

There was no effect of breed on CLA in the early lactation study (Paper III), while in the whole lactation study SR cows had more days until CLA than SH cows (Paper IV). Some previous studies performed on the same herd have given similar indications of SR cows being less fertile than SH cows (O'Hara *et al.*, 2016; Andrée O'Hara *et al.*, 2019). Other studies that have included many more cows from several herds have instead found that SR cows have much better fertility than SH cows (Muuttoranta *et al.*, 2019; Andrée O'Hara *et al.*, 2020). There were no differences in BCS between the breeds in Papers III and IV, but SR cows had higher BCS than SH cows in the studies by Andrée O'Hara *et al.* (2016; 2019). High BCS around calving could explain the poorer reproductive performance in SR cows (Roche *et al.*, 2009).

Fertility generally declines with age in dairy cows (Muuttoranta *et al.*, 2019). However, there was no difference in CLA between primiparous and multiparous cows in the early lactation study (Paper III), or for any of the fertility measures in the whole lactation study (Paper IV).

5.5 Feed efficiency

The feed efficiency results are summarised in Tables 5-7. For ECM/DMI and ECM/NEI, there were generally no differences between diets or breeds in the byproduct study (Paper I) or in the early and whole lactation studies (Papers III and IV). However, the cows in the glycerol study (Paper II) had lower

ECM/DMI and were less efficient when fed the glycerol diet than the starch diet, due to higher DMI with the glycerol diet but no difference in ECM between diets. Moreover, SH cows had lower ECM/DMI than SR cows in the byproduct study (Paper I), but with only numerical differences in ECM and DMI. Using NEI as the denominator instead of DMI, the diet with sugar beet pulp and rapeseed meal having a higher value, and thus being more efficient, than any of the other diets fed in the byproduct study (Paper I). Although the value for ECM/NEI was significantly lower, the difference was numerically so small that it is uncertain wether it would have any biological relevance. For both ECM/DMI and ECM/NEI, there were no differences between primiparous cows and multiparous cows (Papers I and III) but second parity cows had higher values than cows in third lactation or older (Paper IV) (Table 5).

Regarding the human-edible efficiency of dairy production, both the HeFCE and net food production values indicated that replacing highly human-edible ingredients such as cereal grain and soybean meal with less human-edible byproducts from feed and fuel industry is beneficial. Dairy cows fed concentrate based on cereal grain and soybean meal consumed more human-edible CP and GE than they produced in the milk (Table 5). In contrast, dairy cows fed concentrate based on byproducts less suitable for human consumption, produced more human-edible CP and GE than they consumed. Replacing cereal grain and soybean meal with byproducts such as sugar beet pulp, distiller's grain, rapeseed meal and bran thus seems to be an efficient way to increase net food production. However, the strategy of replacing cereal grain and pulses with byproducts in dairy cow diets might not be possible if insufficient amounts of byproducts are available on local or global market to maintain high concentrate ratios. Comparing diets with high and low concentrate level in the early and whole lactation studies showed that the efficiency of human-edible food production for CP and GE increased even more than when just replacing the concentrate from high inclusion of human-edibles to low inclusion (Paper III-IV). This was because the grass that replaced concentrate in those studies provided the cows with high-quality nutrients without containing any human-edibles at all. Forage-only systems of course have high efficiency of human-edible food production, but this could come at the cost of lower output, so some concentrate supplementation for high-yielding dairy cows would probably be justified (Wilkinson & Lee, 2018). In the byproduct study (Paper I), the proportion of forage in the diet was 58%, while it was 56% and 67% for the high-concentrate diets in the early and whole lactation studies, respectively. In the low-concentrate diets in Papers III and IV, the proportion of forage was up to 81-82%. In the byproduct study (Paper I) and in the early lactation study (Paper III), there was a significant difference in HeFCE and net food production of both CP and GE between diets, and also in most cases in the whole lactation study (Paper IV). Interestingly, there was no difference in net food production of CP in the whole lactation study (Paper IV), as the higher intake of human-edible CP in the high-concentrate diet compared with the low-concentrate diet seem to be levelled out by numerically higher milk protein yield. In comparison, Ertl *et al.* (2015a) obtained HeFCE values of 5.55 and 4.27 for protein and energy, respectively, when feeding byproduct-based concentrate, and HeFCE values of 1.39 for protein and 1.60 for energy when feeding concentrate based on cereal grain and pulses. In a later study, Ertl *et al.* (2016a) found that the byproduct diet had even higher HeFCE, 8.05 for protein and 7.29 for energy, while the cereal grain and pulse diet had HeFCE of around 1.53 for protein and 1.08 for energy. However, as concluded by Ertl *et al.* (2016a), one challenge is to estimate the potential human-edible fraction of different feedstuffs. In the present thesis, the potential human-edible fraction was roughly estimated according to Wilkinson (2011) as the aim was to compare dairy cow diets and not to compare specific byproducts. Nevertheless, it is important to be aware of this limitation of the results presented in Tables 3-5.

While HeFCE and net food production have been used previously to compare diets or production systems, to my knowledge the effect of breed has not been reported previously. There were no differences between SH and SR cows regarding these two human-edible measures for CP, which aligns with the lack of difference also found in N-efficiency. In the early lactation study (Paper III), the HeFCE and net food production of energy were more beneficial in SH cows compared with SR cows, but there were no differences in the byproduct study (Paper I) and the whole lactation study (Paper IV). Regarding parity classes, it appeard that cows in second lactation and older were more efficient from a human-edible point of view, compared with primiparous cows (Papers I and III), but with no difference between second parity cows and cows in third lactation or older (Paper IV). Takiya *et al.* (2019) found a diet × parity effect for both protein and energy in terms of HeFCE with multiparous cows being more efficient than primiparous cows. These results are to be expected as primiparous cows use a larger part of their nutrient intake for growth rather than for milk production compared with multiparous cows, and in both the present thesis and Takiya *et al.* (2019) only production of milk was accounted for. However, in this thesis there was no interaction of parity × diet for HeFCE or net food production in any of the studies.

As the aim of RFI is to find individual cow differences in feed efficiency for breeding, rather than to compare dairy cow diets, it is perhaps not that surprising that there were no significant differences between diets in Papers I, III and IV. In contrast, the glycerol diet in Paper II emerged as less efficient than the starch diet in terms of RFI. Cows on glycerol diet in Paper II ate more feed, but produced the same amount of ECM. If the extra energy intake of cows when fed the glycerol diet did not go to milk production, then it should reasonably have been stored as body tissue or lost from the cow as undigested matter or enteric CH_4. As there were no detectable differences in BCS change or OMD between diets in Paper II, the CH_4 emissions option remains. Indeed, CH_4 emissions were higher in cows fed the glycerol diet than in cows fed the wheat starch diet. However, this might also be explained by lower energy value of glycerol than reported in feed tables.

In this thesis, a new trait, forage efficiency, was tested. It is defined as forage intake per unit metabolic BW and aims to compare forage efficiency between animals, but not between dietary treatments. The rationale for this trait is that it would be relevant to breed from cows that have good potential to consume forage relative to their size, assuming that forage is converted to milk production. Preliminary results indicate that by combining the traits RFI and forage efficiency, it is possible to identify cows with superior ECM/NEI and IOFC (Karlsson *et al.*, unpublished observations). In the early lactation study (Paper III), SH cows had higher intake of forage per kg metabolic BW compared with SR cows. On the other hand, no difference in forage efficiency between breeds was observed in Paper I. Primiparous cows were less forage efficient than older cows (Table 7). However, it is possible that the observed differences in forage efficiency is an artefact related to BCS, as SR cows had higher BCS than SH cows (Table 2) and primiparous cows had higher BCS than multiparous cows in Paper II (Table 3). Moreover, a measure that also includes ECM yield might be more relevant, as the measure developed in this thesis primarily favours high forage intake and not necessarily efficient use of forage consumed.

Income over feed cost (IOFC) take economic aspects of the diet and production into consideration, but the value of this metric fluctuates depending on prices of milk and feed and the cost of on-farm feed production. Nonetheless, it gives an indication as to the cost for a specific concentrate or a concentrate level that can pay off in terms of milk production. For farmers to estimate which diet is the most profitable with current milk price and feed costs, they need to know what effect on milk production to expect from different diets. That is partly why it is important to study the effect of different diets on dairy cow production on research farms. In the glycerol study (Paper II), the diet was extreme and did not reflect an on-farm feeding situation, as both wheat starch and pure glycerol are expensive, so IOFC was not determined in that study. There were no difference in IOFC between diets in the byproduct study (Paper I), which was expected as the concentrates were quite similar in price, and there were also no effects of diet on ECM or DMI. In Paper III, where all cows were fed the same concentrate, but at two different levels, in early lactation, there was no difference in IOFC between diets. In the whole lactation study (Paper IV), there was no effect of concentrate level or concentrate type (with or without rumen-protected lysine and methionine; 65.3 vs 64.4 SEK/d; P= 0.83) on IOFC although the concentrate with rumen-protected lysine and methionine had a somewhat higher cost and no effect on ECM or feed intake. Henriksson *et al*. (2019) found that feeding more byproducts or offering lower levels of concentrate did not increase feed costs per kg ECM and concluded that on farms which manage to keep forage production costs low, low-concentrate rations might be beneficial from an economic point of view.

There were no effects of breed on IOFC in the byproduct study (Paper I) or the whole lactation study (Paper IV), but SH cows had higher IOFC than SR cows in the early lactation study (Paper III). In that study, SH cows had both higher DMI and ECM than SR cows. However, with the prices used in the present thesis, the higher ECM paid off despite being accompanied by higher DMI. The lack of difference in IOFC between SH and SR cows in the whole lactation study (Paper IV) might be the most relevant finding for farmers, as they generally keep cows for whole lactations and not just early lactation. Based on IOFC for different parity classes in Papers I, III and IV, it can be concluded that multiparous cows are more profitable than primiparous cows. As with the breed effect, higher ECM yield increased IOFC in this thesis.

Table 5. *Impact of diet on daily feed efficiency per cow measured as feed conversion efficiency (kg energy-corrected milk/kg dry matter intake; kg energy-corrected milk/MJ NEI), human-edible feed conversion efficiency (HeFCE; kg milk protein/kg human-edible crude protein intake; MJ gross energy in milk/human-edible MJ gross energy intake), net food production (Net food; kg milk protein – kg human-edible crude protein intake; MJ gross energy in milk - human-edible MJ gross energy intake), residual feed intake (RFI; MJ NE), income-over-feed-cost (IOFC; SEK) and nitrogen efficiency (N-efficiency; (g milk protein/6.38)/(kg crude protein intake/6.25)), presented as least square mean with standard error of the mean (SEM) and P-value, in Papers I-IV. Means within rows and paper with different superscrips differ significantly ($P \leq 0.05$). Dietary treatments are abbreviated in Paper I based on main concentrate ingredients as cereal grain and soybean meal (CG-SBM), sugar beet pulp (SBP), rapeseed meal (RSM) and distiller's grain (DDGS), in Paper II as starch (ST) and glycerol (GL), and in Papers III and IV as low concentrate level (LC) and high concentrate level (HC)*

	Paper I						*Paper II*				*Paper III*				*Paper IV*			
	CG-SBM	SBP-DDGS	SBP-RSM	SBP-RSM-DDGS	*SEM*	*P-value*	ST	GL	*SEM*	*P-value*	LC	HC	*SEM*	*P-value*	LC	HC	*SEM*	*P-value*
ECM/DMI	1.46	1.47	1.51	1.44	*0.047*	*0.32*	1.49	1.32	*0.040*	*0.008*	1.68	1.63	*0.041*	*0.38*	1.42	1.45	*0.034*	*0.64*
ECM/NEI	0.22^{b}	0.22^{b}	0.23^{a}	0.22^{b}	*0.007*	*0.02*	0.23	0.21	*0.006*	*0.03*	0.26	0.25	*0.007*	*0.56*	0.22	0.22	*0.005*	*0.76*
HeFCE, CP	0.73^{b}	2.56^{a}	2.63^{a}	2.68^{a}	*0.059*	*<0.001*	-	-	-	-	7.48	3.59	*0.18*	*<0.001*	8.13	3.97	*0.871*	*0.01*
HeFCE, GE	0.54^{b}	1.62^{a}	1.67^{a}	1.66^{a}	*0.038*	*<0.001*	-	-	-	-	5.44	2.57	*0.119*	*<0.001*	5.48	2.80	*0.635*	*0.02*
Net food, CP	-0.39^{b}	0.62^{a}	0.67^{a}	0.67^{a}	*0.030*	*<0.001*	-	-	-	-	0.93	0.77	*0.034*	*<0.001*	1.06	1.02	*0.033*	*0.47*
Net food, GE	-71.7^{b}	31.8^{a}	34.5^{a}	33.7^{a}	*2.26*	*<0.001*	-	-	-	-	86.7	61.5	*2.93*	*<0.001*	83.9	68.2	*2.84*	*0.002*
RFI, NE	3.85	-0.23	-2.63	0.03	*3.79*	*0.23*	5.53	16.4	*2.77*	*0.01*	3.29	2.97	*4.56*	*0.96*	4.03	3.77	*2.05*	*0.94*
IOFC	56.3	56.2	*60.3*	*57.7*	*3.07*	*0.14*	-	-	-	-	*72.3*	*66.0*	*2.52*	*0.09*	*64.9*	*64.8*	*2.90*	*0.19*
N-efficiency	304^{a}	288^{c}	302^{ab}	291^{bc}	0.8	0.03	355	312	10.1	0.01	359	334	8.3	0.05	284	280	-	0.74

Table 6. *Effect of the breed Swedish Red (SR) and Swedish Holstein (SH) on daily feed efficiency per cow measured as feed conversion efficiency (kg energy-corrected milk/kg dry matter intake; kg energy-corrected milk/MJ NEI), human-edible feed conversion efficiency (HeFCE; kg milk protein/kg human-edible crude protein intake; MJ gross energy in milk/human-edible MJ gross energy intake), net food production (Net food; kg milk protein – kg human-edible crude protein intake; MJ gross energy in milk - human-edible MJ gross energy intake), residual feed intake (RFI; MJ NE), forage efficiency (kg forage dry matter intake/kg body weight*$^{0.75}$*), income-over-feed-cost (IOFC; SEK) and nitrogen efficiency (N-efficiency; (g milk protein/6.38)/(kg crude protein intake/6.25)), presented as least square mean with standard error of the mean (SEM) and P-value, in Papers I, III and IV*

	Paper I				*Paper III*				*Paper IV*			
	SR	SH	*SEM*	*P-value*	SR	SH	*SEM*	*P-value*	SR	SH	*SEM*	*P-value*
ECM/DMI	1.55	1.39	*0.055*	*0.05*	1.65	1.66	*0.042*	*0.89*	1.47	1.40	*0.037*	*0.16*
ECM/NEI	0.23	0.21	*0.008*	*0.06*	0.25	0.25	*0.007*	*0.99*	0.22	0.21	*0.006*	*0.15*
HeFCE, CP	2.17	2.13	*0.058*	*0.62*	5.46	5.61	*0.178*	*0.59*	5.89	6.22	*0.948*	*0.81*
HeFCE, GE	1.40	1.35	*0.038*	*0.42*	3.82	4.18	*0.121*	*0.05*	4.12	4.15	*0.691*	*0.98*
Net food, CP	0.41	0.38	*0.031*	*0.58*	0.82	0.88	*0.034*	*0.25*	1.00	1.08	*0.036*	*0.12*
Net food, GE	8.66	5.45	*2.483*	*0.40*	68.7	79.5	*2.960*	*0.02*	75.0	77.1	*3.10*	*0.66*
RFI, NE	-5.21	5.74	*4.192*	*0.09*	1.35	4.91	*4.607*	*0.60*	0.30	7.50	*2.232*	*0.03*
Forage efficiency	0.10	0.11	*0.005*	*0.24*	0.10	0.12	*0.004*	*0.02*	0.13	0.13	*0.002*	*0.21*
IOFC	61.4	53.8	*3.49*	*0.16*	65.3	73.0	*2.54*	*0.05*	65.8	63.9	*3.16*	*0.69*
N-efficiency	308	284	*9.1*	*0.08*	355	337	*8.37*	*0.15*	288	277	-	*0.40*

Table 7. *Effect of parity classes primiparous (1; first lactation) and multiparous (2+; second lactation and older) in Papers I, II and III, or second parity (2; second lactation) and multiparous (3+; third lactation and older) in Paper IV on daily feed efficiency per cow measured as feed conversion efficiency (kg energy-corrected milk/kg dry matter intake; kg energy-corrected milk/MJ NEI), human-edible feed conversion efficiency (HeFCE; kg milk protein/kg human-edible crude protein intake; MJ gross energy in milk/human-edible MJ gross energy intake), net food production (Net food; kg milk protein – kg human-edible crude protein intake; MJ gross energy in milk - human-edible MJ gross energy intake), residual feed intake (RFI; MJ NE), forage efficiency (kg forage dry matter intake/kg body weight$^{0.75}$), income-over-feed-cost (IOFC; SEK) and nitrogen efficiency (N-efficiency; (g milk protein/6.38)/(kg crude protein intake/6.25)), presented as least square mean with standard error of the mean (SEM) and P-value*

	Paper I				*Paper II*				*Paper III*				*Paper IV*			
	1	2+	*SEM*	*P-value*	1	2+	*SEM*	*P-value*	1	2+	*SEM*	*P-value*	2	3+	*SEM*	*P-value*
ECM/DMI	1.49	1.45	*0.057*	*0.62*	1.34	1.45	*0.047*	*0.11*	1.61	1.69	*0.042*	*0.22*	1.55	1.32	*0.059*	*0.04*
ECM/NEI	0.22	0.22	*0.008*	*0.91*	0.19	0.21	*0.007*	*0.13*	0.25	0.26	*0.007*	*0.19*	0.24	0.20	*0.009*	*0.03*
HeFCE, CP	2.02	2.28	*0.061*	*0.002*	-	-	-	-	5.39	5.68	*0.177*	*0.28*	6.42	5.69	*1.51*	*0.79*
HeFCE, GE	1.29	1.46	*0.040*	*0.003*	-	-	-	-	3.89	4.11	*0.120*	*0.22*	4.25	4.03	*1.094*	*0.91*
Net food, CP	0.32	0.46	*0.032*	*0.003*	-	-	-	-	0.72	0.99	*0.034*	*<0.001*	1.15	0.96	*0.058*	*0.15*
Net food, GE	1.47	12.6	*2.60*	*0.003*	-	-	-	-	61.5	86.6	*2.95*	*<0.001*	84.6	67.5	*4.98*	*0.06*
RFI, NE	-1.31	1.85	*4.39*	*0.60*	15.2	7.91	*3.194*	*0.14*	0.81	5.44	*4.57*	*0.49*	-1.75	9.56	*3.587*	*0.09*
Forage efficiency	0.09	0.11	*0.005*	*0.007*	-	-	-	-	0.10	0.12	*0.004*	*0.001*	0.13	0.12	*0.004*	*0.22*
IOFC	53.3	61.9	*3.65*	*0.09*	-	-	-	-	58.0	80.3	*2.54*	*<0.001*	74.3	55.4	*5.08*	*0.05*
N-efficiency	295	295	*9.5*	*0.87*	311	337	*10.6*	*0.11*	340	353	*8.36*	*0.27*	303	263	-	*0.11*

5.6 Nitrogen efficiency

Nitrogen efficiency is a valuable indicator used for reducing the negative consequences of eutrophication from *e.g.* animal production such as dairy. Diets low in CP are usually more N-efficient, but too low dietary CP can cause a decrease in milk production. In the byproduct study (Paper I), the cows had lower N-efficiency when fed concentrate based on sugar beet pulp and distiller's grain than when fed concentrate that did not contain any distiller's grain (Table 5). This effect is probably linked to the lower milk protein yield when the cows were fed concentrate based on sugar beet pulp and distiller's grain, since there were no differences in CP intake between cows fed different concentrates. The production process for ethanol manufacturing can reduce the digestibility of the CP fraction in distiller's grain (Böttger & Südekum, 2018). However, there was no difference in total tract apparent CP digestibility between the three byproduct-based concentrates in Paper I that could explain the lower N-efficiency when cows were fed concentrate based on sugar beet pulp and distiller's grain.

In the Glycerol study, the higher N-efficiency when feeding the starch diet was not related to milk protein yield (1.0 kg/d with starch diet and 0.94 kg/d with glycerol diet; P=0.15), but rather higher CP intake from higher total DMI when feeding the glycerol diet (Paper II).

The optimal CP content in dairy cow diets is around 165 g/kg DM in typical North American diets based on maize silage, maize and soybean (Colmenero & Broderick, 2006). In the grass silage-based diets common in Northen Europe, the optimal CP content in dairy cow diets is also around 160-170 g/kg DM (Nadeau *et al.*, 2007). Some studies have concludeed that, in practice, dairy cows should not be fed diets with CP content lower than 130-140 g/kg DM (Huhtanen, 2013). The CP level in the diets in the byproduct study (Paper I) ranged between 154 and 157 g/kg DM, while in the glycerol study (Paper II) both diets contained 140 g CP/kg DM. In the early lactation study (Paper III), the low-concentrate diet contained 147 g CP/kg DM while the high-concentrate diet contained 158 g CP/kg DM. In the whole lactation study (Paper IV), all diets, high- and low-concentrate and with/ without rumen-protected amino acids, contained 162-163 g CP/kg DM.

One reason for feeding rumen-protected amino acids such as lysine and methionine is to enable use of diets even lower in CP than otherwise optimal, to increase N-efficiency further without losing milk production. In the whole lactation study (Paper IV), supplementing byproduct-based concentrate with rumen-protected lysine and methionine had no effect on feed intake, milk production, milk composition, EB or blood plasma concentrations of NEFA, glucose, insulin, and IGF-1 (Table 8). Cows fed concentrate with rumen-protected lysine and methionine had higher plasma concentration of BHB than cows fed concentrate without rumen-protected lysine and methionine. There was also a concentrate type × lactation week interaction (P=0.002) where early lactation cows fed rumen-protected lysine and methionine had higher plasma concentrations of BHB (1.23 mmol/L) than mid-lactation cows (0.73 mmol/L) or cows fed concentrate without rumen-protected lysine and methionine (0.82 mmol/L). The methionine used (MetaSmart) does not affect plasma concentration of BHB in lactating cows (Osorio *et al.*, 2013). Therefore, it could be speculated that the increase in BHB concentration was related to lysine supplementation. It has been shown in mice that dietary supplementation with lysine stimulates liver β-oxidation by activating carnitine palmitoyltransferase 1a (Sato *et al.*, 2018). In ruminants, this is a key enzyme facilitating transport of NEFA into the mitochondria for β-oxidation and ketogenesis (Herdt, 2000). This would potentially increase ketogenesis and decrease esterification of NEFA to form triglycerides, thereby lowering the risk of fat infiltration into the liver in early-lactation dairy cows. In contrast to the results in the whole lactation study (Paper IV), lysine supplementation of a maize-based diet fed to transition cows have been shown to increase DMI and decrease BHB in early lactation (Girma *et al.*, 2019).

The overall lack of effect of supplementation with rumen-protected lysine and methionine in the whole lactation study (Paper IV) might be becasue both diets contained sufficient amounts of essential amino acids and because the total CP level in the diets was not low enough to require supplementation with rumen-protected lysine and methionine. Another explanation can be that histidine, rather than lysine or methionine, is the first limiting amino acid in milk production in dairy cows fed grass silage-based diets (Vanhatalo *et al.*, 1999; Kim *et al.*, 1999; Korhonen *et al.*, 2000). Unfortunately, there is no commercially available rumen-protected histidine available today for comparative studies (Giallongo & Hristov, 2017).

Table 8. *Treatment effect on daily feed intake, milk yield, milk composition, body weight (BW), body condition score (BCS), blood plasma concentrations of glucose, insulin, non-esterified fatty acids (NEFA), β-hydroxybutyrat (BHB) and insulin-like growth factor 1 (IGF-1), and nitrogen (N) efficiency presented as least square mean with standard error of the mean (SEM) and P-value for concentrate either supplemented with rumen-protected lysine and methionine (WithAA) or not (NoAA) (Paper IV)*

	WithAA	NoAA	SEM	P-value
Total dry matter intake, kg DM/d	24.4	24.6	0.35	0.79
Forage intake, kg DM/d	18.2	18.3	0.35	0.90
Concentrate intake, kg DM/d	6.25	6.33	0.048	0.19
Milk yield, kg/d	33.1	32.4	1.02	0.62
Energy-corrected milk yield, kg/d	35.0	35.2	1.08	0.88
Milk fat, g/kg	43.8	45.3	0.89	0.21
Milk protein, g/kg	35.0	35.7	0.46	0.25
Milk lactose, g/kg	47.3	47.2	0.32	0.80
BW change, kg/week	1.52	1.64	0.679	0.98
BCS change, kg/week	-0.005	-0.025	0.0127	0.25
Energy balance, MJ NE/d	3.47	3.69	2.694	0.95
Glucose, mmol/L	2.98	3.03	0.084	0.65
Insulin (log10)	-0.87	-0.94	0.068	0.45
Insulin antilog, µg/L	0.14	0.12	-	-
NEFA (log10)	-0.54	-0.57	0.031	0.45
NEFA antilog, mmol/L	0.29	0.27	-	-
BHB (log10)	0.04	-0.08	0.030	0.003
BHB antilog, mmol/L	1.09	0.83	-	-
IGF-1 (log10)	1.86	1.91	0.031	0.25
IGF-1 antilog, ng/ml	73.2	81.8	-	-
N-efficiency (log10)	2.45	2.45	0.014	0.75
N-efficiency antilog, g/kg	284	280	-	-

5.7 Methane from enteric fermentation

Enteric fermentation leads to methane emissions, which contribute to global warming. Therefore, there is intensive research on strategies to lower methane emissions per kg of milk in dairy production. Werner Omazic *et al.* (2015) have shown that glycerol is absorbed over the rumen wall in significant amounts, and should then enter the blood fast and not be available for fermentation in the rumen. In the glycerol study (Paper II), the hypothesis tested was that feeding glycerol to dairy cows would provide the animals with energy without contributing much to enteric CH_4 production. However, compared with wheat starch, adding pure glycerol (200 g/kg DM) to a grass silage-based TMR fed to mid-lactation dairy cows did not decrease enteric CH_4 emissions. In fact, daily CH_4 emissions were higher in cows fed the glycerol diet. There were no differences in CH_4/DMI or OMD, so it can be assumed that the increase in CH_4 was mainly a result of higher feed intake, rather than an effect of the glycerol itself. These results indicate that when glycerol is mixed with other feedstuffs, it is less available for direct absorption over the rumen wall, which suppors findings that oral drenching of glycerol increases blood plasma concentrations of glucose and insulin, but not mixing glycerol with the feed (Linke *et al.*, 2004). Another contributing factor could be that when glycerol, instead of *e.g.* carbohydrates from starch, is fermented to propionate, H_2 is produced (Avila-Stagno *et al.*, 2014). This H_2 can then be used by methanogens to reduce CO_2 to CH_4. Propionate from carbohydrate fermentation is instead a H_2 sink (Moss *et al.*, 2000), leading to reduced CH_4 production.

5.8 Methodological considerations

Reliable recording of forage intake is important when studying feed intake in dairy cows. In the whole lactation study (Paper IV), it was found that the recorded forage intake data for some individual cows deviated from expected pattern. Further investigation revealed that the behaviour of some cows influenced the forage intake measurements, *e.g.*some cows were throwing silage out of the forage troughs. When a cow started this behaviour, it was difficult to get them to stop. It also appeared that if the cows learned these behaviours from each other, resulting in more cows with these behaviours as time went on. The whole lactation study had been running for quite some time when the problem was detected, and thus it was not possible to replace the cows with problematic behaviour, while removing those cows would have left with very few cows in total. At the end of the whole lactation study, the design of the forage troughs was modified with the aim of alleviating the problem.

The problem with forage intake data quality was handled as follows in the whole lactation study: The intake for feeding occasions with intake rate >8.28 g/s of fresh weight (95% confidence level of all eating occasions for all cows included in the study) was replaced by individual intake estimates derived from daily average intake rate <8.28 g/s. Forage DMI and total DMI were treated as missing values for days when total DMI divided by metabolic BW was above 0.22 kg/kg (95% confidence level). In the early lactation study (Paper III), a similar approach was used but with the threshold level for adjusting intake rate set at 30 g/s (95% confidence level of all eating occasions for all cows included in the study). In Paper III, forage DMI and total DMI were treated as missing values for those days when the total DMI was above 34 kg DM (95% confidence level). No adjustments was considered necessary in the byproduct study (Paper I) or in the glycerol study (Paper II), as cow feeding behaviour problems were not observed in those studies.

The definition of what is "human-edible" is not clear and definitive. Rather, it is a diffuse concept that depends on the situation and location. Not all cereal grains and pulses are classified as human-edible, depending on quality and access to other food products. Today, the highest-quality crops are used as food, while lower quality crops are used as animal feed. However, in a crisis situation that involves food shortages, humans might not be as selective about what quality of grain they consume. From a local perspective, high use of cereal grain and pulses as animal feed can act as a reserve supply of food for humans. On a global scale, on the other hand, the sustainability of using land suitable for producing human food directly for producing animal feed instead can be questioned as long as there is insufficient food to end hunger among all people.

In the present thesis, the potential human-edible fraction was roughly estimated according to Wilkinson (2011), mainly because that approach is simple to apply when the aim is to compare diets used for production animals. However, it might be more correct to define the human-edible proportion based on more precise mass allocation between main product and byproducts of different crops. For example, it has been estimated for Swedish conditions that the proportion of byproduct from crops based on mass is 58% for rapeseed meal, 50% for distiller's grain, 20% for sugar beet pulp, 10% for molasses, 17% for wheat bran and 11% for wheat flour of feed quality (Flygsjö *et al.*, 2008). However, as demonstrated by Ertl *et al.* (2015b, 2016a), there is a range of possible proportions of human-edibles for different products. It is also possible to define the human-edible proportion in terms of the amount of human-digestible energy, protein or amino acids that ends up in the main product and in the byproducts (Patel *et al.*, 2017; Ertl *et al.*, 2016b).

In the two studies with a randomised experimental design (Papers III and IV), there were quite large numerical differences between diets as regards *e.g.* milk yield and ECM yield, but no statistically significant differences were found. The lack of statistical power is probably related to the relatively low number of animals, but also to the study design. In the whole lactation study (Paper IV), relatively high numbers of animals were excluded due to clinical or sub-clinical mastitis. In total, 51 cows entered that study, but only 37 made it through the whole lactation without any events that were judged to influence the results, or because cows had to be moved to another pen to reduce the risk of spread of *Staphylococcus aureus* infection (*S. aureus*, n = 7; mastitis, n = 3; feeding mistakes, n = 2; other health issues, n = 2). In the whole lactation study (Paper IV), both the unbalanced design and the 2 × 2 factorial design probably influenced the statistical power of the study. The numbers of cows allocated to each treatment were unbalanced due to a parallel genetic study on the cows fed the low-concentrate diet. The 2 × 2 factorial design, with both concentrate level (high or low) and concentrate type (with or without supplementation of rumen protected amino acids), might have been more appropriate to include in the early lactation study (Paper III), but with a larger number of animals and much lower overall CP level in the diets.

Whole lactation studies with dairy cows are quite rare. Most dietary studies on dairy cows are conducted during the most challenging time of a dairy cow's life, *i.e.* the transition period from late gestation to peak lactation, or during their most stable period, in mid-lactation. However, whole lactation studies give a valuable overall picture that is important from a practical point of view, since farmers do not keep cows for early or mid-lactation, but for whole lactations. The whole lactation study (Paper IV) revealed *e.g.* that the cows may lose BCS in early lactation but manage to recover over the course of lactation (Figure 7). In addition, whole lactation studies can verify the quality of input data, since *e.g.* if data on feed intake or milk production are erroneous, then the EB or BCS would probably not show reasonable values over the course of a whole lactation.

6 Conclusions

Replacing cereal grain and soybean meal with the byproducts sugar beet pulp, rapeseed meal, distiller's grain and wheat bran in the diet of dairy cows in mid-lactation did not affect feed intake or milk production when fed together with grass silage of high digestibility. However, feeding human-inedible byproducts substantially increased the efficiency of human food production. Increasing the amount of forage in the byproduct-based diets increased the efficiency of human food production even further. When low levels of byproduct-based concentrate were combined with highly digestible grass-clover silage in dairy cow diets, total feed intake seemed to decrease somewhat, but with no effect on energy-corrected milk yield compared with feeding higher levels of byproduct-based concentrate. There were no effects of feeding different levels of byproduct-based concentrate on estimated energy balance, on its indicator milk fatty acids or on metabolic status indicators such as blood plasma concentration of glucose, non-esterified fatty acids, and β-hydroxybutyrate. However, in a whole lactation study with only multiparous cows, cows fed a higher level of concentrate had higher concentrations of insulin and insulin-like growth factor 1 in blood plasma, which can be related to higher energy intake. In summary, high-producing Swedish Red and Swedish Holstein dairy cows fed low levels of byproduct-based concentrate and high-digestibility grass-clover silage can manage to perform well on this diet, without compromising milk production, energy balance or feed efficiency, thereby contributing to more sustainable food production.

Feeding glycerol instead of starch to dairy cows was tested as a way of further improving sustainability by reducing enteric methane emissions, based on the assumption that direct absorption of glycerol over the rumen wall would provide dairy cows with energy, but leave less available for fermentation. However, it was found that replacing wheat starch (200 g/kg of DM) with glycerol in a grass silage and barley-based total mixed ration did not decrease enteric methane emissions.

7 Practical implications

From a practical perspective, increasing the use of byproducts and grass-clover silage in diets for high-yielding dairy cows may add value to dairy products from a consumer perspective. Labelling milk as a resource efficient, low-input and high-output product that also contributes to ecosystem services through grass production could increase profitability for dairy farmers. The challenge is to compete with many other consumer labels and brands claiming to benefit different aspects of food production.

The results in this thesis indicate that, with better knowledge of production responses from diets high in forage and virtually without any human-edible ingredients, farmers and advisors would be able to estimate and calculate economic outcomes for a wider range of possible diets. The knowledge of the effects of using only byproducts could make formulation of feed rations much more flexible, resilient and cost-efficient. The use of possible feedstuffs from industrial byproducts needs to be continually explored and updated as new products arise from changes in production methods or new plant varieties.

8 Future perspectives

This thesis focused mainly on the effects of high-forage and byproduct-based diets, and to some extent also on the effect of breed and parity, in improving the sustainability of dairy production. In future studies, it would be interesting to explore the variation between individual cows in terms of efficient use of diets high in forage and complemented with byproducts. A first step, could be to investigate whether there are any phenotypes related to efficient use of diets high in forage, later on potentially investigating different genotypes. However, genetic evaluations require good-quality data from many individual cows that have been fed fixed rations with low concentrate levels in combination with *ad libitum* access to forage and accurate recording of forage intake. Development of a metric that indicates truly forage-efficient cows would facilitate the search for dairy cows that can produce much milk on diets high in forage, have low total feed intake in relation to their milk production and at the same time maintain healthy body condition.

Another area to explore is development of useful and informative measures of how dairy production could contribute *e.g.* to sustainable food production. Clear and accurate metrics would facilitate the work of improving sustainability. However, dairy and other food production systems are complex interactions between biology, technology and economic, and also politics and other societal aspects, so developing all-embracing measures may be challenging.

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Popular science summary

This thesis shows that high-producing dairy cows can perform well on a diet virtually without any human-edible ingredients, and thereby contribute to sustainable food production. In studies comparing the effects of feeding dairy cows different byproducts instead of human-edibles or different levels of byproduct-based concentrate, it was found that the cows managed to produce much milk on diets where human-edibles such as wheat, barley, oats or beans were replaced with small amounts of byproducts from the production of sugar, ethanol, rapeseed oil and baking flour together with large quantities of grass-based silage. Feeding dairy cows products inedible for humans would increase net production of food, as grain otherwise used as cow feed instead could feed the growing human population.

The grains fed to livestock globally today could potentially feed around 3.5 billion humans. In future, even a greater human population will need to be fed using the limited area of land available and suitable for crop production. However, there is competition for land to grow crops for feed, fuel and food. Land that is less suited for crop production can be used to produce food by growing forage that is fed to animals, or biofuel crops for bioenergy production.

When dairy cows are fed diets with a large proportion of cereal grains and pulses, they consume more human-edibles than they manage to produce as milk. Net food production from energy and protein consumption is then negative. However, when cereal grains and pulses in dairy cow diets are replaced with byproducts such as sugar beet pulp, distiller's grain, rapeseed meal and bran, dairy cows produce much more human-edibles than they consume.

Feeding byproducts instead of wheat, barley, oats, beans or peas is therefore an efficient way of increasing net food production and using finite resources wisely. However, the amount of byproducts available will not be sufficient if large amounts of concentrate continue to be fed to animals. An alternative strategy for increasing net food production from dairy cows is therefore to increase the proportion of grass in their diets. Grass production is also good for the environment in multipleways, *e.g.* it improves carbon storage in the soil and reduces the need for chemicals for controlling pests and weeds, and is thus good for soil quality and for the invironment.

Grass has a high fibre content and usually has a lower content of energy and protein than concentrates. However, by harvesting grass at an early growth stage, it is possible to make silage with a high content of energy and protein. When dairy cows were fed diets based on small amounts of byproducts along with large amounts of grass-clover silage in this thesis, the cows managed to produce a lot of milk, while still maintaining a healthy weight and body condition. In addition, there were no signs of reduced fertility in cows fed a diet based on small amounts of byproducts and large amounts of grass-clover silage.

It is important to reduce methane production within cow feed digestion, since methane is a potent greenhouse gas. A study was performed to test whether feeding glycerol instead of wheat starch to dairy cows would reduce methane emissions, based on the assumption that the glycerol would be absorbed directly from the rumen into the blood and thus less available to rumen microbes that produce methane when digesting feed. However, replacing wheat starch with glycerol, a byproduct from bio-diesel production, was found to have no effect in reducing methane production in dairy cows.

Populärvetenskaplig sammanfattning

Denna avhandling visar att kor som producerar mycket mjölk kan anpassa sig till en foderstat som praktiskt taget helt baseras på sådant som inte människor äter. Detta bidrar till en hållbar livsmedelproduktion. Korna producerade mycket mjölk även när spannmål och soja inte ingick i fodret. Kraftfodret bestod istället av biprodukter från tillverkning av socker, etanol, rapsolja och mjöl istället för potentiella livsmedel som vete, korn, havre och bönor. Korna hade alltid fri tillgång till gräsensilage. Resultaten baseras på fyra studier där effekten av att utfodra mjölkkor med antingen olika biprodukter jämfört med spannmål eller olika mängder biproduktbaserat kraftfoder. När man utfodrar mjölkkor med produkter som inte passar som livsmedel så ökar nettoproduktionen av mat, eftersom spannmålet och bönorna som annars används som foder istället kan användas i större utsträckning som mat till människor. Det är viktigt eftersom vi blir fler och fler människor på jorden som behöver mat.

Idag utfodras djur med många produkter som lika gärna hade kunnat användas som livsmedel. Den mängd spannmål som boskap utfodras med idag skulle räcka som mat till omkring 3,5 miljarder människor. I framtiden behöver vi kunna producera livsmedel till den växande befolkningen från den åkermark vi har idag. Det är dessutom konkurrens om vad som ska odlas på åkermarken, och om vad det som odlats ska användas till – mat, foder eller bränsle. Mark som inte passar för odling av spannmål kan producera mat via odling av gräs som utfodras till djur eller bränsle via odling av biobränsle.

När mjölkkor utfodras med mycket spannmål och bönor äter de mer potentiellt livsmedel än vad de producerar i form av mjölk. Men om man istället ersätter spannmål och bönor med biprodukter som betfiber, drank, rapsmjöl och kli till mjölkkor så producerar de mycket mer livsmedel än vad de äter.

Mängden biprodukter som passar som ko-foder kommer inte räcka om vi fortsätter att utfodra med mycket kraftfoder. Ett annat sätt för att öka nettoproduktionen av livsmedel är därför begränsa kraftfodergivan, då äter mjölkkorna mer gräs och klöver. Dessutom är odling av gräs bra för miljön på flera sätt då det ökar kolinlagringen i marken och därmed är bra för klimatet och jordkvalitén, samt att det minskar behovet av kemikalier som används för att bli av med skadedjur och ogräs.

Gräs innehåller mycket fibrer och ofta mindre energi och protein jämfört med kraftfoder. Men om man skördar gräset tidigt så är det möjligt att göra ensilage som innehåller mycket energi och protein. Mjölkkor som utfodrades med en relativt liten andel biprodukter och mycket ensilage av gräs och klöver producerade mycket mjölk utan att vikt och hull påverkades negativt. Vi såg inga tecken på att fruktsamheten blev sämre när korna utfodrades lite biprodukter och mycket ensilage av gräs och klöver.

Det är viktigt att minska metanproduktionen från kors fodersmältning eftersom metan är en potent växthusgas. En studie utfördes för att undersöka om man genom att utfodra kor med glycerol istället för med vetestärkelse skulle kunna minska metanutsläppen, baserat på antagandet att glycerolen skulle absorberas direkt från våmmen in i blodet och därmed vara mindre tillängligt för metanproducerade mikrober i våmmen. Att ersätta vetestärkelse med glycerol, en biprodukt från biodieselproduktion, resulterade dock inte i en minskad metanproduktion hos mjölkkor.

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This thesis shows that high-yielding dairy cows can perform well on high-forage diets complemented with small amounts of byproduct-based concentrate. Replacing human-edibles such as cereal grain or soybean meal with forage and byproducts increased net food production, while it did not impair milk production, feed efficiency or energy balance. This feeding regime can therefore contribute to sustainable food production.

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