Extrusion of grass silage and its effect on feed intake, milk production and ingestive behaviour of dairy cows

Markos Managos

Degree project • (30 hp)
Swedish University of Agricultural Sciences, SLU
Department of Animal Nutrition and Management
Animal Science – Master's Programme
Uppsala 2020

Extrusion of grass silage and its effect on feed intake, milk production and ingestive behaviour of dairy cows

Markos Managos

Supervisor:	Bengt-Ove Rustas, SLU, HUV
Assistant supervisor:	Torsten Eriksson, SLU, HUV
Examiner:	Horacio Gonda, SLU, HUV

Credits:	30 Credits
Level:	Second cycle
Course title:	Independent project in Animal Science
Course code:	EX0870
Programme/education:	Animal Science - Master's Programme
Course coordinating dept:	Department of Animal Breeding and Genetics
Place of publication:	Uppsala
Year of publication:	2020

Keywords: Extrusion, Grass silage, Dairy cows, Milk production, Milk composition, Rumen pH, Ingestive behaviour.

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file.

If you are more than one author you all need to agree on a decision. You can find more information about publishing and archiving here: https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.

Abstract

Grass and clover ley is the main forage crop in Sweden, however, its restrictive role in feed intake limits the total amount included in the diet of a dairy cow. This study examined the effect of extrusion on grass silage intake, milk production, ingestive behaviour and rumen pH. Eight Swedish Red dairy cows in mid/late lactation were fed grass silage of early or late harvest, extruded or control, in a 4x4 Latin square design with four periods of three weeks. Diets were supplemented with a mix of soybean meal, compound feed and minerals. Extrusion increased daily silage dry matter (DM) intake by 1.84 kg/d ($p < 0.001$), neutral detergent fibre (NDF) intake by 1.04 kg/d ($p < 0.001$) and decreased physical effective NDF ($peNDF_8$) intake by 1.37 kg/d ($p < 0.001$). Total DM intake increased by 1.74 kg/d ($p < 0.001$) while the dietary DM percentage of $peNDF_8$ decreased by 6.9 units ($p < 0.001$). Milk yield increased by 1.32 kg/d ($p = 0.008$), Energy corrected milk (ECM) yield increased by 1.87 kg/d ($p = 0.004$), milk protein concentration increased by 0.09 percentage units ($p < 0.001$) while total fat and protein production increased by 72.4 g/d ($p = 0.015$) and 73.7 g/d ($p < 0.001$), respectively. Extrusion decreased average rumen pH by 0.1 units ($p = 0.008$). The time rumen pH was below 5.8 increased by 2.97 h/d ($p = 0.038$) while the curve area below this cut-off point was not affected ($p = 0.166$). Rate of intake of silage DM and NDF was increased by 20.3 and 11.3 g/min respectively ($p < 0.001$), daily silage eating time decreased by 0.6 h/d ($p = 0.006$) and daily rumination time decreased by 1.96 h/d ($p < 0.001$). Daily chewing time decreased by 2.49 h/d ($p < 0.001$), with rumination and chewing time per kg of silage NDF intake, decreased by 18.9 and 26.3 min/kg ($p < 0.001$) respectively. In conclusion, extrusion increased silage intake, eating rate and milk production but decreased chewing activity.

Keywords: Extrusion, Grass silage, Dairy cows, Milk production, Milk composition, Rumen pH, Ingestive behaviour.

Sammanfattning

Vall är den huvudsakliga grovfodergrödan i Sverige men vallfodrets egenskaper begränsar konsumtionen och därmed även den totala mängden som kan ingå i mjölkkons totalfoderstat. Syftet med denna studie var att undersöka hur extrudering (en metod för intensiv bearbetning och finfördelning) av vallensilage påverkar foderkonsumtion, mjölkproduktion, ätbeteende och våm-pH. Åtta SRB-kor utfodrades med gräsensilage från förstaskörd, slaget vid två tillfällen, som utfodrades hackat eller extruderat, i en romersk kvadrat med fyra behandlingar och fyra försöksperioder. Grovfodret kompletterades med kraftfoder bestående av färdigfoder, sojamjöl och mineralfoder. Extrudering ökade intaget av ensilage med 1,84 kg torrsubstans (TS) / dag ($p < 0,001$) och intaget av fiber (Neutral Detergent Fiber, NDF) med 1,04 kg / d ($p < 0,001$) medan intaget av peNDF8 (ett mått på struktureffekt som innefattar NDF-halt och partikelstorleksfördelning) minskade med 1,37 kg/d ($p < 0,001$). Det totala TS-intaget ökade med 1,74 kg / dag ($p < 0,001$) medan andelen $peNDF_8$ i foderstaten minskade med 6,9 procentenheter ($p < 0,001$). Mjölkavkastningen ökade med 1,32 kg / d ($p = 0,008$), energikorrigerad mjölk (ECM) ökade med 1,87 kg / d ($p = 0,004$), mjölkproteinhalten ökade med 0,09 procentenheter ($p < 0,001$) medan den totala dagliga produktionen av fett och protein ökade med 72,4 g / d ($p = 0,015$) respektive 73,7 g / d ($p < 0,001$). Extrudering minskade pH-värdet i våmmen med 0,1 enheter ($p = 0,008$). Tiden med våm-pH under 5,8 ökade med 2,97 h / d ($p = 0,038$), medan kurvarean under detta tröskelvärde inte påverkades ($p = 0,166$). Äthastigheten för torrsubstans och NDF i ensilage ökade med 20,3 respektive 11,3 g / min ($p < 0,001$), den dagliga ättiden för ensilage minskade med 0,6 h / d ($p = 0,006$) och den dagliga idisslingstiden minskade med 1,96 h / d ($p < 0,001$). Den totala tuggtiden minskade med 2,49 h / d ($p < 0,001$), medan idisslingstid och tuggtid för NDF från grovfoder minskade med 18,9 respektive 26,3 min / kg ($p < 0,001$). Sammanfattningsvis ökade extrudering ensilageintag, äthastighet och mjölkproduktion men minskade tuggningsaktiviteten.

Nyckelord: Extrudering, Gräsensilage, Mjölkkor, Mjölkproduktion, Mjölksammansättning, Våm-pH, Ätbeteende.

Table of contents

List of tables

List of figures

Abbreviations

CP	Crude Protein
DIAAS	Digestible Indispensable Amino Acid Score
DIM	Days in Milk
DM	Dry Matter
DMI	Dry Matter Intake
EC	Early harvest Control
ECM	Energy Corrected Milk
EE	Early harvest Extruded
FPS	Feed Particle Size
GE	Gross Energy
IAAS	Intestinal Amino Acid Supply
IVOMD	*In Vitro* Organic Matter Digestibility
LC	Late harvest Control
LE	Late harvest Extruded
ME	Metabolizable Energy
MEI	Metabolizable Energy Intake
MUFA	Mono Unsaturated Fatty Acids
NDF	Neutral Detergent Fibre
OM	Organic Matter
peNDF	Physical Effective Neutral Detergent Fibre
PUFA	Poly Unsaturated Fatty Acids
SARA	Sub-Acute Ruminal Acidosis
SD	Standard Deviation
SFA	Saturated Fatty Acids

1. Introduction

Grass silage from temporal leys is the major feed for Swedish dairy cows. Silage is the conservation method of choice since it minimizes the loss of nutrients compared with dry forages due to easier storing, feeding and handling (Grant & Ferraretto 2018). However, forage-based diets, due to their filling effect limit feed intake and cannot satisfy the high energy and protein requirements of dairy cows (Allen 2000).

Limiting the forage filling effect and increasing fibre digestibility presents an opportunity for the dairy industry. Increased dry matter intake (DMI) and milk yield combined with decreased manure production are expected to result in increased profitability (Hernandez-Urdaneta *et al.* 1976; Allen *et al.* 2009; Adesogan *et al.* 2019). Grass-based diets present important environmental benefits. Grasslands have a key role in the prevention of soil erosion, immobilization of leaching materials and pesticides, regularization of water regimes and act as a carbon reservoir (Rodriguez *et al.* 2017). According to Murphy *et al.* (2011), 50 % of the energy requirements of agriculture are related to fertilizer production, 22 % for machinery cost, 15 % for transportation and 13 % for pesticide production. Grass production requires less tillage, crop seedings, fertilizing, herbicides and pesticides compared with crop production resulting in lower energy input requirements per hectare (Murphy *et al.* 2011). Additionally, grasslands present an opportunity for wildlife habitat and improved attractiveness of the landscape (Carlier *et al.* 2009; Rodriguez *et al.* 2017).

Livestock production has an important role in human nutrition, income generation and livelihoods (Smith *et al.* 2013). Ruminants possess the unique ability to convert fibrous feeds, that cannot be utilized by humans, such as forages, into high-quality food protein (Mottet *et al.* 2017). Increasing fibre digestibility will allow for greater inclusion of forage in the rations of dairy cows satisfying the demand of locally produced crops with good traceability in animal feeding (Mendowski *et al.* 2020).

Ruminal fermentation of fibrous feeds results in higher methane production with detrimental effects for the dairy sector (Adesogan *et al.* 2019). Methane is produced at the expense of energy that could be utilized for milk production. The increased methane production combined with the decreased milk yield result in a higher carbon footprint of the final product. Furthermore, environmental reasons are one of the main arguments for vegan diets (Adesogan *et al.* 2019). Increasing fibre digestibility, present an opportunity for decreased methane emissions. Additionally, decreasing forage filling effect will result in decreased use of concentrates thus lowering the competition among nonruminants, bio-fuel production and human food sector (Adesogan *et al.* 2019).

2. Forage fibre

Forages can make up from 40% to 100% of the ratio of a dairy cow and are characterized by higher fibre and lower energy content compared to concentrates (Hernandez-Urdaneta *et al.* 1976). They are sources of neutral detergent fibre (NDF) and through their physical and chemical characteristics, they stimulate chewing, rumination and reticulorumen motility. Increased salivation buffers and keeps rumen environment healthy, maintaining animal welfare, health and productivity (Mertens 1997; Zebeli *et al.* 2012; Adesogan *et al.* 2019). Additionally, they pose an important energy source for the ruminants and through microbial fermentation produce milk fat precursors.

The ruminal mat is the result of the extensive stratification of the reticuloruminal content with fibre acting as a supporting frame (Adesogan *et al.* 2019). It facilitates the digestion of solid feed particles through particle retention and optimizes the production and harvest of fermentation end products by optimizing the rumen micro-environment (Clauss *et al.* 2011; Adesogan *et al.* 2019). Particle density is an important factor affecting the sorting mechanism inside a ruminants forestomach (Clauss *et al.* 2011). Younger and larger fibre particles are, due to buoyancy, positioned into “lag-fermentation” flow-paths and are subjected to rumination. As feed particle size (FPS) decreases, they become less efficient of entrapping the fermentation gasses, their density increases and eventually they escape the rumen (Ellis *et al.* 2005). Consequently, FPS, chemical characteristics and density affect the reticulorumen motility and the rumen retention time (Allen 1996).

3. Feed intake

Feed intake is affected by animal factors including the energy status of the animal (Jensen *et al.* 2016) and dietary factors including feed availability, concentrate/forage ratio, NDF content, organic matter (OM) digestibility, FPS and palatability (Allen 1996, 2000; Oba & Allen 2000; Grant & Ferraretto 2018). These factors affect the chewing time that can be the main limiting factor under time-restricted feeding regimes or conditions of high feed competition. However, under *ad libitum* feeding of high forage diets, feed intake is limited by the distention of the reticulorumen (Adesogan *et al.* 2019).

Reticuloruminal distension is affected by the removal of its content by digestion, absorption or passage (Allen 2000). Retention time in the rumen and the total digestive tract are known to be negatively correlated to FPS and influenced by the functional specific gravity (Clauss *et al.* 2011; Dufreneix *et al.* 2019). The functional specific gravity refers to the particle density associated with the volume occupied by liquid and gases produced during the steps of feed hydration and digestion (Wattiaux *et al.* 1992). For low producing animals, a feed particle density of 1.1 to 1.4 and a FPS of 1.13 to 3.35 mm minimizes the retention time in the rumen and the whole digestive tract (Maulfair *et al.* 2011). Higher intake levels, on the other hand, can positively affect the passage rate through the digestive tract, decreasing ruminal retention time and increasing the probability that particles escape the rumen before extensive microbial degradation. High producing dairy cow have increased intake and a FPS of 3 to 4 mm and a density of 1.2 to 1.3 minimizes rumen and total digestive tract retention time (Dufreneix *et al.* 2019). Therefore, decreased FPS and increased density directly affect and increase dry matter (DM) intake (Allen 2000).

Feed digestibility and fragility can also affect passage rate (Udén 1984; Udén & Sutton 1994; Allen 1996). Higher fibre digestibility and higher fragility will lead to faster FPS reduction limiting the filling effect of NDF (Allen & Mertens 1988). The higher fragility of legumes results in lower filling effect compared to grass despite their lower DM and NDF digestibility (Weiss & Shockey 1991; Oba & Allen 2000). Increased forage FPS and the consequent decrease of available surface area, increased buoyancy can result in slower fibre fermentation rates, greater retention times thus limiting feed intake (Allen 1996; Zebeli *et al.* 2012).

The physical effective NDF (peNDF) as a concept combines the physical and chemical properties of the diet as FPS and NDF content respectively (Humer *et al.* 2018b). High peNDF levels are linked with higher dietary chewing index which can in term negatively affect DMI (Jensen *et al.* 2016). Diets on the other hand with insufficient amounts of peNDF and high starch content can suppress rumination, ruminal mat formation and chewing activity leading to lower ruminal pH. Lower ruminal pH creates suboptimal conditions for the cellulolytic bacteria and leads to decreased appetite, fibre digestion, rumen motility, microbial yield and milk fat (Allen 1997; Adesogan *et al.* 2019). Under these conditions, diet digestibility can be affected potentially increasing the filling effect and decreasing feed intake.

The rumen volatile fatty acid (VFA) pattern can also affect satiety and voluntary intake. Propionate is a VFAproduced mainly during starch digestion by rumen bacteria but can also be present in small amounts in the silage. Propionate and unsaturated fatty acids from incomplete biohydrogenation have the greater effect on feed intake compared to other organic acids according to the hepatic oxidation theory (Harvatine & Allen 2006; Allen & Voelker Linton 2007; Allen *et al.* 2009). According to this theory diets with high levels of ruminal available starch result in greater production of propionate. The produced VFA are absorbed by the rumen epithelial and through the blood, reach the liver. Once the liver's glucogenic capacity is exceeded the surplus of propionate is oxidized resulting in increased adenosine triphosphate (ATP) production and cerebral stimulation of the vagus nerve resulting in satiety (Allen *et al.* 2009; Grant & Ferraretto 2018).

Diet palatability is affected by FPS, DM content and chemical composition (Nasrollahi *et al.* 2015; Grant & Ferraretto 2018). Low dietary DM content negatively affects palatability and has been reported in silage. Silage DM content affects the fermentation process resulting in a different composition of end products such as organic acids or nitrogenous compounds. These end products are known to affect behaviour and feed consumption of dairy cows. The main fatty acid during silage fermentation is lactic acid however acetate, propionate, ethanol, butyrate, ammonia-N and amines are also present at various concentrations. Butyrate is a result of fermentation of sugars and lactic acid by Clostridia and can suppress feed intake (Grant & Ferraretto 2018). Acetate may also contribute to intake regulation while ethanol does not appear to influence feed intake. Nitrogenous compounds are the result of proteolysis, can occur even under adequate management conditions and undergo rapid and extensive ruminal degradation (Grant & Ferraretto 2018). However, in silage-based diets, these nitrogenous compounds can increase blood ammonia-N and suppress intake through an increase in gamma-aminobutyric acid (Scherer *et al.* 2015).

4. Forage digestibility

Forage digestibility is depended upon many parameters including the chemical composition of the forage cell wall and the plant maturity stage. The main components of the plant cell wall are cellulose, hemicelluloses and lignin. Their proportions and how they interact with each other defines the degree of digestibility of the plant material.

4.1. Forage chemical composition

Cellulose is the most abundant biopolymer on earth and is polymerized by β-glucose disaccharide (cellobiose). It is a linear homo-polysaccharide that functions as the major structural component of the plant cell wall (Hon 1994). It is characterized by a high degree of polymerization that can reach up to 500-15.000 glucan units linked by β-1,4-glycosidic bonds (Holtzapple 2003a; Abraham *et al.* 2020). Cellulose molecules run parallel in the same direction from nonreducing to reducing ends and are interlinked by hydrogen and covalent bonds and Van der Waals forces leading to a highly crystalline cellulose microfibril (Holtzapple 2003a; Kim *et al.* 2013). Crystalline or amorphous regions of cellulose are present in the plant cell wall and they are a result of the different orientation of the cellulose molecules (Atalla & Vanderhart 1984; Holtzapple 2003a). The large size of these microfibrils and the hydrogen bonds that hold the crystalline structure render them insoluble in water and resistant to biological degradation (Holtzapple 2003a; Bodvik *et al.* 2010). The amorphous regions, on the other hand, are more porous allowing water to penetrate and increase the reactivity to acid or enzymatic hydrolysis (Lindman *et al.* 2010).

Hemicelluloses, the world's second most abundant carbohydrates, comprise 20-30% of the plant cell wall (Holtzapple 2003b). They include arabinoxylans, xyloglucans, glucomannans, galactomannans and β-glucans (Holtzapple 2003b; Hamaker & Tuncil 2014). They are short, highly branched heteropolysaccharides that are composed of 50-200 monomers such as pentoses (xylose and arabinose), hexoses (glucose, galactose and mannose) and sugar acids (Holtzapple 2003b). Most hemicelluloses have a continuous β-1,4-linked backbone that may be simple (one monosaccharide and few linkage types) or very complex (many monosaccharides, many linkage types, and varying length of branches), except for β-glucans that are not branched and have both β-1,4 and β-1,3 linkages in the backbone (Hamaker & Tuncil 2014). Hemicelluloses are more susceptible to physical and biological degradation due to their lower degree of polymerization and their amorphous structure (Li *et al.* 2015). Their branched nature allows them to form strong bonds with cellulose (hydrogen bonds) and lignin (covalent bonds) acting as a matrix that increases the rigidity of the plant material (Holtzapple 2003b). Hemicelluloses have many variations of structures depending on the plant sources and genotype, the growing environment, the anatomical parts of the plant and variation within polymers (Scheller & Ulvskov 2010). Loosely branched hemicelluloses with smaller size can be solubilized in water and can be easily digested by bacteria (Hamaker & Tuncil 2014).

Lignin is a complex, amorphous, hydrophobic, heteropolymer composed mainly of cross-linked aromatic components (trans-coniferyl, trans-sinapyl and trans-p-coumaryl alcohols). It closely associates with cellulose and hemicelluloses and forms a complex matrix around cellulose microfibrils forming the rigid structure of plant cell wall (Holtzapple 2003c; Adesogan *et al.* 2019). Strong carbon-carbon and ether linkages and the insolubility of lignin render it undigestible under the anaerobic conditions of the rumen. Additionally, the formation of crosslinks with hemicelluloses creates a physical barrier against cellulolytic microorganisms lowering accessibility and digestibility of cell wall carbohydrates (Hatfield & Jung 2007; Liu *et al.* 2018; Adesogan *et al.* 2019).

4.2. Plant maturity

The digestibility of the different plant tissues is affected by the changes of the chemical composition that take place as the plant matures and strongly affects animals performance (Akin 1989; Adesogan *et al.* 2019). As the plant matures tissues such as the sclerenchyma, the epidermis, the xylem, the non-clorenchymatous parenchyma and the parenchyma bundle sheath cells become highly lignified and have lower digestibility while the mesophyll, the collenchyma and the phloem are more digestible (Akin 1989; Adesogan *et al.* 2019). The xylem, the lignified vascular tissue and the middle lamella are the greatest physicals constraints of microbial degradation (Akin 1989; Adesogan *et al.* 2019). Leaves are of greater nutritional value due to the higher proportion of non-lignified tissues (Akin 1989; Adesogan *et al.* 2019) while the lignified stem acts as a barrier to fibre digestion (Adesogan *et al.* 2019). The degree of lignification can affect the total chewing and rumination time, while the lower FPS reduction can affect the ruminal passage rate (Adesogan *et al.* 2019).

5. Physical-Mechanical treatments

Physical-mechanical treatments such as milling and maceration do not use any external compounds and rely on physical force to increase forage digestibility (Saini *et al.* 2015; Rodriguez *et al.* 2017). Extrusion is based on the action of a single screw or double screws that co- or anti-rotate, forcing the feed through a tight barrel subjecting it to a combination of heating, mixing and shearing while as it exits the extruder the high abrasion and the sudden drop of pressure lead to the evaporation of the intracellular water resulting in cells rupture (Lee *et al.* 2009; Duque *et al.* 2017; Rodriguez *et al.* 2017; Abraham *et al.* 2020). It is a continuous process that acts both on the interior and exterior of the feed. The shearing forces act and remove the exterior surfaces of the feed, exposing the interior thus enhancing feed accessibility to microorganisms and increasing digestibility (Lamsal *et al.* 2010). The rise of temperature can be due to the friction or due to supplemental heat provision and can vary from 50^{o} to 250^{o} C with the majority of the studies reporting temperatures between 140^{o} to 160^{o} C (Mendowski *et al.* 2019, 2020; Abraham *et al.* 2020). The opening size at the end of the barrel, feed moisture and the screw speed modify the intensity of the extrusion process (Rodriguez *et al.* 2017).

The structural changes induced by different mechanical treatments can be classified as Class I and Class II (Leu & Zhu 2013). Class I changes do not compromise significantly the crosslinks between the fibre's microfibrils and the structural integrity of the cell wall. Increasing the processing intensity can cause fibres to be cut, separated, fragmented and slightly defibrillated leading to the increased external surface but minor cell wall deconstruction. On the other hand, Class II changes cause a significant breakup of the microfibrils crosslinks, internal defibrillation, structural cell wall deconstruction to a micro or nanofibril level. Compared to Class I changes that only increase the external surface, Class II changes result in a more porous structure and decreased cellulose crystallinity.

Extrusion is differentiated by other mechanical treatments since it causes Class II structural changes and fibre disintegration at a cellular level see Figure 1 (Lee *et al.* 2009; Duque *et al.* 2017). The thermal and mechanical forces exerted on the feed by the screws disintegrate the quaternary and tertiary structure and the interactions between food components (Redgwell *et al.* 2011; Robin *et al.* 2012; Chen *et al.* 2014; Alam *et al.* 2016; Duque *et al.* 2017). Higher mechanical energy input, higher temperature, or lower liquid/solid ratio will lead to the greater FPS reduction and finer skewness of the FPS distribution (Redgwell *et al.* 2011; Zhang *et al.* 2011; Robin *et al.* 2012; Um *et al.* 2013; Chen *et al.* 2014; Duque *et al.* 2017). Extruded cellulose fibre bundles are shortened, and defibrillated resulting in increased aspect ratio (length/diameter), porosity, WHC, and surface area (Chen *et al.* 2014; Duque *et al.* 2017; Gallos *et al.* 2017). Additionally, extrusion affects or removes hemicelluloses or lignin that wrap the cellulose (Duque *et al.* 2017). These effects facilitate enzymatic attack and accelerate the hydrolysis rate of hemicellulose and cellulose (Hjorth *et al.* 2011; Chen *et al.* 2014).

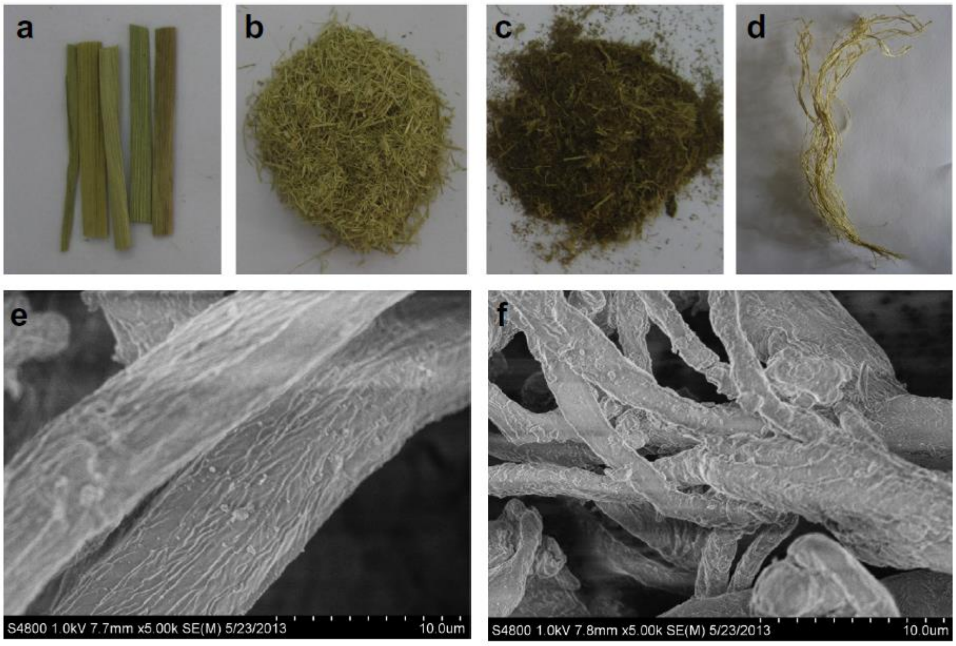

Figure 1*. Effect of different pretreatment methods on Rice Straw: (a) Unpretreated Rice Straw,(b) milling pre-treated rice straw, (c and d) Extruded pre-treated rice straw, (e and f) Scanning Electron Microscopy micrographs of unpretreated and extrude rice straw respectively (Chen* et al. *2014).*

5.1. Effect on digestibility

Enzymatic hydrolysis and ruminal degradation of NDF require the physical access and attachment of the fibrolytic bacteria (Ellis *et al.* 2005). Surface sites are colonized first and then erosive degradation of the subsurface takes place (Ellis *et al.* 2005). The speed of these processes is determined by the number and the spatial distribution of these sites and is related to FPS (Ellis *et al.* 2005). The available surface can be distinguished into the external surface that depends on FPS and shape and the internal that depends on the microstructure of the fibers. Physical treatment methods such as milling lead to FPS reduction and increase in the external surface (Leu & Zhu 2013). Extrusion, however, leads to increased surface both externally, due to FPS reduction (Johnson *et al.* 1999; Adesogan *et al.* 2019), and internally through the increase in porosity and reduction of cellulose crystallinity. The forces developed during extrusion break down the hydrogen bonds between hemicellulose, lignin and proteins (Lamsal *et al.* 2010; Hjorth *et al.* 2011). Altering or breaking down of lignin or hemicelluloses can also increase the solubility and the accessibility of the feed resulting in faster hydrolysis by the rumen bacteria and higher digestibility (Mosier 2005; Yang *et al.* 2015).

Extrusion has been used to decrease rumen degradability of proteinous seeds while maintaining high intestinal digestibility, leading to improved nutritive value (Paula *et al.* 2018; Mendowski *et al.* 2019, 2020). Numerous studies on biogas production report that extrusion as a pretreatment method leads to increased digestibility and increased methane yields (Abraham *et al.* 2020). Extruded material presented faster degradation of slowly degradable compounds and improved the degradability of some otherwise non-degradable compounds (Hjorth *et al.* 2011). Enzymatic digestibility is enhanced and accessibility of carbohydrates due to a combination of FPS reduction, increased surface area, number of pores and changes in biomass composition (Duque *et al.* 2017). Under high temperature and/or shearing forces extrusion is reported to affect the lignin fraction of the biomass, causing condensation or pseudo-lignin complex formation (Duque *et al.* 2017).

Prolonged heat exposure during extrusion can decrease digestibility due to extensive Maillard reactions, the formation of toxic compounds (Fernández-Cegrí *et al.* 2012; Mendowski *et al.* 2019). Moderate extrusion conditions prevent the formation of toxic compounds resulting from oxidation of lignin (Olsson & Hahn-Hägerdal 1996).

6. Background

A limited number of studies that investigated forage extrusion for non-ruminant species were found in the literature. Feeding extruded alfalfa silage to laying hens did not present any benefits (Wüstholz *et al.* 2017). Sows fed with extruded corn silage lost more body weight and backfat thickness during lactation and inclusion of 30% of extruded corn silage lead to a non-statistically significant increase in litter weight gain (Weng 2019). Ponies fed different extruded grass species presented higher apparent digestibility coefficient for acid detergent fibre but lower digestibility coefficient for other feed components (Feltre *et al.* 2019).

No studies investigating the effect of inclusion of extruded forage or silage in the diets of dairy cows were found. A study by Oliveira *et al*. (2018) compared the inclusion of corn silage with extruded sugar cane. However, the difference in the nutrient content between the diets and the experimental design used do not allow clear conclusions. Agbossamey *et al*. (2000) used maceration before ensiling to improve the characteristics of alfalfa silage. However, due to rainy conditions and prolonged wilting and soil contamination, the quality of the silage declined with increased levels of mechanical treatment potentially affecting feed intake.

Results from *in vitro* studies have been inconsistent. Williams *et al.* (1997) did not find extrusion treatment to lead to any positive change in the fermentation and the total gas production in corn silage and wheat straw with VFA production being lower for extruded substrates. On the other hand, rumen fermentability of precision chopped grass and clover silage has been reported to be increased by extrusion (Yang *et al.* 2018).

7. Aim and Hypothesis

Grass silage of different maturity stages was intensively mechanically processed using a bio-extruder and fed to dairy cows. The experiment aimed to investigate the effect of extrusion on milk yield, milk composition, intake and ingestive behaviour of dairy cows. The hypothesis was that extrusion will increase feed intake and milk production without adverse effects on dairy cows ingestive behaviour.

8. Materials and Methods

The study was conducted in SLU facilities (Swedish Livestock Research Centre, Lövsta, Uppsala) from January till April 2020. The experimental design and all handling of the animals were approved by the Uppsala Local Ethics Committee (Uppsala, Sweden), ID-No: 5.8.18-12171/2018.

8.1. Search terms

The information presented in this thesis was based on studies retrieved from the literature with search keywords that included (“extruder, extrusion” etc.) as well as keywords (“forage, silage, roughage, grass, alfalfa, hay”). Papers were selected based on the relevance to the topic. Search engines used included Google Scholar and Web of Science and notification alerts were created at the time of the search. Additional, papers were included based on the information needed.

8.2. Animals, housing, feeding and study design

Eight multiparous lactating Swedish Red cows, four fistulated (Days in Milk, mean 143 ± 38 SD) and four intact (DIM mean 68 ± 10 SD) were used for this experiment. Mean lactation number was 2.8 ± 0.96 for fistulated cows and 2.5 ± 0.58 for intact cows. The cows were cannulated not later than 5 months before the experimental start while one of the fistulated animals had an incidence of mastitis in a previous lactation and had one non-functional udder quarter.

The cows were housed in individual tie-stalls (width: 1.6 m, length: 1.8m, an additional 0.6 m platform was added to the tie-stall to provide additional space) with rubber mats covered with sawdust bedding, with an empty stall between each cow. The stable was temperate with temperatures between 8 to 15^{o} C. Each animal had individual automatic water bowls equipped with water meters (model P-50; Schlumberger Ltd., Montrouge, France). Forage, concentrates and mineral feed were fed in separate troughs while a salt licking block was available for each animal. Animals were moved into the individual tie stalls 7 days before the start of the experiment to allow for acclimatization to the new housing conditions. Milking took place twice per day at 7:00 and 17:30 in the individual tie stalls.

A change-over design (Latin square) with 2 blocks (fistulated or intact) was used with 4 periods and 4 treatments. Each experimental period lasted 21 days. The first 14 days were used as an adaptation period to the new diet and measurements were collected during the last 7 days. Cows were offered *ad libitum* silage (processed or unprocessed) of different maturity stage (early or late harvest) in a 2x2 factorial arrangement with 4 diets. Animals were randomly allocated to treatment at the beginning of the experiment and changed treatments according to the Latin square design.

In addition to silage, cows were receiving a concentrate mix consisting of minerals, pelleted compound feed (Komplett Norm 180, Lantmännen Malmö, Sweden, Table 1) and soybean meal at a flat rate throughout the experiment. Intact animals, being earlier in lactation and having higher milk yield received 10 kg of concentrates per day (8 kg compound, 2 kg Soybean Meal) while fistulated ones received 8 kg/d (6 kg compound feed, 2 kg Soybean Meal). Concentrates were offered four times per day (at 6:00, 11:00, 15:00 and 19:00) and silage was offered three times per day (at 6:00, 11:00 and 19:00). If needed additional silage was offered to assure ad libitum feeding (minimum 10% leftovers). Refusals were collected before the morning feeding.

Table 1. *Pelleted compound feed composition*

Ingredients	Composition %
Barley	36.3
Rapeseed meal	24.1
Wheat bran	15
Oats	10
Wheat	5
Molasses sugar beet	2
Sugar beet pulp	1.9
Fat	1.9
Vitamins and minerals	3.8

8.3. Silage production

Silage was produced from the primary growth of a long-term grass-dominated ley near Uppsala, Sweden (18^{o} E, 60^{o} N). Cutting took place on the 13th of June 2019 and will be referred to as early harvest and on the 23rd of June that will be referred to as late harvest. The grass was wilted to a DM content of 40-50% and preserved as bale silage (diameter 130 cm, height 110 cm) and 8 layers of plastic were used. Bales were stored in an external concrete area close to the barn and covered by a net to protect against birds. Before harvest, samples were collected for botanical analysis and the average composition of the ley was determined to be 70 % Timothy, 26 % Tall Fescue, 3 % Red Clover and 1 % weeds. The maturity stage was estimated according to Pomerleau-Lacasse *et al.* (2017) with 22 % of Timothy being at elongation stages E4-E5 and 78 % at the reproductive stage (42 % R0, 19 % R1, 17 % R2-R3). Bale silage core samples were taken from 2 bales for early and late harvest respectively before the experiment, three cores per bale were drilled and pooled into one sample per bale for chemical analyses. The results, presented in Table 2, were used for intake calculations.

Table 2. *Chemical composition of experimental feeds*

	Silage		Concentrate	
	Early harvest	Late harvest	Soybean Meal[2]	Compound feed[1]
DM, g/kg	361	436	876	880
Chemical composition, g/kg DM				
Ash	127	107	74	60
NDF	550	561	135	225
CP	127	107	487	180
Sugar			121	60
Starch			62	310
IVOMD[3]	806	679		
ME, MJ/kg DM	10.0[3]	8.26[3]	13.4	13.4

[1]Tabulated value from the manufacturer

[2]Feedipedia (INRA *et al.* 2020)

[3]Metabolizable energy values were calculated based on Spörndly R. (2003)

NDF = Neutral Detergent Fibre, CP = Crude Protein, IVOMD = *In Vitro* organic matter digestibility, ME = Metabolizable energy.

8.4. Extrusion Conditions

During the experiment, silage was processed twice per week. Bales were opened and inspected for the presence of signs of mal-fermentation before further processing. Affected areas were discarded and in case of extensive areas, the whole bale was discarded. Bales were then chopped using a vertical TMR feed mixer (SILOKING TrailedLine Classic Premium 14). Speed of blades was set to 30 revolutions per minute (RPM). A single bale was processed for a total of 30 minutes while for two bales total time was 60 minutes. Additive (ProMyr^{TM} TMR

Performance), to improve the aerobic stability (4 litres/tonne) was added at half-way during processing. Extrusion of grass silage took place in a twin-screw co-rotating bio-extruder (Bio-Extruder MSZ-B15e, two 11 kW electrical motors, LEHMANN Maschinenbau GmbH). Rotation speed was set at 6 (54 RPM) while the opening at the end of the extruder was set at 50%. A handheld infrared non-contact thermometer gun (STANLEY STHT77365 Infrared Thermometer) was used during the last day of period 4 to measure temperature during processing for early harvest extruded (EE) (4 recordings) and late harvest extruded (LE) (3 recordings) silage. The temperature was recorded on the silage as it exited the extruder (LE mean 57º C ± 3 SD, EE 52º C ± 2 SD), at the middle of the barrel of the extruder (LE mean 54º C ± 5 SD, EE mean 43º C ± 4 SD), the temperature of the barrel at the exit point (LE mean 63º C ± 2 SD, EE 60º C ± 3 SD). At the time of extrusion, the control silage temperature was 11.7º C for early harvest and 10.3 º C for late harvest while the environmental temperature as measured on the wall of the facility was 12.6 º C.

8.5. Measurements and Sampling

Offered feed and leftover weights (forage and concentrates) on fresh matter basis was recorded daily throughout the experiment. Milk yield was recorded in each milking (DelPro MU 480 with a MM25WC milk meter; DeLaval International AB, Tumba, Sweden). Milk samples (4 samples) were collected from the evening milking of the 16th day until the morning milking of the 18th day of each period and stored at 4º C. Animal behaviour was video recorded during the whole 24h throughout the experiment.

Samples including silage, compound feed, soybean meal and mineral feed were collected from day 15 till day 19 of each period (day 16 to day 19 in period 1). Concentrate leftovers were collected from day 16 to day 20. All samples were stored at -20 ºC until the end of the experiment. Each period silage samples (day 15 till day 19) and refusals samples (day 16 till day 20) were collected for DM determination. Dried samples were stored in plastic bags for future analyses.

Spot samples of urine and faeces were collected from day 15 to day 19 at 8:00 and 16:00. Urine samples were divided into acidified (5 ml of urine with 1 ml of HCl 3.87 M) and diluted (1 ml of urine in 9 ml of water) sub-samples. Faeces (450ml) were collected in plastic bags. Both urine and faeces were frozen at -20º C for future analyses

Ruminal liquid samples were collected at 20 different times during days 15 to 19, to represent the entire 24-h cycle. Samples in the interval 22:00 to 6:00 were only obtained bihourly. A 50-mL tube was manually inserted in the rumen approximately 20 cm below the surface and filled with rumen sample. The samples were strained, and pH was measured (pH 1000 H VWR® pHenomenal®).

8.6. Sample handling and analyses

Offered silage and refusal samples were collected daily and used for DM determination at 60° C for 24 hours till constant weight was achieved. Offered and refused silage samples were collected on 4 occasions during the experiment and sieved using the Penn State Particle Separator (Nasco, Fort Atkinson, WI, USA) consisting of two sieves, 19 mm and 8 mm, and a bottom pan results presented in Table 3. Concentrate leftover samples were pooled within animal and period. The pooled samples were then milled on a (DAVIDE 4V, Novital S.r.l, Lonate Pozzolo, Italy) using a (0.4 mm screen) screen and analysed for DM, ash, acid insoluble ash and Kjedahl-N.

***Table 1**. Percentage of particles retained in the sieves of Penn State Particle Separator per experimental silage*

Sieve size	Early Extruded	Early Control	Late extruded	Late control
19 mm	0 %	19% ± 11%	0 %	25% ± 10%
8 mm	48% ± 12%	45% ± 5%	42% ± 14%	41% ± 6%
Bottom pan	52% ± 12%	36% ± 6%	58% ± 14%	34% ± 5%

Silage samples were collected daily and stored at -20 °C. A sub-sample (0.5 kg) was stored at -20 °C for future analyses and the rest of the silage samples were pooled within period and treatment. More specifically, 0.5 kg of frozen from each day was used to create a pooled sample. The pooled sample was milled through a (13 mm screen) on a meat mincer while still frozen and divided into 3 subsamples. A sample (400gr) was analysed for DM, ash, minerals, acid insoluble ash, NDF, IVOMD and Kjedahl-N. A 20 g sub-sample was mixed with an equal weight of water and stored at 4° C overnight. Silage liquid was extracted using a hydraulic press and the extracted liquid was analysed for Kjedahl-N, NH_4, pH, VFA, lactic acid and alcohols.

Soybean meal, compound concentrate and mineral samples were pooled at the end of the experiment. Two subsamples were created (250 g for soybean meal and compound feed and 100 g for mineral) within feed type and period. One of the subsamples was stored at -20° C while the other was analysed for DM, ash, acid insoluble ash, Kjedahl-N and NDF.

Faecal samples were thawed and pooled gently within animal and period. A subsample of 0.5 kg was stored at -20° C for future analyses. Water was added to the rest of the pooled sample (10 % of total weight) an electric hand drilling machine was used to mix it vigorously. A subsample of 1 kg was collected and stored at -20° C and an average of 180 g was weighed into two big Petri dishes for freeze-drying and analyses of ash and acid-insoluble ash.

Milk samples were analysed with an infrared analyser (FT 120; Foss, Hillerød, Denmark) for fat, protein, lactose, solids, somatic cell count, and fatty acids categories (saturated, monounsaturated and polyunsaturated, C16:0, C18:0, C18:1C9, C14:0).

Video recordings (24 h) of the 21st day of each collection period were scanned at 5-minute intervals and 1 min of each 5-minute interval was observed. Behaviour was classified into: Eating forage, eating concentrate, ruminating standing, ruminating laying, idle standing, idle laying, drinking, and licking salt block. The activities were assumed to persist for the entire 5 min period. The results were used to calculate total eating time, total ruminating time and the total time the animal was idle (Beauchemin *et al.* 2003).

8.7. Calculations

Daily DM intake for silage and concentrate was calculated using the information collected from DM content, fresh weight offered and weight of refusals. Daily DMI for day 15 till day 19 was used to calculate the average DMI per period for each cow. While calculating average silage DMI per cow, intake information from one day of period 1, for a fistulated cow (cow 734) receiving early harvest extruded (EE) diet, was excluded since the animal appeared depressed and there was a sudden drop in feed intake (unusual observation: 15.8 kg/d, average 20.8 kg/d ± 0.7 SD). Additionally, the weekly average DMI information for an intact cow (cow 653), under EE treatment, for period 4 was discarded since the cow presented difficulty while laying and intake was negatively affected the last 9 days of the period.

Daily milk yield was calculated by summing the yield from both milkings per day. Average milk yield per period was calculated using daily milk yield from day 15 till day 19. Average milk composition was determined using the results from the infrared analysis combined with the milk yield at the time of sampling. Energy corrected milk (ECM) was calculated as: ECM (kg) = milk yield (kg) × {[38.30 × fat content (g/kg) + 24.20 × protein content (g/kg) + 16.54 × lactose content (g/kg) + 20.7]/3.140} (Sjaunja *et al.* 1990). Feed efficiency was calculated by dividing the daily milk yield with the total DMI and the daily ECM yield with the total DMI.

Eating activity (min/d) and Eating rate (g/min) were calculated for total DMI and silage DMI using the intake information and the behavioural observations collected on day 21 of each period. Since biting activity could not be differentiated during the video analysis, it was included in the eating activity time. Rumination time (min/kg) for silage DMI and NDF intake was calculated using Rumination time (min/d) and intake information. Idle time (min/d) and Total Chewing time (min/d) were calculated with chewing time being the sum of eating and ruminating time. Chewing time (min/kg) for total DMI and silage DMI was calculated using the intake information. NDF intake and the particle distribution (particles bigger than 8mm) of the diets were used to calculate the $peNDF_8$ as kg per day and as a percentage of total DM intake. The average, median, minimum and maximum values of ruminal pH value were calculated per cow per period. Additionally, time and area under the curve that pH was lower than the thresholds of 6 and 5.8 was calculated (Zebeli *et al.* 2012; Humer *et al.* 2018a).

8.8. Statistical Analysis

Statistical analysis was performed with Minitab® 18.1 Statistical Software (2017) State College, PA: Minitab, Inc.(www.minitab.com) using ANOVA General Linear Model. Cow (nested in Block), Period, Harvest, Treatment, Block and the interaction of Harvest × Treatment were considered as factors in the model. Residuals were tested for normality using the Anderson–Darling test. Significance level was set at $p < 0.05$, while value of $0.05 < p < 0.1$ were considered as tendency. Behaviour observations for an intact cow under EE for period 4 was excluded since the cow presented difficulty while laying affecting her daily activities. Additionally, behaviour observations of an intact cow on period 1, receiving late control (LC) diet, were discarded because the animal spent an unusual amount idle. Behaviour observation was performed on day 20 instead.

9. Results

9.1. Intake

As expected, extrusion increased intake of silage DM, OM and NDF by 11.3 % while silage crude protein (CP) and silage metabolizable energy (ME) intake increased by 11.0 % ($p < 0.001$). These values are presented in Table 4 and correspond to an additional intake of 1.87 kg DM, 1.72 kg OM, 1.04 kg NDF, 0.21 kg CP and 16.7 MJ ME per day. Extrusion decreased $peNDF_8$ intake by 22.9 % (1.37 kg/d). Harvest did not affect silage DM, OM or NDF intake. Early harvest diets resulted in increased silage CP, ME and $peNDF_8$ intakes by 21.0 % (0.39 kg CP), 23.7 % (33.9 MJ ME) and 4.6% (0.24kg/d), respectively ($p < 0.001$). A tendency in the interaction between Harvest × Treatment was observed for silage DM ($p = 0.063$), OM ($p = 0.056$) and NDF ($p = 0.053$) intakes. Extrusion increased silage DM intake by 8.9 % (1.48 kg) for early harvest and by 14.0 % in the late harvest (2.27 kg) while silage OM and NDF intake increased by 8.7 % (1.35 kg OM and 0.82 kg NDF) and by 14.0 % (2.09 kg OM and 1.27 kg NDF) for early and late harvest respectively. Extrusion decreased $peNDF_8$ intake by 18.4% (1.10 kg/d) in early harvest and by 27.5% (1.65 kg/d) in late harvest.

Concentrate DM intake was decreased in extruded diets by 1.7 % (0.13 kg) while in diets based on early harvest it decreased by 0.7 % (0.06 kg). Extrusion increased total DM intake by 7.2 % (1.74 kg), total NDF intake by 9.4 % (1.02 kg), total CP intake by 4.7 % (0.18 kg) and ME intakes by 5.8 % (14.94 MJ). However, extrusion decreased the percentage of $peNDF_8$ on total DMI intake by 28.2 % (6.9 percentage units). Diets based on early harvest increased total CP intake by 9.9 % (0.38 kg/d), ME intake by 13.4 % (33.16 MJ) and had a higher percentage of $peNDF_8$ by 2.8 % (0.6 percentage units). An interaction between Harvest × Treatment and a tendency towards interaction was observed for total NDF ($p = 0.05$) and total DM intake respectively ($p = 0.055$). Extrusion increased total DM intake by 5.4 % (1.33 kg) and total NDF intake by 7.2 % (0.78 kg) for early harvest diets while for late harvest diets the increase was 9.0 % (2.16 kg) for total DM intake and 11.7% (1.25 kg) for total NDF intake. Finally, an interaction between Harvest and Treatment ($p < 0.001$)

resulted in a decrease of $peNDF_8$ content of the diets by 22.7 % for early harvest and by 33.5 % for late harvests.

***Table 2**. Effect of Treatment and Harvest on Silage, Concentrate and Total Intake*

	Early harvest		Late Harvest			P-value		
	Extruded	Control	Extruded	Control	SED[1]	Harvest	Treatment	Harvest × Treatment
Silage								
kg/d								
DMI	18.4	17.0	18.5	16.2	0.29 (0.27)	0.102	< 0.001	0.063
OMI	16.8	15.5	17.1	15.0	0.26 (0.25)	0.512	< 0.001	0.056
NDFI	10.1	9.33	10.4	9.10	0.159 (0.151)	0.983	< 0.001	0.053
$peNDF_8I$	4.87^b	5.97^a	4.36^c	6.01^a	0.070 (0.067)	< 0.001	< 0.001	< 0.001
CPI	2.34	2.15	1.98	1.74	0.035	< 0.001	< 0.001	0.256
MEI (MJ/day)	185	170	153	134	2.8 (2.7)	< 0.001	< 0.001	0.3
Concentrate DMI								
kg/d	7.61	7.76	7.69	7.80	0.035	0.034	< 0.001	0.415
Total								
kg/d								
DMI	26.1	24.7	26.2	24.0	0.29 (0.28)	0.172	< 0.001	0.055
NDFI	11.7^a	10.9^b	12.0^a	10.7^b	0.160 (0.152)	0.903	< 0.001	0.05
CPI	4.23	4.08	3.89	3.67	0.038 (0.036)	< 0.001	< 0.001	0.212
$peNDF_8$ %	18.7^c	24.1^b	16.6^d	25.0^a	0.18 (0.17)	< 0.001	< 0.001	< 0.001
MEI (MJ/day)	287	274	256	238	2.9 (2.8)	< 0.001	< 0.001	0.26

DMI = Dry Matter Intake. OMI = Organic Matter Intake. NDFI = Neutral Detergent Fibre Intake. CPI = Crude Protein Intake. MEI = Metabolizable Energy Intake, $peNDF_8I$ = Physical Effective NDF Intake, $peNDF_8$ = Percentage of $peNDF_8$ intake of total DM intake.

[1]Standard Error of the Difference. Minimum values presented in parenthesis.

[abcd] Means within each row with different superscripts were significantly different from each other ($P \leq 0.05$).

9.2. Milk production

Extrusion affected milk yield with the results presented in Table 5. Milk yield increased by 4.2 % (1.32 kg) and ECM yield by 5.4 % (1.89 kg). Milk fat and lactose content were not affected by extrusion, but protein content increased by 2.5

% (0.09 percentage units). Daily total milk solid production was also affected by extrusion with total fat production increasing by 5.2 % (72.4 g) and total protein production increasing by 6.8 % (73.7 g). Diets based on early harvest caused greater milk yield, ECM yield and total milk protein production by 3.5% (1.1 kg), 3.8 % (1.34 kg) and 3.8 % (42.4 g) respectively while they tended ($p < 0.1$) to increase milk fat content. Extrusion tended ($p < 0.1$) to affect milk fatty acid composition, more specifically SFA tended to increase by 1.1 %, while MUFA and PUFA decreased by 2.5 % and 5.5 %.

***Table 3**. Effect of Treatment and Harvest on Milk yield, ECM and milk composition*

	Early harvest		Late Harvest			P-value		
	Extruded	Control	Extruded	Control	SED[1]	Harvest	Treatment	Harvest × Treatment
Yield kg/d								
Milk	33.0	32.5	32.7	30.6	0.62	0.022	0.008	0.069
ECM	36.9	36.2	36.8	33.7	0.80	0.029	0.004	0.054
Milk composition %								
Fat	4.55	4.51	4.57	4.48	0.061	0.88	0.143	0.573
Protein	3.62	3.51	3.59	3.52	0.024	0.391	< 0.001	0.269
Lactose	4.58	4.56	4.56	4.54	0.027	0.245	0.384	0.911
Fatty acids g/kg								
SFA	2.982	2.938	3.000	2.902	0.0497	0.795	0.058	0.451
MUFA	0.926	0.935	0.930	0.943	0.0174	0.609	0.379	0.873
PUFA	0.127	0.127	0.124	0.134	0.0048	0.583	0.152	0.190
Daily solid production g/d								
Fat	1459	1440	1459	1333	38.1	0.066	0.015	0.063
Protein	1167	1126	1158	1051	25.6	0.031	0.001	0.088
Milk efficiency								
Milk/kg DMI	1.26	1.32	1.25	1.27	0.024 (0.023)	0.114	0.038	0.398
ECM/kg DMI	1.42	1.47	1.41	1.40	0.031 (0.029)	0.108	0.261	0.23

ECM = Energy Corrected Milk. DMI = Dry Matter Intake. SFA = Saturated Fatty Acids, MUFA = Mono Unsaturated Fatty Acids, PUFA = Poly Unsaturated Fatty Acids.

[1]Standard Error of the Difference. Minimum values presented in parenthesis.

A tendency in the interaction between Harvest × Treatment was observed for milk yield ($p = 0.069$), ECM yield ($p = 0.054$) and total milk solids production (fat production $p = 0.063$; protein production $p = 0.088$). Extrusion of early harvest increased milk yield by 1.4 % (0.47 kg), ECM yield by 2 % (0.72 kg), daily milk fat production by 1.3 % (18.9 g) and daily protein production by 3.6 % (41 g). Extrusion of late harvest instead increased milk yield by 7.1 % (2.16 kg), ECM yield by 9.1 % (3.05 kg), daily fat production by 9.4 % (125.8 g) and protein production by 10.1 % (106.5 g). The efficiency of milk production (milk yield per kg DM intake) decreased with extrusion by 2.9 % (0.04 kg), however, the efficiency of ECM production (ECM yield per DM intake) was not affected by treatment.

9.3. Ruminal pH

Extrusion decreased average ruminal pH by 1.7 % (0.10 units) and minimum ruminal pH by 1.5 % (0.09 units) (Table 6 and Figure 2). Time rumen pH was below 5.8 increased by 151.4 % (178.1 min) while time below rumen pH 6.0 increased by 92.6 % (402.2 min). The area under pH value of 5.8 was not affected by treatment while the area under pH value of 6.0 increased by 168.7 % (74.4 pH × minutes). There was tendency ($p = 0.066$) for extrusion to increase maximum pH by 1.2 % (0.07 units). Diets of early harvest lowered maximum rumen pH by 1.7 % (0.1 units).

Table 4*. Effect of Treatment and Harvest on ruminal pH*

	Early harvest		Late Harvest			P-value		
	Extruded	Control	Extruded	Control	SED[1]	Harvest	Treatment	Harvest × Treatment
Ruminal pH								
Average	5.99	6.09	6.01	6.11	0.038	0.486	0.008	0.991
Median	5.96	6.09	6.00	6.12	0.053	0.399	0.016	0.846
Minimum	5.69	5.75	5.66	5.78	0.048	0.935	0.041	0.377
Maximum	6.44	6.37	6.55	6.48	0.047	0.018	0.066	0.925
Time (min/d) under								
5.8	262	89.3	330	146	95.20	0.392	0.038	0.939
6.0	868	433	806	436	136.0	0.769	0.006	0.748
Area (pH × min) under								
5.8	21.7	1.74	18.3	9.27	13.0	0.828	0.166	0.576
6.0	120	36.6	117	51.6	31.60	0.807	0.016	0.694

[1]Standard Error of the Difference.

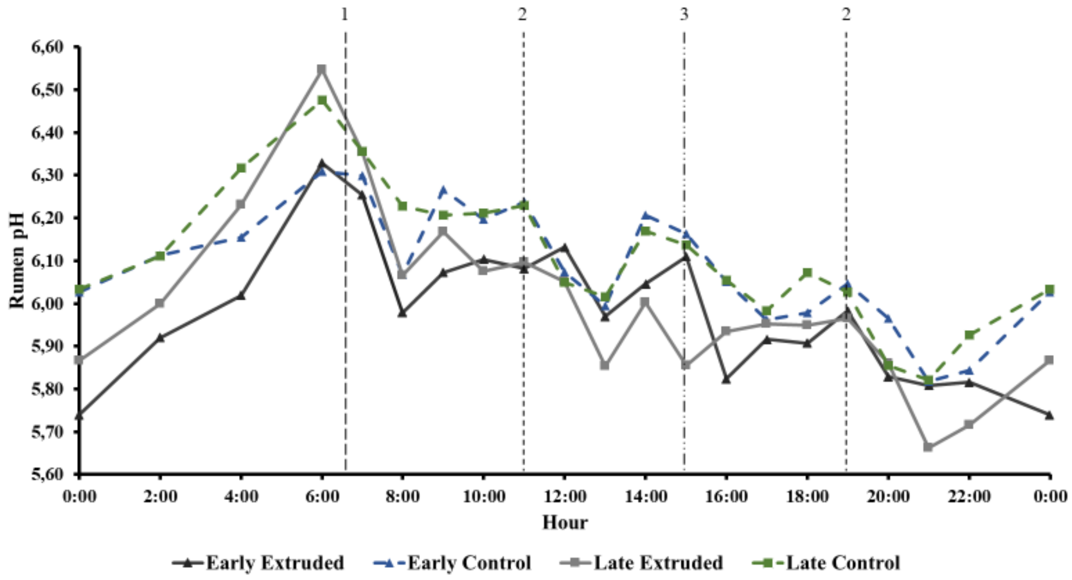

Figure 2. *Rumen pH variation over 24 hours. 1= feeding of forage, 2=feeding of forage and concentrate, 3=feeding of concentrate.*

9.4. Ingestive behaviour

Extrusion affected daily ingestive behaviour as shown in Table 7. Daily silage eating time and total eating time decreased by 13.2 % (36 min) and 10.3 % (31.8 min) respectively. Eating rate (g/min) increased with extrusion by 33.1% for silage DM (20.3 g/min) and for silage NDF (11.3 g/min). Extruded diets decreased rumination time by 19.9 % (117.8 min) with rumination time per kg silage DM and kg silage NDF intake decreasing by 29.1 % (10.5 min/kg DM; 18.9 min/kg NDF respectively). Extrusion, decreased total chewing time by 16.6% (149.6 min) with chewing time per kg silage DM and kg silage NDF intake decreasing by 26.7 % (14.6 min/kg DM; 26.3 min/kg NDF respectively). Harvest did not affect ingestive behaviour, while a tendency in the interaction between Harvest × Treatment was observed in total rumination time ($p = 0.084$) with extrusion resulting in a decrease of 23.2 % (140.6 min/d) for early harvest and 16.4 % (95 min/d) for the late harvest.

Table 5. *Effect of Treatment and Harvest on ingestive behaviour*

	Early harvest		Late Harvest			P-value		
	Extruded	Control	Extruded	Control	SED[1]	Harvest	Treatment	Harvest × Treatment
Eating time (min/d)								
Silage	228	267	245	279	16.7 (15.9)	0.22	0.006	0.827
Total	266	300	287	315	16.9 (16.1)	0.144	0.014	0.808
Eating rate (g/min)								
Silage DMI	82.1	63.8	81.0	58.8	4.70 (4.46)	0.362	< 0.001	0.541
Silage NDF	45.1	35.1	45.5	33.0	2.61 (2.47)	0.619	< 0.001	0.505
Rumination (min/d)	465	605	485	580	18.0 (17.1)	0.846	< 0.001	0.084
Unitary time (min/kg)								
Silage NDFI	46.8	65.4	45.2	64.3	2.48 (2.35)	0.448	< 0.001	0.895
Silage DMI	25.7	36.0	25.4	36.1	1.38 (1.31)	0.899	< 0.001	0.814
Chewing time (min/d)	731	906	772	896	23.0 (21.8)	0.343	< 0.001	0.123
Unitary time (min/kg)								
Silage NDFI	73.4	98.0	71.5	99.6	2.99 (2.84)	0.945	< 0.001	0.409
Silage DMI	40.4	53.9	40.1	55.9	1.67 (1.58)	0.461	< 0.001	0.348

[1]Standard Error of the Difference. Minimum values presented in parenthesis.

10. Discussion

10.1. Intake and eating time

According to our hypothesis, the animals receiving extruded diets had higher silage DM intake compared to control diets. These results are in agreement with a meta-analysis by Nasrollahi *et al.* (2015) and indicate that decreasing forage FPS improves intake capacity and lowers eating time. However, the magnitude of the effect in the present study is bigger since DM intake increased by 1.74 kg/d compared to the increase of 0.5 kg/d (minimum 0.3 kg/d; maximum 0.75 kg/d) for high forage diets expected in the meta-analysis.

Extrusion resulted in a reduction of FPS (Table 3) which is expected to increase available surface area affecting digestibility, passage rate and filling effect. Increasing available surface area is expected to increase the accessibility of cellulose and hemicellulose by the fibrolytic bacteria and increase the speed of FPS reduction affecting passage rate. Additionally, the decreasing FPS is expected to increase rumen passage rate and decrease the filling effect of the diet (Allen 2000; Nasrollahi et al. 2015). Extrusion increased silage DMI more in the late harvest diets (Extruded 18.49 kg/d; Control 16.22 kg/d) compared to early harvest diets (Extruded 18.44 kg/d; Control 16.96 kg/d). However, silage DMI intake did not differ between extruded diets (early harvest 18.4 kg/d, late harvest 18.5 kg/d) despite the difference in maturity. This indicates that the chemical composition was not the limiting factor. Intake might have been limited instead by the rumen pool size and the FPS reduction achieved by the extrusion intensity selected for this project. On the contrary, control diets differed in DMI between early harvest (17.0 kg/d) and late harvest (16.2 kg/d). This difference can be explained by the lower digestibility of the late harvest and the higher degree of lignification resulting is slower passage rate, increased reticulorumen distention and satiety. Total DMI intake also followed this trend with extruded diets not differing (early extruded 26.1 kg/d, late extruded 26.2 kg/d) compared to control diets (early control 24.7 kg/d, late control 24.0 kg/d). The effect of extrusion on the filling effect is also observed in the NDF intake. Despite, late harvest diets having higher NDF content, extrusion resulted in increased NDF intake by 1.25 kg/d for late harvests compared to 0.78

kg/d for early harvests. According to Nasrollahi *et al.* (2015) decreasing FPS is expected to increase NDF intake by 0.17 kg/d, but in the present study, the total NDF intake increased by 1.02 kg/d indicating that extrusion resulting in higher NDF intake compared with other mechanical treatment methods. According to Zebeli *et al.* (2012) increasing the percentage of $peNDF_8$ on total DMI beyond 14.9 % suppress DMI due to filling effect. In the present experiment, $peNDF_8$ was 17.6 % for extruded and 24.6% for control diets, indicating that the higher DMI of extruded diets was due to decreased filling effect. Additionally, the decrease of $peNDF_8$ with extrusion follows the corresponding changes of DMI with the value for late harvest (16.6 %) being the lowest and resulting in the highest increase in DMI followed by early harvest (18.7 %). This indicates that extrusion is an efficient method of manipulating FPS to increase feed intake.

The physical changes caused by extrusion on silage resulted in decreased silage eating time by 0.6 h/d while the total eating time decreased by 0.5 h/d. The decreased eating time combined with the increased intake resulted in higher eating rates for extruded diets. These results are in agreement with studies on the effect of FPS reduction on eating time and eating rate, however, the magnitude of the effect is bigger since a decrease of 0.3 h/d was expected (Nasrollahi *et al.* 2016).

10.2. Milk yield

According to a meta-analysis by Nasrollahi *et al.* (2015) decreasing FPS is expected to increase milk yield by 0.54 kg/d (minimum 0.35k kg/d, maximum 0.73 kg/d). In the present experiment, extrusion resulted in a greater increase in milk yield (1.32 kg/d). The increase was more profound in the late harvest (2.16 kg/d) compared to early harvest (0.47 kg/d). It is particularly interesting that the milk yield of animals receiving late extruded (LE) diets (32.7 kg/d), did not differ statistically but was numerically higher than for animals receiving early control (EC) diets (32.5 kg/d). Some milk components were affected by treatment while others were not. Protein content was statistically significantly higher in extruded diets (Extruded 3.61 %, Control 3.52 %) corresponding to an increase of protein content by 0.09 %. These results indicate that extrusion is more efficient in increasing protein content compared to other methods that result in FPS reduction as indicated by the expected increase in protein content of 0.01 % (minimum -0.005 %, maximum 0.024 %) (Nasrollahi *et al.* 2015). The difference in milk solids resulted in increased ECM yield for extruded diets (36.8 kg/d) compared to control diets (35.0 kg/d). ECM yield increased more in the late harvest (3.05 kg/d) compared to early harvest (0.72 kg/d) and it is particularly interesting that LE diets produced more ECM daily compared with EC diets (LE: 36.8 kg/d, EC: 36.2 kg/d). Total milk fat production increased by 72.4 g/d compared with the expected decrease of 5 g/d (minimum -20 g/d, maximum 9 g/d) and total milk protein production increased by 73.7 g/d compared with expected increase of 20 g/d (minimum 10 g/d, maximum 30 g/d) (Nasrollahi *et al.* 2015). Fat and lactose content was only numerical higher in extruded diets compared to control, but no statistically significant difference was observed. These improvements in milk production can be explained by the increased intake from extruded diets resulting in enhanced energy and nutrient balance (Table 8).

The dietary chewing index (minutes per kg DMI) is negatively linearly related to Net Energy intake (Jensen *et al.* 2016). In the present study, extrusion decreased the dietary chewing index and increased DMI providing more energy for milk production. However, the increased DM intake by 1.74 kg/d does not explain the increase in milk yield by 1.32 kg/d. This is also observed in the amount of DM consumed per kilogram of milk produced. Extrusion resulted in a decrease of 0.04 kg of milk per kg of DM intake. However, due to the increased milk-solid production ECM yield per DM intake was not affected. Additionally, the amount of saturated fatty acids did not differ significantly between treatments indicating that there was no-significant increased fat tissue mobilization. Unfortunately, due to time constraints, faecal analyses were not performed so there is no information regarding digestibility and passage rate.

Table 6. Metabolizable Energy and Intestinal Amino Acid Supply

		Early harvest		Late Harvest	
		Extruded	Control	Extruded	Control
Required					
	ME MJ/d	264	260	263	245
	IAAS g/d	2004	1974	2000	1865
Intake					
	ME MJ/d	287	275	257	240
	IAAS g/d	2442	2368	2332	2206
Balance					
	ME MJ/d	23	15	-6	-5
	IAAS g/d	438	394	332	341
Expected BW change kg/d					
	Based on ME balance	0.64	0.42	-0.19	-0.18
	Based on IAAS balance	1.75	1.58	1.33	1.36

ME = Metabolizable energy, IAAS = Intestinal Amino Acid Supply

Calculated based on Fodertabeller för idisslare 2003

10.3. Rumen pH

Cows receiving extruded diets presented decreased average rumen pH (pH 6.0) compared with animals receiving control diets (pH 6.1). Minimum ruminal pH was also decreased while maximum ruminal pH tended to increase. These results are in agreement with the meta-analysis by Nasrollahi *et al.* (2016) which indicated that decreasing FPS in silage based diets will result in decreased rumen pH. Several signals can be used to detect Sub Acute Ruminal Acidosis (SARA), however, rumen pH is the most reliable (Humer *et al.* 2018a). Different cut-off points can be used depending on available methods. When a single measurement is used, the cut-off point for SARA is 5.5 (Humer *et al.* 2018a), in the present experiment the minimum pH value of all animals was above this cut-off point. Another method of accessing the risk of SARA is by calculating the time ruminal pH drops below certain cut-off points. Plaizier *et al.* (2008) suggested that ruminal pH below 5.6 for more than 3 h per day can be used, in the present experiment ruminal pH did not drop below this cut-off point. Zebeli *et al.* (2008), on the other hand, suggested that a cut-off point of 5.8 for more than 5.24 h/d indicates an increased risk of SARA. Extrusion increased significantly the time rumen pH was below this cut-off point (4.9 h/d) compared to control diets (2.0 h/d), yet, the values are not within the range indicating increased risk of SARA. Late extruded diets had an average time of 5.5 h/d being marginally higher that the limit of increased SARA risk, however, when calculating the Area Under Curve for this cut-off point no statistical difference was observed between treatments. The increased SARA risk for late extruded diets can also be explained by the $peNDF_8$ intake. According to Zebeli *et al.* (2012), diets should contain more than 18.5 % $peNDF_8$ to minimize the risk of SARA. In the present experiment extruded diets had $peNDF_8$ values of 16.6 % and 18.7 % for late and early harvest respectively explaining the slightly lower ruminal pH in late harvest.

Changes in rumen pH can be explained by the altered ingestive behaviour. Extrusion resulted in a decrease of total rumination time by 2.0 h/d while total chewing time decreased by 2.5 h/d. The decrease of total chewing time is in agreement with studies on the effect of FPS reduction, however, the expected decrease according to these studies is 0.7 h/d (Nasrollahi *et al.* 2016). Decreased total chewing time is explained by the physical changes of diet that can also be observed in the $peNDF_8$ intake. Extruded diets, resulted in decreased $peNDF_8$ intakes (17.6 %) compared to control (24.6 %) diets. The decrease in $peNDF_8$ intake was higher in late extruded diets indicating that the treatment effect is stronger on the more mature and lignified plant tissues.

10.4. Strengths and weaknesses

The results of this study indicate that forage extrusion is an effective method for improving the nutritional quality of forage with interesting future applications. However, as every study, it presents some strengths and is subject to some limitations. The chosen housing system (tie-stalls) have been a subject to criticism for animal welfare reasons in many parts of the world since in restricts voluntary movement possibilities and social interaction of dairy cows (Robbins *et al.* 2019). Nevertheless, under good management practices allowing dairy cows to exercise improves animal welfare quality and does not necessarily indicate that tie-stalls will result in poor welfare (Popescu *et al.* 2013). Unfortunately, in the present experiment animals were not allowed to exercise since it would affect the sampling protocols and accessing to pasture would complicate the estimation of their feed intake. However, the distance between the tie stalls allowed for physical contact between the cows and the expression of affiliative behaviours such as grooming. Tie stalls resulted in high internal validity since they allowed to monitor with high precision the reaction of each individual to the offered treatments while eliminating feed competition. The external validity of the effect of extrusion on DMI is high in farms practising tie-stall housing system. On the other hand, selection of this housing system might underestimate the effect of extrusion on the DMI, feed sorting and the productivity of a group of animals (Grant & Ferraretto 2018).

The selection of the experimental design (Latin square) increased the internal validity of the experiment and resulted in a smaller mean square for error. The number of animals was also decreased according to the Reduction principle of the 3 R's (*NC3Rs*). Although the use of fistulated animals facilitated the collection of samples, a study with a greater number of intact animals will allow to verify the results of the present experiment and detect additional differences between the treatments.

Extrusion decreased the average ruminal pH and increased time under a pH cut-off point of 5.8. However, ruminal pH variation within 24 h was estimated using information from ruminal liquid samples that were collected at 20 different occasions for 5 days. This indicates that a degree of variation in ruminal pH may have not been detected. Despite this, the available information combined with the productivity and behaviour of the animals indicates that there was no risk of SARA. Furthermore, the adaptation period to the new diets was two weeks, which might be insufficient for the microbial population of the rumen to adjust to dietary changes. Finally, silage was offered *ad libitum* while concentrates were offered on restricted amounts separately. Offering concentrates separately might have affected the ruminal fermentation pattern, resulting in excessive fermentation compared to a total mixed ration (Humer *et al.* 2018a).

This study was focused on mid and late lactation animals and utilized diets of lower nutritional quality than the ones normally used in a dairy farm. Despite the short duration of the experiment (84 days), a clear effect of treatment was observed in milk production and milk compositions. Furthermore, due to time limitations, this study is based on the available information that could be provided within one month from the end of the experiment. Information regarding rumen pool size, passage rate, digestibility, microbial protein production, detailed chemical analysis of the offered feeds etc. had to be excluded from the current work. However, the available results support the hypothesis that extrusion of silage results in improved DMI and milk production without compromising ingestive behaviour.

10.5. Implementation

The results of this thesis indicate that adoption of extrusion as a mechanical treatment method for silage can be beneficial for milk production. Extruded diets resulted in increased milk production 32.9 kg/d compared to control diets 31.5 kg/d. Grass silage used in the present experiment had relatively low ME content of 10.0 MJ/kg DM for Early harvest and 8.26 MJ/kg DM for Late harvest. Despite this difference, extruded diets resulted in non-statistically significant different milk yield (early harvest 33.0 kg/d, late harvest 32.7 kg/d) and ECM yields (early harvest 36.9 kg/d, late harvest 36.8 kg/d). Consequently, extrusion presents an opportunity in situations where forage of lower nutritional quality is available since it can sustain or even increase the production levels. Cases like this include unforeseen drawbacks of the harvest resulting in forage of higher maturity stage.

Delaying harvest is expected to result in increased DM yield per hectare at the expense of forage nutritional quality. Decreasing the number of harvests might substitute for the extrusion cost and combined with the increased DM yield will result in decreased production cost per kilo of forage DM. Forage extrusion can then be implemented as a method to increase feed intake and milk production without increasing the overall cost for milk production. This approach can be beneficial for animals with lower productivity such as dairy cows at late lactation or small ruminants.

Diets in the present experiment were silage-based with an average forage to concentrate ratio of 70:30. Despite the high forage inclusion and the lower nutritional quality, extrusion resulted in increased milk production and silage DMI while decreasing concentrate DMI. This effect can be utilized by organic farms, providing an opportunity for increased use of farm-grown forages and lower need for concentrates.

Ingestive behaviour was also affected by extrusion resulting in decreased eating and rumination time and consequently increase in eating rate. These effects were observed in the tie-stalls where there was no feed competition and additionally the cows were milked in the stall. The increased eating rate can be particularly beneficial under farm conditions and may facilitate feed bunk management (Grant & Ferraretto 2018). Additionally, the particle size decrease and the more homogenous diet created by extrusion is expected to allow, all individuals in a group to consume sufficient amounts of silage and improve the supply of nutrients to the rumen (Table 9, Appendix). This will result in increased production of the group since all intake of all individuals will improve.

In the present experiment decreased eating and rumination time allowed the cows to spend more time idle. The time budget of a dairy cow under farm conditions differs from the one observed in the present experiment since animals need to move to and from the milking parlour etc. Eating time is in an inelastic relation with resting time. Decreasing eating time without suppressing rumen pH can be beneficial under farm conditions (Grant & Ferraretto 2018).

Finally, increasing forage consumption at the expense of concentrates while increasing milk production can be beneficial for the overall net food productions. Forages are a source of nutrients indigestible to human in contrast with the ingredients of certain concentrates as indicated by human edible proportion (Ertl *et al.* 2016). Adopting diets, high in silage and low in human-edible concentrates presents an opportunity for improved and sustainable animal production.

10.6. Future research

The results of the present experiment show that extrusion of silage can affect feed intake and result in increased milk production. However, to enlighten the causative conditions more research is needed. Silage extrusion affected FPS, indicating that passage rate and digestibility have also been altered. Meticulous sieving of extruded silage will allow for a more precise determination of the effect on FPS reduction and correlate the effect with the intensity of the extrusion process and the physical characteristics of the silage. Use of imaging techniques, such as Scanning Electron Microscopy or Light Microscopy will allow to identify changes in the microstructure of the feed material and correlate them with the observed effect.

Extrusion, through the mechanical energy, affects the behaviour of feed particles with water (Redgwell *et al.* 2011; Robin *et al.* 2012; Alam *et al.* 2016; Huang & Ma 2016; Bader Ul Ain *et al.* 2019). Determination of fibre water solubility and water absorption index will provide information regarding this interaction (Oikonomou & Krokida 2012), explaining potential differences in digestibility and its effect on rumen microbiota and rumen fermentation. The effect of extrusion on feed accessibility can be evaluated through estimations of porosity, water retention index and by estimating the absorption of different substances such as stains or nitrogen (Leu & Zhu 2013; Chen *et al.* 2014). Performing these analyses on faeces and rumen content will provide additional information regarding the digestion and the behaviour of the particles throughout the digestive tract. Additionally, since extruded substrates have increased water holding capacity and solubility and since the reduction of FPS is expected to result in faster passage rate and a slight decrease in NDF digestibility, we can assume that the higher NDF content in the faeces combined with the increased water holding capacity may affect the viscosity of the faeces. This might affect farm hygienic conditions.

The result of extrusion is affected by many parameters including the setting of the extruder (speed, opening size, feed rate) but it is also affected by the characteristics of the diets such as DM content and FPS (Duque et al. 2017). According to Zheng *et al.* (2015), the screw profile can generate local temperature spikes even under low-temperature conditions, like the ones in the present experiment, resulting in lignin relocalization affecting digestibility. These characteristics can differ significantly between silage, increasing the variation in the extrusion conditions (temperature, pressure etc.). Extrusion parameters should be optimized based on the characteristics of the diets to be extruded such as NDF content, FPS and DM to attain repeatable results. Additionally, based on the production stage of the animal different processing intensities might be required to achieve the ideal FPS reduction and the consequent passage rate increase.

The present experiment followed a fraction of the lactation. A study on the whole lactation will provide more information on the effect of extrusion on milk production, feed intake and additionally its effects on reproduction and energy balance. Adopting extrusion as a strategy to increase DMI in dairy cows in early lactation, may assist in easing the effects of negative energy balance through increased nutrient supply. Improved energy balance will improve the health and welfare of the dairy cows, additionally, it might affect the lactation curve and improve reproductive performance.

Extrusion presents an opportunity for improving the nutritional quality of different feeds. The results of this experiment indicate that the filling effect of the diet is decreased. This can be particularly beneficial in feeds and by-products high in NDF content and CP. By increasing DMI and passage rate more nutrients will escape rumen degradation and be absorbed in the small intestines increasing the productivity of the animals. Extrusion, however, might be beneficial also in silage of higher nutritional quality through decreased FPS, increased digestibility and passage rate. In the present experiment, extrusion resulted in a significant increase in DMI in both early and late harvest. Animals consuming EE diets consumed 1.48kg/d more indicating that extrusion might increase intake also in silage of lower maturity stage compared with the one used.

Future research should aim to provide recommendations on how to implement the acquired knowledge on-farm conditions. A study on TMR extrusion, or silage extrusion and then the formation of TMR diets would be beneficial. Additionally, the cost of extrusion combined with the benefits from improvements in feed nutritional quality, milk production and potential effects on reproduction, carcass composition etc. should be considered.

Intensively processed silage is expected to interact and affect rumen microbiota. Rumen samples can be analysed using sequencing techniques for microbiota composition or transcriptome. Detecting changes in the microbiota composition and identify metabolic pathways resulting in methane production will clarify the manner they are being affected by the changes in digestibility and passage rate.

Last but not least, the current results indicate that extrusion can contribute to a more sustainable food production system by decreasing human-animal competition. Farm animals have been often criticized for consuming ingredients that could otherwise be consumed by humans. However, some of these claims often do not take into consideration that the diet of a dairy cow is mostly based on materials that are indigestible by a human. In the present experiment, extrusion resulted in increased silage intake and milk production while concentrate intake decreased. Milk protein is according to the Digestible indispensable amino acid score (DIAAS) recommended by (FAO 2013) of excellent nutritional value with a score higher than 100. On the contrary, plant-based proteins such as soy protein isolate, soy flour or wheat have a DIAAS score of 84.89 and 45 respectively (Mathai *et al.* 2017). Implementation of this system in the calculations of milk protein production will allow a more accurate estimation of the net food production and will estimate the changes in the amino acid profile (Tables 13, 14, 15 in Appendix).

11. Conclusions

This study investigated the effect of extrusion on intake, milk production and ingestive behaviour. Extrusion increased silage DM intake while decreasing concentrate DM intake. Extruded diets increased milk and ECM yields and resulted in higher daily milk solid production. No statistically significant difference was found in milk yield between early harvest control diet and late harvest extruded diets. Silage eating rate increased while eating time and rumination time decreased. The decreased total chewing time resulted in a slightly lower average rumen pH without increasing the risk of SARA. Extrusion can be utilized as a mechanical treatment for forage and silage resulting in increased intake and milk production without adverse effects in ingestive behaviour.

References

Abraham, A., Mathew, A.K., Park, H., Choi, O., Sindhu, R., Parameswaran, B., Pandey, A., Park, J.H. & Sang, B.-I. (2020). Pretreatment strategies for enhanced biogas production from lignocellulosic biomass. *Bioresource Technology*, vol. 301, p. 122725

Adesogan, A.T., Arriola, K.G., Jiang, Y., Oyebade, A., Paula, E.M., Pech-Cervantes, A.A., Romero, J.J., Ferraretto, L.F. & Vyas, D. (2019). Symposium review: Technologies for improving fiber utilization. *Journal of Dairy Science*, vol. 102 (6), pp. 5726–5755

Agbossamey, Y.R., Savoie, P., Seoane, J.R. & Petit, H.V. (2000). Effect of intensity of maceration on digestibility and intake of alfalfa hay and silage fed to sheep. *Canadian Journal of Animal Science*, vol. 80 (1), pp. 113–121

Akin, D.E. (1989). Histological and Physical Factors Affecting Digestibility of Forages. *Agronomy Journal*, vol. 81 (1), pp. 17–25

Alam, M.S., Kaur, J., Khaira, H. & Gupta, K. (2016). Extrusion and Extruded Products: Changes in Quality Attributes as Affected by Extrusion Process Parameters: A Review. *Critical Reviews in Food Science and Nutrition*, vol. 56 (3), pp. 445–473

Allen, M.S. (1996). Physical constraints on voluntary intake of forages by ruminants. *Journal of Animal Science*, vol. 74 (12), p. 3063

Allen, M.S. (1997). Relationship Between Fermentation Acid Production in the Rumen and the Requirement for Physically Effective Fiber. *Journal of Dairy Science*, vol. 80 (7), pp. 1447–1462

Allen, M.S. (2000). Effects of Diet on Short-Term Regulation of Feed Intake by Lactating Dairy Cattle. *Journal of Dairy Science*, vol. 83 (7), pp. 1598–1624

Allen, M.S., Bradford, B.J. & Oba, M. (2009). BOARD-INVITED REVIEW: The hepatic oxidation theory of the control of feed intake and its application to ruminants. *Journal of Animal Science*, vol. 87 (10), pp. 3317–3334

Allen, M.S. & Mertens, D.R. (1988). Evaluating Constraints on Fiber Digestion by Rumen Microbes. *The Journal of Nutrition*, vol. 118 (2), pp. 261–270

Allen, M.S. & Voelker Linton, J.A. (2007). In vivo methods to measure digestibility and digestion kinetics of feed fractions in the rumen. Pp. 72-88, Proc. International Symposium on Advances in Ruminant Nutrition Research, Departmento de Nutricão e Producão Animal Faculdade de Medicna Veterinania e Zootecnia, University of São Paulo, Pirassununga, Brazil

Atalla, R.H. & Vanderhart, D.L. (1984). Native Cellulose: A Composite of Two Distinct Crystalline Forms. *Science*, vol. 223 (4633), pp. 283–285

Bader Ul Ain, H., Saeed, F., Ahmed, A., Asif Khan, M., Niaz, B. & Tufail, T. (2019). Improving the physicochemical properties of partially enhanced soluble dietary fiber through innovative techniques: A coherent review. *Journal of Food Processing and Preservation*, vol. 43 (4), p. e13917

Beauchemin, K.A., Yang, W.Z. & Rode, L.M. (2003). Effects of Particle Size of Alfalfa-Based Dairy Cow Diets on Chewing Activity, Ruminal Fermentation, and Milk Production. *Journal of Dairy Science*, vol. 86 (2), pp. 630–643

Bodvik, R., Dedinaite, A., Karlson, L., Bergström, M., Bäverbäck, P., Pedersen, J.S., Edwards, K., Karlsson, G., Varga, I. & Claesson, P.M. (2010). Aggregation and network formation of aqueous methylcellulose and hydroxypropylmethylcellulose solutions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 354 (1–3), pp. 162–171

Carlier, L., Rotar, I., Vlahova, M. & Vidican, R. (2009). Importance and functions of grasslands. *Notulae Botanicae, Horti Agrobotanici, Cluj-Napoca*, vol. 37 (1), pp. 25–30 University of Agricultural Sciences and Veterinary Medicine.

Chen, X., Zhang, Y., Gu, Y., Liu, Z., Shen, Z., Chu, H. & Zhou, X. (2014). Enhancing methane production from rice straw by extrusion pretreatment. *Applied Energy*, vol. 122, pp. 34–41

Clauss, M., Lechner, I., Barboza, P., Collins, W., Tervoort, T.A., Südekum, K.-H., Codron, D. & Hummel, J. (2011). The effect of size and density on the mean retention time of particles in the reticulorumen of cattle (*Bos primigenius* f. *taurus*), muskoxen (*Ovibos moschatus*) and moose (*Alces alces*). *British Journal of Nutrition*, vol. 105 (4), pp. 634–644

Dufreneix, F., Faverdin, P. & Peyraud, J.-L. (2019). Influence of particle size and density on mean retention time in the rumen of dairy cows. *Journal of Dairy Science*, vol. 102 (4), pp. 3010–3022

Duque, A., Manzanares, P. & Ballesteros, M. (2017). Extrusion as a pretreatment for lignocellulosic biomass: Fundamentals and applications. *Renewable Energy*, vol. 114, pp. 1427–1441

Ellis, W.C., Mahlooji, M., Lascano, C.E. & Matis, J.H. (2005). Effects of size of ingestively masticated fragments of plant tissues on kinetics of digestion of NDF. *Journal of Animal Science*, vol. 83 (7), pp. 1602–1615

Ertl, P., Klocker, H., Hörtenhuber, S., Knaus, W. & Zollitsch, W. (2015). The net contribution of dairy production to human food supply: The case of Austrian dairy farms. *Agricultural Systems*, vol. 137, pp. 119–125

Ertl, P., Steinwidder, A., Schönauer, M., Krimberger, K., Knaus, W. & Zollitsch, W. (2016). Net food production of different livestock: A national analysis for Austria including relative occupation of different land categories / Netto-Lebensmittelproduktion der Nutztierhaltung: Eine nationale Analyse für Österreich inklusive relativer Flächenbeanspruchung. *Die Bodenkultur: Journal of Land Management, Food and Environment*, vol. 67 (2), pp. 91–103

FAO (ed.) (2013). *Dietary protein quality evaluation in human nutrition: report of an FAO expert consultation, 31 March-2 April, 2011, Auckland, New Zealand*. Rome: Food and Agriculture Organization of the United Nations. (FAO food and nutrition paper; 92)

Feltre, K., Balieiro, J.C. de C., Fukumasu, H. & Gobesso, A.A. de O. (2019). Digestive effects and intestinal health of ponies fed a complete single diet, thermally processed and containing long fiber. *Livestock Science*, vol. 223, pp. 151–156

Fernández-Cegrí, V., Ángeles De la Rubia, M., Raposo, F. & Borja, R. (2012). Effect of hydrothermal pretreatment of sunflower oil cake on biomethane potential focusing on fibre composition. *Bioresource Technology*, vol. 123, pp. 424–429

Gallos, A., Paës, G., Allais, F. & Beaugrand, J. (2017). Lignocellulosic fibers: a critical review of the extrusion process for enhancement of the properties of natural fiber composites. *RSC Advances*, vol. 7 (55), pp. 34638–34654

Grant, R.J. & Ferraretto, L.F. (2018). Silage review: Silage feeding management: Silage characteristics and dairy cow feeding behavior. *Journal of Dairy Science*, vol. 101 (5), pp. 4111–4121

Hamaker, B.R. & Tuncil, Y.E. (2014). A Perspective on the Complexity of Dietary Fiber Structures and Their Potential Effect on the Gut Microbiota. *Journal of Molecular Biology*, vol. 426 (23), pp. 3838–3850
Harvatine, K.J. & Allen, M.S. (2006). Effects of Fatty Acid Supplements on Feed Intake, and Feeding and Chewing Behavior of Lactating Dairy Cows. *Journal of Dairy Science*, vol. 89 (3), pp. 1104–1112
Hatfield, R.D. & Jung, H.-J.G. (2007). Nutritional Chemistry of Forages. p. 20
Hernandez-Urdaneta, A., Coppock, C.E., McDowell, R.E., Gianola, D. & Smith, N.E. (1976). Changes in Forage-Concentrate Ratio of Complete Feeds for Dairy Cows. *Journal of Dairy Science*, vol. 59 (4), pp. 695–707
Hjorth, M., Gränitz, K., Adamsen, A.P.S. & Møller, H.B. (2011). Extrusion as a pretreatment to increase biogas production. *Bioresource Technology*, vol. 102 (8), pp. 4989–4994
Holtzapple, M.T. (2003a). CELLULOSE. In: Caballero, B. (ed.) *Encyclopedia of Food Sciences and Nutrition (Second Edition)*. Oxford: Academic Press, pp. 998–1007.
Holtzapple, M.T. (2003b). HEMICELLULOSES. In: Caballero, B. (ed.) *Encyclopedia of Food Sciences and Nutrition (Second Edition)*. Oxford: Academic Press, pp. 3060–3071.
Holtzapple, M.T. (2003c). LIGNIN. In: Caballero, B. (ed.) *Encyclopedia of Food Sciences and Nutrition (Second Edition)*. Oxford: Academic Press, pp. 3535–3542.
Hon, D.N.S. (1994). Cellulose: a random walk along its historical path. p. 25
Huang, Y.-L. & Ma, Y.-S. (2016). The effect of extrusion processing on the physiochemical properties of extruded orange pomace. *Food Chemistry*, vol. 192, pp. 363–369
Humer, E., Aschenbach, J.R., Neubauer, V., Kröger, I., Khiaosa-ard, R., Baumgartner, W. & Zebeli, Q. (2018a). Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. *Journal of Animal Physiology and Animal Nutrition*, vol. 102 (2), pp. 380–392
Humer, E., Petri, R.M., Aschenbach, J.R., Bradford, B.J., Penner, G.B., Tafaj, M., Südekum, K.-H. & Zebeli, Q. (2018b). Invited review: Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. *Journal of Dairy Science*, vol. 101 (2), pp. 872–888
INRA, CIRAD, AFZ & FAO (2020). *Feedipedia—Animal Feed Resources Information System*. *Feedipedia*. Available at: https://www.feedipedia.org/content/about-feedipedia [2020-06-07]
Jensen, L.M., Markussen, B., Nielsen, N.I., Nadeau, E., Weisbjerg, M.R. & Nørgaard, P. (2016). Description and evaluation of a net energy intake model as a function of dietary chewing index. *Journal of Dairy Science*, vol. 99 (11), pp. 8699–8715
Johnson, L., Harrison, J.H., Hunt, C., Shinners, K., Doggett, C.G. & Sapienza, D. (1999). Nutritive Value of Corn Silage as Affected by Maturity and Mechanical Processing: A Contemporary Review. *Journal of Dairy Science*, vol. 82 (12), pp. 2813–2825
Kim, S.H., Lee, C.M. & Kafle, K. (2013). Characterization of crystalline cellulose in biomass: Basic principles, applications, and limitations of XRD, NMR, IR, Raman, and SFG. *Korean Journal of Chemical Engineering*, vol. 30 (12), pp. 2127–2141
Lamsal, B., Yoo, J., Brijwani, K. & Alavi, S. (2010). Extrusion as a thermo-mechanical pre-treatment for lignocellulosic ethanol. *Biomass and Bioenergy*, vol. 34 (12), pp. 1703–1710
Lee, S.-H., Teramoto, Y. & Endo, T. (2009). Enzymatic saccharification of woody biomass micro/nanofibrillated by continuous extrusion process I – Effect of

additives with cellulose affinity. *Bioresource Technology*, vol. 100 (1), pp. 275–279

Leu, S.-Y. & Zhu, J.Y. (2013). Substrate-Related Factors Affecting Enzymatic Saccharification of Lignocelluloses: Our Recent Understanding. *BioEnergy Research*, vol. 6 (2), pp. 405–415

Li, F., Zhang, M., Guo, K., Hu, Z., Zhang, R., Feng, Y., Yi, X., Zou, W., Wang, L., Wu, C., Tian, J., Lu, T., Xie, G. & Peng, L. (2015). High-level hemicellulosic arabinose predominately affects lignocellulose crystallinity for genetically enhancing both plant lodging resistance and biomass enzymatic digestibility in rice mutants. *Plant Biotechnology Journal*, vol. 13 (4), pp. 514–525

Lindman, B., Karlström, G. & Stigsson, L. (2010). On the mechanism of dissolution of cellulose. *Journal of Molecular Liquids*, vol. 156 (1), pp. 76–81

Liu, Q., Luo, L. & Zheng, L. (2018). Lignins: Biosynthesis and Biological Functions in Plants. *International Journal of Molecular Sciences*, vol. 19 (2), p. 335

Mathai, J.K., Liu, Y. & Stein, H.H. (2017). Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). *British Journal of Nutrition*, vol. 117 (4), pp. 490–499

Maulfair, D.D., Fustini, M. & Heinrichs, A.J. (2011). Effect of varying total mixed ration particle size on rumen digesta and fecal particle size and digestibility in lactating dairy cows. *Journal of Dairy Science*, vol. 94 (7), pp. 3527–3536

Mendowski, S., Chapoutot, P., Chesneau, G., Ferlay, A., Enjalbert, F., Cantalapiedra-Hijar, G., Germain, A. & Nozière, P. (2019). Effects of replacing soybean meal with raw or extruded blends containing faba bean or lupin seeds on nitrogen metabolism and performance of dairy cows. *Journal of Dairy Science*, vol. 102 (6), pp. 5130–5147

Mendowski, S., Chapoutot, P., Chesneau, G., Ferlay, A., Enjalbert, F., Cantalapiedra-Hijar, G., Germain, A. & Nozière, P. (2020). Effects of pretreatment with reducing sugars or an enzymatic cocktail before extrusion of fava bean on nitrogen metabolism and performance of dairy cows. *Journal of Dairy Science*, vol. 103 (1), pp. 396–409

Mertens, D.R. (1997). Creating a System for Meeting the Fiber Requirements of Dairy Cows. *Journal of Dairy Science*, vol. 80 (7), pp. 1463–1481

Mosier, N. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, vol. 96 (6), pp. 673–686

Mottet, A., de Haan, C., Falcucci, A., Tempio, G., Opio, C. & Gerber, P. (2017). Livestock: On our plates or eating at our table? A new analysis of the feed/food debate. *Global Food Security*, vol. 14, pp. 1–8

Murphy, J., Braun, R., Weiland, P. & Wellinger, A. (2011). Biogas from Crop Digestion. p. 24

Nasrollahi, S.M., Imani, M. & Zebeli, Q. (2015). A meta-analysis and meta-regression of the effect of forage particle size, level, source, and preservation method on feed intake, nutrient digestibility, and performance in dairy cows. *Journal of Dairy Science*, vol. 98 (12), pp. 8926–8939

Nasrollahi, S.M., Imani, M. & Zebeli, Q. (2016). A meta-analysis and meta-regression of the impact of particle size, level, source and preservation method of forages on chewing behavior and ruminal fermentation in dairy cows. *Animal Feed Science and Technology*, vol. 219, pp. 144–158

Oba, M. & Allen, M.S. (2000). Effects of Brown Midrib 3 Mutation in Corn Silage on Productivity of Dairy Cows Fed Two Concentrations of Dietary Neutral

Detergent Fiber: 1. Feeding Behavior and Nutrient Utilization. *Journal of Dairy Science*, vol. 83 (6), pp. 1333–1341

Oikonomou, N.A. & Krokida, M.K. (2012). Water Absorption Index and Water Solubility Index Prediction for Extruded Food Products. *International Journal of Food Properties*, vol. 15 (1), pp. 157–168

Oliveira, K.A., Macedo Junior, G.D.L., Silva, S.P. da, Araújo, C.M., Varanis, L.F.M. & Sousa, L.F. (2018). Nutritional and metabolic parameters of sheep fed with extrused roughage in comparison with corn silage. *Semina: Ciências Agrárias*, vol. 39 (4), p. 1795

Olsson, L. & Hahn-Hägerdal, B. (1996). Fermentation of lignocellulosic hydrolysates for ethanol production. *Enzyme and Microbial Technology*, vol. 18 (5), pp. 312–331

Paula, E.M., Broderick, G.A., Danes, M.A.C., Lobos, N.E., Zanton, G.I. & Faciola, A.P. (2018). Effects of replacing soybean meal with canola meal or treated canola meal on ruminal digestion, omasal nutrient flow, and performance in lactating dairy cows. *Journal of Dairy Science*, vol. 101 (1), pp. 328–339

Plaizier, J.C., Krause, D.O., Gozho, G.N. & McBride, B.W. (2008). Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *The Veterinary Journal*, vol. 176 (1), pp. 21–31

Pomerleau-Lacasse, F., Seguin, P., Tremblay, G. & Mongrain, D. (2017). Developmental stages of timothy and alfalfa. *Minister of Agriculture and Agri-Food, (2017)*, p. 23

Popescu, S., Borda, C., Diugan, E.A., Spinu, M., Groza, I.S. & Sandru, C.D. (2013). Dairy cows welfare quality in tie-stall housing system with or without access to exercise. *Acta Veterinaria Scandinavica*, vol. 55 (1), p. 43

Redgwell, R.J., Curti, D., Robin, F., Donato, L. & Pineau, N. (2011). Extrusion-Induced Changes to the Chemical Profile and Viscosity Generating Properties of Citrus Fiber. *Journal of Agricultural and Food Chemistry*, vol. 59 (15), pp. 8272–8279

Robbins, J.A., Roberts, C., Weary, D.M., Franks, B. & von Keyserlingk, M.A.G. (2019). Factors influencing public support for dairy tie stall housing in the U.S. (Loor, J. J., ed.) *PLOS ONE*, vol. 14 (5), p. e0216544

Robin, F., Schuchmann, H.P. & Palzer, S. (2012). Dietary fiber in extruded cereals: Limitations and opportunities. *Trends in Food Science & Technology*, vol. 28 (1), pp. 23–32

Rodriguez, C., Alaswad, A., Benyounis, K.Y. & Olabi, A.G. (2017). Pretreatment techniques used in biogas production from grass. *Renewable and Sustainable Energy Reviews*, vol. 68, pp. 1193–1204

Saini, J.K., Saini, R. & Tewari, L. (2015). Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. *3 Biotech*, vol. 5 (4), pp. 337–353

Scheller, H.V. & Ulvskov, P. (2010). Hemicelluloses. *Annual Review of Plant Biology*, vol. 61 (1), pp. 263–289

Scherer, R., Gerlach, K. & Südekum, K.-H. (2015). Biogenic amines and gamma-amino butyric acid in silages: Formation, occurrence and influence on dry matter intake and ruminant production. *Animal Feed Science and Technology*, vol. 210, pp. 1–16

Sjaunja, L.O., Baevre, L., Junkkarinem, L., Pedersen, J. & Setälä, J. (1990). *A Nordic proposal for an energy corrected milk (ECM) formula*. *International Comitte for Recording the productivity of milk animals*. Available at: https://www.researchgate.net/publication/284193091_A_Nordic_proposal_for_an_energy_corrected_milk_ECM_formula [2020-05-06]

Smith, J., Sones, K., Grace, D., MacMillan, S., Tarawali, S. & Herrero, M. (2013). Beyond milk, meat, and eggs: Role of livestock in food and nutrition security. *Animal Frontiers*, vol. 3 (1), pp. 6–13

Spörndly, R. (2003). Fodertabeller för idisslare (Feed tables for ruminants). Report 257. Dep. of Anim. Nutr. and Management, Swedish Univ. of Agric. Sci., Uppsala, Sweden.

The 3Rs | NC3Rs. Available at: https://www.nc3rs.org.uk/the-3rs [2020-05-31]

Udén, P. (1984). The effect of intake and hay: Concentrate ratio upon digestibility and digesta passage. *Animal Feed Science and Technology*, vol. 11 (3), pp. 167–179

Udén, P. & Sutton, J.D. (1994). Retention of Cr-labelled grass hay and silage in different segments of the gastrointestinal tract of dairy cows. *Livestock Production Science*, vol. 37 (3), pp. 297–309

Um, B.-H., Choi, C.H. & Oh, K.K. (2013). Chemicals effect on the enzymatic digestibility of rape straw over the thermo-mechanical pretreatment using a continuous twin screw-driven reactor (CTSR). *Bioresource Technology*, vol. 130, pp. 38–44

Wattiaux, M.A., Satter, L.D. & Mertens, D.R. (1992). Effect of microbial fermentation on functional specific gravity of small forage particles. *Journal of Animal Science*, vol. 70 (4), pp. 1262–1270 Oxford Academic.

Weiss, W.P. & Shockey, W.L. (1991). Value of Orchardgrass and Alfalfa Silages Fed with Varying Amounts of Concentrates to Dairy Cows. *Journal of Dairy Science*, vol. 74 (6), pp. 1933–1943

Weng, R.-C. (2019). The effects of dietary replacement with extruded whole plant forage corn silage on gestation and lactation performances of sow and litter. *Journal of Agricultural and Crop Research*, vol. 7 (8), pp. 137–147

Wilkinson, J.M. (2011). Re-defining efficiency of feed use by livestock. *animal*, vol. 5 (7), pp. 1014–1022

Williams, B.A., van der Poel, A.F.B., Boer, H. & Tamminga, S. (1997). The Effect of Extrusion Conditions on the Fermentability of Wheat Straw and Corn Silage. p. 8

Wüstholz, J., Carrasco, S., Berger, U., Sundrum, A. & Bellof, G. (2017). Silage of young harvested alfalfa (Medicago sativa) as home-grown protein feed in the organic feeding of laying hens. *Organic Agriculture*, vol. 7 (2), pp. 153–163

Yang, H., Rustas, B.-O. & Eriksson, T. (2018). Rumen in vitro total gas production of timothy, red clover and the mixed silage after extrusion. *Proceedings of Nordic Feed Science Conference*, June 2018. pp. 181–183. SLU

Yang, L., Xu, F., Ge, X. & Li, Y. (2015). Challenges and strategies for solid-state anaerobic digestion of lignocellulosic biomass. *Renewable and Sustainable Energy Reviews*, vol. 44, pp. 824–834

Zebeli, Q., Aschenbach, J.R., Tafaj, M., Boguhn, J., Ametaj, B.N. & Drochner, W. (2012). Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. *Journal of Dairy Science*, vol. 95 (3), pp. 1041–1056

Zebeli, Q., Dijkstra, J., Tafaj, M., Steingass, H., Ametaj, B.N. & Drochner, W. (2008). Modeling the Adequacy of Dietary Fiber in Dairy Cows Based on the Responses of Ruminal pH and Milk Fat Production to Composition of the Diet. *Journal of Dairy Science*, vol. 91 (5), pp. 2046–2066

Zhang, M., Bai, X. & Zhang, Z. (2011). Extrusion process improves the functionality of soluble dietary fiber in oat bran. *Journal of Cereal Science*, vol. 54 (1), pp. 98–103

Zheng, J., Choo, K. & Rehmann, L. (2015). The effects of screw elements on enzymatic digestibility of corncobs after pretreatment in a twin-screw extruder. *Biomass and Bioenergy*, vol. 74, pp. 224–232

Acknowledgements

I would like to express my thanks to my supervisors Bengt-Ove Rustas and Torsten Eriksson for their help and feedback. I would also like to thank the people that were involved and assisted during this experiment.

Appendix

Particle size

The information regarding the particle size is presented in Table 9. Extrusion resulted in a noted decreased in FPS with no particles retained on the upper sieve (pore size 19 mm). In extruded diets, the majority of the particles were collected in the bottom pan indicating an average FPS of less than 8 mm.

Table 7. *Percentage of particles retained in the sieves of Penn State Particle Separator per experimental silage and silage refusals*

Sieve size	Early Extruded	Early Control	Late extruded	Late control
Offered silage				
19 mm	0 %	19 % ± 11 %	0 %	25% ± 10%
8 mm	48 % ± 12 %	45 % ± 5 %	42 % ± 14 %	41% ± 6%
Bottom pan	52 % ± 12 %	36 % ± 6 %	58 % ± 14 %	34% ± 5%
Silage refusals				
19 mm	0%	21 % ± 11 %	0 %	25 % ± 9 %
8 mm	60 % ± 7 %	45 % ± 6 %	48 % ± 16 %	42 % ± 6 %
Bottom pan	40 % ± 7 %	34 % ± 5 %	52 % ± 16 %	33 % ± 3 %

Energy and protein requirements

The available information regarding the average bodyweight of the animals, their milk yield and their milk composition were used to calculate the expected energy and protein requirements. The calculations were performed according to Spörndly (2003) and are presented in Table 10.

Table 8. *Calculation of Metabolizable energy and Intestinal Amino Acid requirements.*

	Early harvest		Late Harvest	
	Extruded	Control	Extruded	Control
ME Requirements (MJ/d)				
BW kg	650	650	650	650
$ME_m = 0.507\ MJ/BW^{0.75}$	65.3	65.3	65.3	65.3
ECM yield kg/d	36.9	36.2	36.8	33.7
$ME_l = 5$ MJ / kg ECM	184.5	181	184	168
Corrected ME requirements = $1.11 \times (ME_m + ME_l) - 13.6$	263.7	259.8	263.1	245.4
IAAS requirements (g/d)				
IAAS (g/d) = ME × 7.6	2004	1974	2000	1865

ME_m = Metabolizable energy maintenance, ME_l = Metabolizable energy lactation, IAAS = Intestinal Amino Acid Supply, BW = Body Weight, ECM = Energy Corrected Milk

Calculated based on Spörndly (2003)

The metabolizable energy content of the diets, the Intestinal Amino Acid Supply and the Rumen Nitrogen Balance are presented in Table 11. Calculation was based on Spörndly (2003).

Table 9. *Calculation of diet Metabolizable energy, Intestinal Amino Acid Supply and Rumen Nitrogen Balance*

	Compound feed	Soybean Meal	Concentrates			Early Harvest	Late harvest
			Fistulated	Intact	Average		
ME MJ/kg DM	13.4	13.8	13.5	13.48	13.49	10	8.26
IAAS g/kg DM	114	292	158.5	149.6	154.05	69	62
RNB g/kg DM	13	79	29.5	26.2	27.850	14	-1

ME = Metabolizable energy, IAAS = Intestinal Amino Acid Supply, RNB = Rumen Nitrogen balance

The results from tables 10 and 11 were used to calculate the Metabolizable energy Balance, the Protein Balance and the expected Body Weight (Table 12). It is interesting to note that animals receiving late extruded diets are in a similar negative energy balance as animals receiving late control diets (Late extruded -6.55 MJ/d; Late Control -6.37) despite having significantly higher milk yield (Late extruded 32.7 kg/d; Late Control 30.6 kg/d).

Table 10. *Energy, Protein Balance and expected Body Weight change*

	Early harvest		Late Harvest	
	Extruded	Control	Extruded	Control
Silage DM intake kg/d	18.4	17	18.5	16.2
Silage ME intake MJ/d	184	170	152.81	133.81
Silage IAAS intake g/d	1269.6	1173	1147	1004.4
Silage RNB g/d	257.6	238	-18.5	-16.2
Concentrate DM intake kg/d	7.61	7.76	7.69	7.8
Concentrate ME intake MJ/d	102.66	104.68	103.74	105.22
Concentrate IAAS intake g/d	1172.32	1195.43	1184.64	1201.59
Concentrate RNB g/d	211.94	216.12	214.17	217.23
Total Intake				
ME MJ/d	286.66	274.68	256.55	239.03
IAAS g/d	2441.92	2368.43	2331.64	2205.99
RNB g/d	469.54	454.12	195.67	201.03
Balance				
ME MJ/d	22.96	14.88	-6.55	-6.37
IAAS g/d	437.92	394.43	331.64	340.99
Expected BW change kg/d				
Based on ME balance[1]	0.64	0.42	-0.19	-0.18
Based on IAAS balance	1.75	1.58	1.33	1.36

ME = Metabolizable energy, IAAS = Intestinal Amino Acid Supply, RNB = Rumen Nitrogen Balance

[1]Metabolizable energy per 1 kg gain Bodyweight 35.8 MJ, 1 kg loss of Body Weight 34.5 MJ

[2]Intestinal Amino Acid Supply per 1 kg gain Bodyweight 250 g, 1 kg loss of Body Weight 185g

Calculated based on Spörndly (2003)

Human food production

Livestock traditionally has been used as a means of converting feeds into useful animal products of high nutritional value. The increasing use of grains in the nutrition of farm animals has, however, resulted in increasing criticism by the public regarding the potential competition between humans and animals for the same resources. One common argument during these debates is the high feed conversion ratio of livestock. However, this term is a simplification that fails to consider that most of the feed consumed by livestock is inedible by humans. The term human-edible fraction has been used by Wilkinson (2011) and Ertl (2015, 2016) to describe the fraction of gross energy and crude protein of the feedstuff that would be available for human consumption through processing. On Table 13 the human edible fractions of feedstuffs and the milk are presented for different processing scenarios. The Digestible Indispensable Amino Acid Score (DIAAS) is a method of evaluating the protein quality proposed by FAO (2013). Comparisons based on crude protein content can result in misleading conclusion since they do not take into consideration the amino acid composition and the digestibility of the proteins.

***Table 11**. Human-edible fractions (% of protein and energy) and DIAAS of feedstuffs (Wilkinson 2011; Ertl* et al. *2016).*

Feed	Protein %		Energy %		Energy = Protein	DIAAS %
	Minimum	Maximum	Minimum	Maximum		
Barley	40	80	40	80	80	47.2
Rapeseed meal	30	87	26	47	20	70.2
Wheat bran	0	20	0	20	20	48.8
Oats	50	75	50	75	80	56.7
Wheat	60	100	60	100	80	40.2
Molasses (sugar beet)	0	80	0	80	20	n.d.
Sugar beet pulp	-	20	-	20	20	n.d.
Plant fats	-	-	0	80	20	n.d.
Vitamins and minerals	0	0	0	0	0	n.d.
Soybean Meal	50	92	42	65	80	97
Grass silage	0	0	0	0	0	n.d.
Milk	100	100	100	100	100	115.9

DIAAS = Digestible Indispensable Amino Acid Score. E = Energy, P = Protein, n.d. = not determined.

Human-edible fractions (% of protein and energy) of feedstuffs for minimum and maximum scenario are taken from Ertl *et al.* (2016) while Energy = Protein is taken from Wilkinson *et al.* (2011) and correspond to an equally efficient extraction for both the protein and energy fraction of the feedstuff. DIAAS (%) according to Ertl *et al.* (2016)

Values for “Minimum” and “Maximum” scenarios were taken from the “Low” and “High” scenarios of Ertl et al., (2015, 2016). The minimum scenario represents human edible fraction that can be easily achieved, through the processing of these feeds without high-end technology. The maximum scenario assumes high extraction rates due to the development of novel technologies or change in eating habits (consumption of whole grain products etc.).

Data on gross energy (GE) values for feeds was used from Feedipedia database (INRA *et al.* 2020) while energy output was calculated using the gross energy using the caloric factors 23 kJ/g (protein), 38.9 kJ/g (fat), and 17.2 kJ/g (carbohydrates) according to Ertl *et al.*, (2016). Net food production, as MJ of GE/d for energy or g of CP/d for protein, was calculated as the human edible content in the milk minus the human edible content in the feed consumed according to Ertl *et al.*, (2016).

Table 12. *Effect of Extrusion and Harvest on Net Food production.*

	Early harvest		Late harvest			Effect (p -value)		
	Extruded	Control	Extruded	Control	SED	Harvest	Treatment	Harvest x Treatment
HeCP production g/d								
Minimum	308	250	290	171	27.5 (26.1)	0.02	< 0.001	0.128
E = P	97.0	34.3	76.5	-45.8	27.5 (26.1)	0.016	0.001	0.092
Maximum	-465	-539	-491	-622	27.7 (26.3)	0.011	< 0.001	0.157
DIAAS production g/d								
Minimum	611	549	593	459	31.9 (30.3)	0.024	< 0.001	0.122
E = P	429	363	409	272	31.9 (30.2)	0.021	< 0.001	0.126
Maximum	-56.6	-132	-81.9	-226	32 (30.3)	0.015	< 0.001	0.14
Gross energy MJ/d								
Minimum	54.3	50.8	53.0	43.0	2.57 (2.44)	0.02	0.001	0.087
E = P	29.3	25.2	27.7	17.3	2.56 (2.43)	0.017	< 0.001	0.134
Maximum	19.3	15.1	17.6	7.2	2.57 (2.43)	0.014	0.001	0.095

HeCP = Human edible Crude Protein production, DIAAS = Digestible Indispensable Amino Acid Score. E = Energy, P = Protein. SED = Standard Error of the Difference.

Table 13. *Main effect of Treatment and Harvest on Net food production*

	Treatment		Harvest		
	Extruded	Control	Early	Late	SED
Net HeCP production g/d					
Minimum	299	211	279	231	19
E = P	86.7	-5.75	65.6	15.3	18.9
Maximum	-478	-580	-502	-556	19.1
Net Protein production based on DIAAS g/d					
Minimum	602	504	580	526	22
E = P	419	317	396	340	22.0
Maximum	-69.3	-179	-94.3	-154	22
Net Gross Energy production MJ/d					
Minimum	53.6	46.9	52.5	48.0	1.77
E = P	28.5	21.3	27.2	22.5	1.77
Maximum	18.5	11.1	17.2	12.4	1.77

HeCP = Human edible Crude Protein production, DIAAS = Digestible Indispensable Amino Acid Score. E = Energy, P = Protein. SED = Standard Error of the Difference.

Negative values on Tables 14 and 15 indicate that the total Net Protein or Energy production is negative since the animal output is less than the feed input. These values account only for milk production and don’t take into consideration other aspects of animal products such as meat production. It appears that with the current technology and eating habits, dairy production based on high forage diets significantly contributes the total food production. Finally, processing of forages with extrusion will allow higher intakes of silage and forages further decreasing the need for concentrates and human edible feedstuff. This will result in decreased resource competition and more sustainable animal production.