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Effects of extruded linseed on methane emission and milk production of dairy cows

Effekter av extruderat linfrö på metanemission och mjölkproduktion hos mjölkkor

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Innehållsförteckning

Abbreviations

AMS-automatic milking system

CIP-Competitive and innovation frame work programme

CO_2-eq-carbon dioxide equivalence

de novo- “from the new”

DIM-days in milk

DM-dry matter

DMI-dry matter intake

EL-extruded linseed

FA-fatty acid

FFA-free fatty acids

FPCM-fat and protein corrected milk

FTIR-Fourier Transform Infra-red analyse. Analyse distributed by Agrosom via Eurofins Steins laboratory.

FATtot- fat concentration in milk for cows in testgroup. Analyse from FTIR

FApr- protein concentration in milk for cows in testgroup. Analyse from FTIR

ECM-Energy corrected milk

GC-Gas *Chromatography*

GHG-greenhouse gas

GLM-General linear model

in vitro-experiment done in laboratory

in vivo-experiment done inside the body

LCFA-long-chain fatty acids

LMD-laser methane detector

MF-milk fat content from official Swedish milking recording scheme distributed via VäxaSverige

MP-milk protein content

MY-milk yield

MUFA-monounsaturated fatty acid

NDF-Neutral detergent fibre

Per-period

PUFA-poly unsaturated fatty acids

PMR-partly mixed ration

SFA-saturated fatty acids

TMR-total mixed ration

UFA-unsaturated fatty acids

VFA-volatile fatty acids

VLDL-very-low density lipoprotein

Abstract

The agriculture sector is one of the major sources to produce greenhouse gases of today. One big contributor is methane (CH_4) generated during rumen digestion of the feed carbohydrates. One approach to lower CH4 output from the agriculture sector, is to change the diet for the cows. Diets with extruded linseed (EL) have in earlier studies showed to have a lowering effect on CH_4 output from cows. Previous studies have shown correlations between CH_4 output and fatty acid (FA) profile of milkfat. EL has also shown in previous studies to influence the FA profile in milkfat. The objects of this study were to evaluate if CH_4 output can be reduced if diets are supplemented with extruded linseed, if CH_4 increases the concentration of unsaturated FA in milk fat, and the effect of EL on milk yield.

In this study a total of 177 cows from five herds were individually scanned for CH_4 output, milk fatty acid profile and milk yield. Cows were studied while fed a control diet, during the treatment diet with EL and when going back to the control diet again. EL diets had no effect on milk yield but increased the unsaturated FA in the milk. EL also decreased the CH_4 output from the cows with 22% from the first control period to the treatment diet, however there was no increase of CH_4 output between treatment period and second control period. Conclusion of this master thesis is that Extruded linseed did not affect the milk yield. EL had an effect on milk fat composition. Due to the unreliable measuring method of methane this study could not point out an obvious decrease of CH_4 due to EL.

Key words: extruded linseed, methane output, fatty acid, milk yield.

Sammanfattning

Lantbruket är en betydande källa till dagens globala utsläpp av växthusgaser. Växthusgasen metan produceras i kons våm när fodret smälts. Ett sätt att minska dessa utsläpp från lantbrukssektorn är att förändra foderstaten för boskapen. Att inkludera extruderat linfrö (EL) i foderstaten har i tidigare försök visat sig minska metanutsläppet. Tidigare försök har även visat att det finns en korrelation mellan metanutsläpp och mjölkfettets fettsyreprofil samt att EL påverkar mjölkfettets fettsyreprofil. I denna studie har totalt 177 kor från 5 gårdar medverkat för att undersöka ELs effekt på mjölkavkastning, fettsyrasamansättningen i mjölken och metanutsläpp från kor. Kornas metanutsläpp mättes totalt tre gånger under testperioden. Första gången under en kontrollfoderstat andra gången under behandlingsfoderstat med EL och slutligen under ytterligare en kontrollfoderstat. Denna studie visar att EL inte hade någon effekt på mjölkavkastningen. EL ökade de omättade fettsyrorna och minskade de mättade fettsyrorna i mjölken. EL minskade metan utsläppen per kg ECM med 22 % mellan första kontrollperioden och den följande perioden med behandlingsfoderstaten. Dock erhölls ingen förändring i metanutsläpp då korna åter fick kontrollfoderstaten. Slutsatsen av denna

studie är att EL hade en effekt på mjölkfettsyraprofilen. På grund av mätmetoden kunde inte studien visa på ett tydligt minskat metanutsläpp beroende på EL.

1. Introduction and background

Global warming is a big threat for the world today. The underlying reason is the increasing concentration of greenhouse gases (GHG) in the atmosphere. These GHG are among others carbon oxide (CO_2), CH_4 and nitrous oxide (NO2) that “traps” the heat in the atmosphere and results in global warming (Lovett et al., 2005; IPCC, 2007 (Climate Change 2007: Synthesis Report). An increased global temperature has already resulted in increased melting of ice and rising of average sea levels (IPCC 2007). Output of GHG have a natural or an anthropogenic origin (Bousquet 2006). Example of natural GHG output is volcanic eruptions and wetlands (IPCC Arturo 2007 chapter 2). Leading anthropogenic GHG output is energy supply, 25.9 %, industry, 19.4 %, forestry and deforestation, 17.4 % and on fourth place agriculture sector with 13.5 % (IPCC 2007). The global dairy sector alone stands for of 4 % of the total GHG emissions however large differences exists between different parts of the world (FAO, 2010).

There are many potential ways to decrease GHG emissions from the agricultural sectors. They involve further intensifications of livestock production by breeding, feeding and management that results in using less animals (FAO, 2006).

Feeding strategies aiming at lowering CH_4 include the use of fat supplements (as reviewed by Toprak (2015) and Knapp et. al (2013). The effects on CH_4 output vary depending on fat source (Chilliard et al, 2009).

Unsaturated fats of linseeds have shown to decrease ruminal CH_4 output depending on how the linseeds are processed (Martin et al, 2008). Fat supplements have further shown to affect the milk fat composition, and a positive correlation between a differed milk fat composition and a decreased CH_4 output (Chillard et al. 2009).

The objects of this study were to evaluate if linseed supplemented to feed could; decrease CH_4 output, increase unsaturated FA in milk fat and increase the milk yield.

Hypotheses:
1) Extruded linseed increases the proportion of unsaturated fat in milk fat and decreases the saturated fat.

2) Extruded linseed increases the milk yield.

3) Extruded linseed supplementation reduces CH_4 emissions per unit milk.

2. Literature review

2.1 Why is methane production a problem?

CH_4 is a smell and colourless gas which is produced during breakdown of organic material (Nationalencyklopedien, 2016). The production is done by microorganisms only in anaerobic environments like soil, swamps and sediment and in the rumen of cattle (Sjaastad, 2010). CH_4 is one of the major greenhouse gases and have the warming effect of 28 (±40%) (IPCC, 2013) times the warming effect of CO_2 on a 100-year time horizon (FAO, 2010: IPCC2013). Different greenhouse gases are often expressed as CO_2-equivalents (-eq), were the GHG is multiplied with its warming potential (IPCC, 2007).

There are many ways to express GHG in literature. One way, which is used by FAO, is to calculate GHG emissions per product or unit. This is also called the carbon footprint (FAO, 2010).

The average carbon footprint of global milk yield is calculated to 2.4 kg CO_2-eq per kg of fat and protein corrected milk (FPCM) (FAO 2010). However, this figure differs due to differences in production circumstances. Regions as Sub-Saharan Africa have a carbon footprint of 7.5 kg CO_2-eq per kg FPCM compared to 1-2 kg CO_2-eq per kg FPCM in industrialised regions like Sweden (FAO, 2010). The biggest contribution, 40 %, of GHG from cattle is the enteric gases (Greber et al. 2013 cited in Danielsson, 2016).

2.2 Why is methane produced in rumen?

2.2.1 Methane production from cows

Every day the rumen produces approximately 2000-4000 litres of different gases. These gases are in order of quantity: 40 % (CO_2), 30-40 % of CH_4, 5 % hydrogen (H_2) and small amounts of oxygen and nitrogen (Sjaastad et al., 2010: Mc Donald, 2011). Gases in the rumen are produced by microbes that ferments the plant carbohydrates. The gases are emitted from the cow through belching, also called eructation. A large portion of the gas is inhaled into the lungs and are then exhaled throughout the nose and mouth (Sjaastad, 2010). Not all gas produced in the rumen exits through eructation. Around 20 % of CH_4 leaves the body through feces (Sjaastad et al, 2010). Enteric emissions are however unavoidable in the process of turning grass and grains into food for humans (Sjaastad, 2010).

2.2.2 Methanogenesis

There are three classes of microbes in the rumen; bacteria, protozoa and fungi. These microbes, and their enzymes, break down dietary carbohydrates to volatile fatty acids (VFA) mainly propionate, acetate and butyrate (Figure 1).

Glucose ($C_6H_{12}O_6$) → 2 pyruvate + 4H

$C_6H_{12}O_6 + 2H_2O \rightarrow 2C2H_4O_2$ (acetate)+ $2CO_2$ + 8H
$C_6H_{12}O_6$ + 4H → 2C3H6 O_2 (propionate) + $2H_2O$
$C_6H_{12}O_6 \rightarrow$ C4H8 O_2 (butyrate)+ $2CO_2$ + 4H

Figure 1. fermentation of glucose to VFA (Boadi et al., 2004; Moss et al., 2000)

The produced VFA is used by the cow as energy and for milk synthesis. The H_2 is accumulating in the rumen and limits further fermentation of feed. However, the methanogenic bacteria use H_2 for CH_4 production (figure 2) and the excessive H_2 is removed from rumen through eructation (McAllister & Newbold, 2008).

Methanogenesis: CH_4+2 $H_2O \leftrightarrow CO_2$+8 H_2

Figure 2. Methanogenesis (Boadi et al., 2004)

There are several types of methanogens that uses other substrate than H_2 for the methanogenesis. The substrate used besides H_2 is example formate and methanol (Liu & Whitman, 2008; Lang et al., 2015). Earlier trial show that type of rumen methanogens can be a reason of variations in CH_4 output. Danielsson (2012) has shown that there might be differences in CH_4 output between individual cows.

Although CH_4 production is an efficient way to reduce H_2 in rumen the process is energy demanding. An alternate way of H_2 could bring, besides reducing CH_4 output, an improved feed efficiency. Acetate and butyrate production produce H_2, however propionate production uses H_2 (Figure1). This dietary arrangement altering the VFA toward a higher production of propionate is one way to decrease CH_4 output (Johnsson & Johnsson 1995).

2.3 Possibilities to reduce methane output through feed ratio

There are a few feed factors that have a significant effect on enteric CH_4 output. One is the positive relationship between DMI and CH_4 output is generally accepted. This is due to the increased amount of substrate in rumen that is fermented (Moss et al., 1995; review by Ramin & Huhtanen, 2013). However, cows with high milk yield generally also have a high DMI

and thus CH_4 output is diluted per unit of product. A high digestibility is correlated with a lowered CH_4 output. Also, here is an increased digestibility connected to high producing cows and gives therefore a lower CH_4 output per unit.

Type of feed affects the CH_4 output. Feed ratios with a high proportion of starch rich concentrate is found to be correlated to a lower CH_4 output and high milk yield (Johnson & Johnson 1995). Starch rich diets have shown to increase propionic acid in rumen (Penner et al., 2009). Production of propionic acid works as a H_2 sink compared to acetic and butyrate acid production as mentioned above. Other factor effecting CH_4 output is how the forage is processed. Silage compared to hay decreases the enteric CH_4. The fermentation process of silage was found to give a variation in acids in the silage. Silage high in total acids or high in acetate will lower the CH_4 production from cows (Ramin & Huhtanen, 2014).

Feed supplements in form of lipids is reviewed as one of the leading strategies to reduce CH_4 output (Beuchemin et al,.2008; Ramin & Huhtanen, 2013).

2.3.1 Dietary fat effect on methane output in general

Fats in different forms and volumes have shown to influence and decrease enteric CH_4 output. Beauchemin et al (2008), gathered data from 17 studies and found that for each supplemented percentage of fat comes a decrease of CH_4 by 5.6 %. However, diets containing higher amount that 5% fat affects the cow by a reduced appetite, decreased fermentation of feed and diminished the motility of the fore stomachs (Sjaastad, 2010). Comparing the supplemented fats, unsaturated fats had a bigger supressing effect on CH_4 output compared to other FA (Patra 2013). The C18:3 had marked inhibitory effect, compared to other FA, on CH_4 production (Patra 2013). The background for the supressing effect is explained by that unsaturated fats are saturated in rumen and act thus as a H_2 sink. However, the bio hydration does not lower the accumulated H2 as efficient as the production of CH_4 (Czwerkawski et al, 1986 quoted in Johnsson & Johnsson 1995). Van Zjiderverld (2011) concludes that saturation of dietary fat of 20 % result in a CH_4 decrease of 1.6 % output (Van Zjiderveld et al., 2011). Bacterial type in cow's rumen can influence the fats depressing effects. Gram positive methanogens was shown to be affected by fat supplementation, although no gram-negative methanogens were affected (Galbraith at al., 1971).

The underlying mechanism of lipids decreasing effects on CH_4 output is versatile and not yet fully understood. Main reasons found in literature are CH_4 inhibition through enhanced propionic acid production (Machmyller 2013; Patra 2013), bio hydration of unsaturated FA as an alternative H_2 sink (Czerkawski et. al, 1966), inhibition of protozoal production (Johnson & Johnson 1995), reduced fibre digestion, and fat to be toxic to

methanogens (Harfoot et al, 1974; review by Topak 2016). It is through the attachment of fat on the fibre and the methanogens that disfavour the methanogens (Machmüller et al., 2003). This is due to that the fat on the membrane of the methanogenic bacteria prevents nutrients to enter. However fat fed in form of free fatty acids (FFA) instead of triacyl glycol have shown to be important for the fat to be able to attach to bacteria (Harfoot et al., 1974)

2.3.2 Linseeds

Linseed *Linum usitatissimum* contains a high part of unsaturated FA (table 1) were 18:3n-3 represents more than 50% of total FA (Chilliard et. al, 2007; table 1). The ratio between omega 3 and omega 6 in linseed fat is approximately 3.6:1 (table 1). Linseed used in experiments differ in form; whole linseed, crushed linseed, linseed oil or extruded linseed (EL). General process of extrusion of linseed are done through short period of cooking followed by crushing of seeds under heat and pressure and quickly heating the crushed linseeds. In this way the content of cyanide is lowered (Martin et al., 2008).

A feeding *in vivo* study where linseed was supplemented to the feed showed a decreased CH_4 output/day. Depending on how the linseeds was processed a reduction of CH_4 output was observed varying from -12 % with crude linseed, -38 % with extruded linseed and -64 % with linseed oil (Martin et. al, 2008). Data expressed as CH_4 per fat corrected milk (FCM) showed a decrease of 23 % due to EL. However, a decrease in DMI (-3.1kg/day) and NDF intake (-6.8 % on average) due to EL was also found in that study (Martin et al. 2008).

In a study from 2011, methane output was determined in 40 cows kept in respiration chamber for five days. No effects of extruded linseed were found compared to control diet. Also, no effect was found in energy balance, digestibility of fibre (NDF) compared to control diet. It was concluded that the importance of confirmation of *in vitro* data with *in vivo* data (Van Zijderveld et al, 2011). In other studies, dietary fat (sunflower oil) was found to decrease digestion of NDF (McGinn et al., 2004).

Table1. Fatty acid composition (g/100g fatty acids) of some common fats and oils. Fatty acid composition of experimental feed Easylin 100/30 (Modified table from McDonald, 2011; Agrosom, 2015).

Fatty acid	Rapeseed	Soya bean	Red clover	Ryegrass	Linseed	Butterfat	Easylin 100/30
16:0	4	10	15.4	12	6	31	4
18:0	1	4	2.3	2	3	10	3.7
20:0	1	Tr	-	-	-	-	-
22:0	Tr	Tr	-	-	-	-	-

16:1	2	Tr	0.1	2	-	2	-
18:1n-9	54	25	-	15	17	23	18
18:2n-6	23	54	20.8	68	13	2	16
18:3n-3	10	7	59.5	-	55	<1	58

2.4. Effects of linseed feed ration on milk yield and fat composition

2.4.1 Milk yield

There are conflicting results in literature of the effect of linseeds on milk yield. Supplementary fat was found to increase milk yield (Patra 2013), linseed oil and extruded linseeds (EL) have been found to increase kg milk yield per day (Bu et al. 2007; Zaucht et. al, 2010). Other results show a decrease in the milk yield (Chilliard et al 2009; Gunthier et al 2005) Other studies show no effect on milk yield (Beuchermin 2009).

In an analysis where many studies with different fat supplements were used, an increase of milk yield was found with increasing fat supplement. However, a decrease of milk yield was found with fat supplements over 3.9-6 % (Amlan K. Patra, 2013). In a study where 5.7 % fat of dietary DM was used a drop of 20 % was found for cows feed EL compared to control group (Martin et al. 2008).

2.5 Milk fat composition

2.5.1 Lipids

Lipids have a crucial role in the body of animals. Lipids are components in the cell membrane, absorber of some vitamins, energy storage and are high in energy and contribute with energy for the animal. Lipids are divided into 4 main groups: FA, glycerides, nonglyceride lipids and complex lipids (Denniston et. al, 2010).

Main constituents of the milk fat fraction are triacyl glycols (figure 3), where glycerol bind three FA (McDonald et al 2011). There are more than 500 different FA in milk (Patton & Jensen 1975).

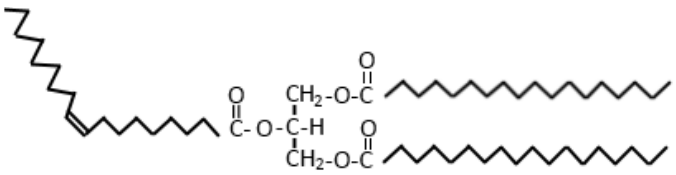

Figure 3. Modified picture from Harvey et al., 2011. A triacyl glycol with single and one double bound.

Short-chain FA consist of a carbon chain of 4 to 8 carbons, medium-chain FA consist of 10 to 14 carbons and long-chain FA contains 16 or more carbons (Bauman & Griinari, 2003). The length and saturation have an impact on the melting point of the FA. It increases with increased number of carbon as well as with saturation level (Deniston et al,). Milk contains mainly medium-chain FA with parts of 16 carbons and the milk FA are foremost saturated. Part of C18:3 in cowmilk is 0.2 % (Lindmark & Mårtensson, 2012).

Linoleic acid, C 18:2, and α-linolenic acid, C 18:3, are essential since they cannot be synthesized by the animal itself (Harvey & Ferrier, 2011). Instead these FA need to be ingested with the feed the feed. Linseed based feed consists of a high part of essential FA as well as grass (table 1).

2.5.2 Dietary fat effect on milk fat composition

Lipids consumed by the cow differ in structure depending of feed source. Lipids in plants often consist of glycolipids where fat in grass have high amounts of polyunsaturated FA (PUFA) (Dewhurst et al 2003). Fat in cell membrane consists of phospholipids and fat stored in the seed consists of triglycerides. The fats in oil seed plants also consists of PUFA. Lipids in dietary fat are degraded by ruminal microbes in rumen through hydrolysis. Freed FA high in PUFA in rumen are bio hydrogenated by ruminal microorganisms where hydrogen is added to the PUFA in a saturation process (McDonald et al, 2011). Almost all linoleic acid (C18:2) and linolenic acid (C18:3) are saturated and converted into stearic acid (C18:0) in rumen (rewired by Doreau & Ferlay 1994). However not all FA is completely saturated but intermediates in form of mono unsaturated FA (MUFA) and PUFA are created. The saturated and unsaturated FA are passed on to duodenum where it is extracted to the blood. The fat is transported in chylomicrons and very-low-density lipoproteins (VLDL) (Chilliard et. al, 2007) in the blood to the udder where it is used for milk fat synthesis (Gonthier et al, 2005).

The main part of VFA is absorbed across the ruminal epithelium and released into the portal blood. Propionate is then converted to glucose in the liver. The main fraction of butyrate is metabolised to the ketone body β-hydroxybutyrate in the ruminal epithelium and released into the portal blood (Sjaastad et al, 2010). Acetate and β-hydroxybutyrate are precursors for *de novo* synthesis of milk fat in the mammary gland. Roughly half of milk FA are produced *de novo* and acetate and β-hydroxybutyrate are the principal precursors. However, the relative contribution from the *de novo* synthesis is generally lower in the early stage of lactation when the cow mobilises long FA from the adipose tissue. The other 50 % of milk fat is produced from preformed FA. Mobilised FA also contributes to the preformed FA. The FA are, in the alveolar cell, esterified into triacyl glycol and transported into the milk by fat globuli

(Sjaastad et al, 2010). Short and medium-chain FA originates from *de novo* synthesis and long chain FA originates from preformed FA. FA with C 16 derives from both *de novo* and preformed FA (Bauman & Griinari, 2003; Lindmark Månsson et al. 2006).

Linseed has, in earlier studies, shown to have an impact on milk fat composition. A diet with linseed increases the unsaturated FA (UFA), C18:3, in the milk fat and decreases the saturated FA (SFA), C16:0, acids in the milk (Zachut et al 2010; Chilliard et al., 2009). At the same time negative correlations (r=-0.86 to -0.90) have been found between increased CH_4 output and elevated levels of unsaturated FA in milk fat (Chilliard et. al, 2009). The elevation of UFA in milk fat is explained by literature as an increased amount of FA passing to the small intestine. The underlying mechanism is the higher uptake of UFA from linseeds in the small intestine (Chilliard et al., 2009). Supporting results was found in a study were EL in diet resulted in decreased levels of medium chain FA and increased levels of long chain FA in plasma (Gonthier et al 2005). However, the milk fat increase of C18:3 was found to be negatively correlated with milk fat percentage and milk yield (Zachut et al., 2010). A risk with diets supplemented with PUFA is a low milkfat syndrome where a heavy decrease of milk fat can be seen. Highest drop in milk fat depression is seen with diets low in fibre and with supplemented PUFA (Griinari et al., 1997).

2.6 How is methane measured from cows?

2.6.1 Methane measurements

CH_4 output is represented as a mass of gas that is released by the cow per unit of time. The released amount of gas varies in time depending on the cows belching. This means that the measuring device should be able to measure both factors (Larios et al., 2016).

CH_4 output from cows can be measured in different ways, both *in vivo* and *in vitro*. The most accurate *in vivo* technique for CH_4 output “the golden standard” is the respiration chamber. The animal is placed in a closed chamber where exhaust air with its gas is sampled with a decided time cycle (Changunda et al., 2013: Ramin & Huhtanen, 2014: Hristov et al., 2015). This method is often used for evaluation of other CH_4 measuring methods (Johnsson & Johnsson 1995; Danielsson et al., 2017).

A widely used technique for measuring methane is the tracer gas technique with sulphur hexafluoride. A permeation tube with sulphur hexafluoride is placed in cows rumen releasing the gas successively with a known releasing rate. Greenhouse gases and sulphur hexafluoride exhaled from the cow is sucked into hoses and transported into a stainless-steel collection sphere. The ratio between sulphur hexabromide and CH4 is determined by a gas chromatograph. Before the

measurements begins the animals need to be trained to wear the sampling equipment on the head. The technique enables measurement on a large number of animals on farm as well as many times/day/animal (Hristov et al., 2015; Boadi et al., 2001).

Other *in vivo* measuring techniques are the GreenFeed-system which is based on an automatic head-chamber system measuring the exhaust air from the cow's muzzle. Here the individual cow is sampled for CH_4 and CO2, during feeding in a portal feed trough. Measurements are done by infra-red sensor and optionally with tracer gas CH4 ratio. The sampling is done during a period of days or weeks resulting in a calculation of CH_4 output per day. Due to many measurements per day this method catches the fluctuations of methane production over the day as well as numerous animals can be measured measurement compared to the respiration camber technique. Measurement of background greenhouse gas can also be made. Tag reader register the animal and data of that specific animal, ex. milk yield, are transferred to the analysing station. Before the measuring begins the animals need to be trained to eat from the portal feed trought (Hristov et al., 2015; Zimmerman et al., 2012).

There is also *In vitro* methods were CH_4 is measured from containers of feed during oxygen fee environment. Indirect estimations of CH_4 exist were correlations with DMI is used for calculations of CH_4 output (Ramin &Huhtanen, 2012 & 2013).

CH_4 output can also be estimated by laser methane detection technique (LMD) which is used in this study. The technique was developed for measurement of CH_4 leaks from gas pipes and started to be used in trials of CH_4 measurements of cows by Changunda et al. in 2009. The LMD measures the CH_4 in the exhaust air from the cow with its laser diode. The method relies on the fact that CH_4 absorbs a specific wavelength of infrared rays. The LMD measures the diffused light back and measures the absorption of the light. The LMD measures the average CH_4 gas density between the detector and the target. One measurement is made every 0.5 second over a duration of a few minutes.

CH_4 output is reported as the product of concentration of CH_4 cloud (measured in parts per million) and path length (measured in meters). Best application is outdoors in strong sunshine. The LMD calibrates itself automatically each time it is turned on (Crowcon production voucher, 2013). With this type of measurement it is possible to measure many cows on farm. The accuracy is however lower compared to respiration chamber which was found in a study of Ricci et al., 2014. In the study, measurements were done on 72 steers, after feeding, with one 4-minute measurement/ animal /day repeated over 3 days. The week after the CH_4 output was measured in a respiration chamber. The CH_4 sample/cow was corrected for background CH_4 measurement where data lower than the lowest measurement was subtracted from then deleted. There was a

strong linear relationship (R^2=0.86) between LDM measurement and the respiration chamber (Ricci et al., 2014) and the relationship of r=0.47 (p=0.001) for dairy cows was found by Changunda et al., 2013.

2.6.2 Influencing factors of methane measuring

CH_4 output was found to be affected by lactation status (lactating or dry cows) and the cow's activity. Depending on the activity of the cow, the pattern of eructation differs. Cows have the highest CH_4 output during rumination, lower CH_4 output during feeding and lowest while staying idle. These facts can influence the results of CH_4 measurements if not corrected for in the experiment (Changunda, 2009).

Time for inurement of cows on the new diet can affect the measurements. In an earlier study an 8 days' time of inurement of linolenic acid in diet appears to be required for the CH_4 measurements to stabilise. A 12 day of re-establish initial CH_4 values was needed for the cows after finished test diet (Zwerkawski, 1966).

3. Materials and Methods

This study was initiated by the feed company Agrosom and was executed as part of an EU-project named "Rolling out of an innovative enteric methane emissions measurement method for cattle" (ECO-methane). The project was performed within the EU-programme "Competitive and innovation frame work programme" (CIP). The design of this project is almost similar to that of the ECO-methane project (ECO-methane, 2014) however there were some modifications of the material and method of this work. These are mentioned under measurements and sampling.

3.1 Experimental design and herds

The study was executed on five conventional dairy herds in Sweden. Three of the participating herds were located in Kalmar län, one in Halland and one in Uppland. Descriptive data of participating herds are presented in table 2.

The study was divided into three periods (Per) where Per 1 was control, Per 2 was treatment, and Per 3 was control. This is also called a switch-back design. During Per 1 all cows were fed control diets, equal to the feeding strategy on the farm. During Per 2 the diets were supplemented with the test feed EL. The EL was fed as an ingredient of commercial compound feed in the ration (table 2 and 3). The goal was to feed each cow 1000 grams of EL per day. Per 3 was a second control period were EL was excluded from the feed ratios and cows returned to their normal feed ratios.

Table 2. Descriptive data of participating herds. PMR=partly mixed ration. TMR= total mixed ration.

Herd:	Breed	Milking system	Feeding system	Feeding system for Extruded Linseed
1	Holstein	AMS	PMR, -feeding wagon	Concentrate in AMS
2	SRB and Holstein	AMS	-PMR, transport band. -Feeding stations.	Concentrate in feeding stations
3	SRB	Milking parlour	TMR two different mixes	Concentrate in mixing wagon
4	SRB, Holstein and crossbreed	AMS	PMR -feeding wagon	Non pelleted in Mixing wagon
5	Holstein	AMS	-PMR -feeding wagon. -feeding stations	Concentrate in feeding station

3.1.1 Treatment feed

The treatment feed containing linseed, Easylin 100/30, was produced in Bremen, Germany. Easylin 100/30 contained proximately 60 % extruded linseeds and 40% rape seed meal. Amount of crude fat in Easylin was 277 g per kg dm. Total fatty acids in Easylin 100/30 was 887 g/kg crude fat. The amount of linolenic acid was 53.2 g/100g (Agrosom, 2016; NorFor, 2018).

The feed rations at the herds were not corrected for different DMI, solubility of feed, parts of carbohydrate, silage process or amount of fat intake.

3.1.2 Selected cows

A test group of 20 cows was created for each herd and period. Criteria for selection for the cows were; days in milk (DIM) within 30-180 days, a health record for at least 2 months back, 25% of the cows was primiparous and 75% were multiparous. These criteria were in accordance with the ECO-methane project (2014). The final selection of the 20 individuals was done at the farms. A list of all potential cows from the herd within the qualifications was created. The first 20 cows from the list that we found in the feeding area was selected to be measured for CH_4 and included in the test group.

3.2 Measurements and sampling

The measurements started in December 2014 and ended in May 2015. None of the cows were on pasture during the trial. The following measurements were done once in every period at every herd as in ECO-project; one direct CH_4 measurement of LMD on the selected cows, one questionnaire per herd directed to the farmer, collection of data from milk sampling and of information of sick and treated cows.

Additional measurements outside the ECO-project sampling, only for this master thesis, were; one-day feeding control per herd and period. For that I collected data on feed consumption and milk yield of all lactating cows for the day we visited the herd and the number of cows in the lactating group. The amount of consumed feed took into account the amount of roughage and concentrate fed and leftover feed from feeding bunker. The leftovers were weighed or estimated by the farmer. Roughage feed sample were taken at the herds the same day as I visited the herd roughage for dry matter measurements. Other analyses on nutrient composition of the feeds were collected from the farmers' own feed analyses. The one-day feeding control was calculated in the computer tool IndividRAM (Växa Sverige, Eskilstuna, Sweden) which is based on the feed evaluation system NorFor (Volden, 2011).

3.2.1 Analyses on feed and milk

Roughage feed samples were analysed for DM (at 60 ºC) at the SLU feed laboratory (Uppsala, Sweden). This sample was used for the one-day feeding control on herd level. The milk samples, collected monthly according to the official Swedish milking recording scheme, were also used for analyses for FA composition. The farmer performed the milk recording for the herd during normal procedures and labelled the sample tubes with cow number for the selected cows (same cows that was measured for CH_4). The labelled tubes were singled out at the Eurofins Steins laboratory (Jönköping, Sweden) and milk samples were analysed on FA with a Fourier Transform Infra-red (FTIR) instrument. Individual milk samples were analysed for the FA: C16:0, C18:0, C18:1, MUFA, PUFA, sum of SFA and sum of UFA, sum of all FA (FAtot) and protein (FApr). Deviation for the FTIR method vary for SFA from -0.07 % to + 0.07 % and for UFA from -0.14 % to +0.12 % (Kaylegian et al., 2009).

Data on milk yield (MY), energy corrected milk (ECM), fat content (MF) and protein content (MP) was collected both on individual cows and on herd level in each period. These data were provided by Växa Sverige through the Swedish milking recording scheme (Eskilstuna Sverige).

The milk fat and protein were analysed by the CombiFoss 6000 equipment (Eurofins Steins, Jönköping, Sweden). According to Nils-Erik Larsson (personal communication), corrections was done for milk fat and protein

content using standard procedures. The corrections were done for herds with milk sampling once per day for the variables; breed, parity, DIM and ratio between the milk recording for the sampling and the milk recording of the complete day. For herds where milk recording, and sampling was done twice per day no corrections were done.

3.2.2 Methane measurement

Direct CH_4 measurement was done with LMD from the trademark Crowcon, production name TGE laser methane mini-Green (LMm-G) (Crowcon production voucher, 2013). The LMD green laser point was focused on the cow's nose during the measurement. Each individual measurement lasted between 3 and 4 minutes resulting in a minimum of 360 individual measurements per cow where one new measurement was done every 0.5 second (figure 3). The LMD measurements was performed by a well-trained person from France.

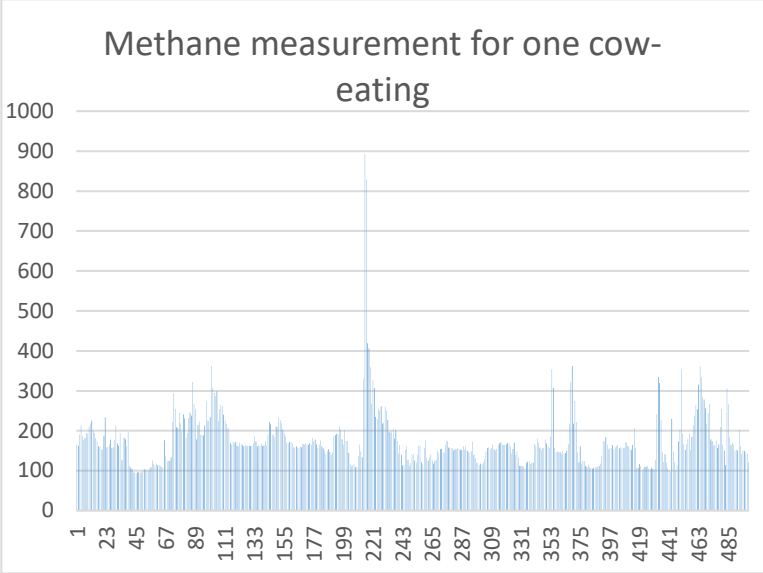

Figure 3. An example of a CH_4 measurement from one individual cow. The x-axis shows the total of 499 measuring points during 250 seconds. The y-axis shows CH_4 output in ppm, when a laser methane detector is pointed towards a cow's nostrils.

The person was standing on the feeding bunker at approximately two meters distance from the cow while he directed the LMD device towards the cow´s nostrils. CH_4 output was measured while the cow was eating. Methane measurements of concentration was specified as parts per million. Each methane measurement lasted for 180 seconds and a total of 360 methane measurements was mace under this time for each cow. A mean of these 360 measurements was calculated for each cow. The individual measured values were then divided by the individual ECM, from milk recording, creating an individual momentary methane output ppm per kg ECM.

3.2.3. Indirect methane estimations with Visiolait

Indirect CH_4 estimation was done on herd level by a feeding ratio tool called Visiolait which estimated the daily CH_4 output in grams per litre of milk of each herd. A patented equation (Chesneau, 2008) was used for the estimate. The equation included information of FA profile, amount of milk yield from tank milk kg per cow and day and DIM of herd. The CH_4 values were used for comparison through correlations with the direct CH_4 measurement.

3.2.4 Indirect methane estimations by NorFor

Indirect CH_4 estimations on herd level were done by NorFor based one-day feeding control calculations. The CH_4 value was estimated on herd level by grams per cow and day. The intention was to create correlations between NorFor estimations and mean of direct CH_4 LMD measurement per kg ECM and CH_4 estimation of Visiolait.

$$CH4 = \frac{1.39 * DMI - 0.091 * FA}{55.65} * 1000$$

Figure 4. Estimation of CH_4 for dairy cows where CH_4=methane g per day, DMI=total feed intake kg DM per day, FA=fatty acid in the diet grams per kg DM, number 55.65 converts CH_4 energy MJ to gram (Nielsen et al. 2013).

3.3 Statistical calculations

An average of DIM was calculated for selected cows in the test groups on each farm and period. Collected data for these cows was used in statistical calculations in Minitab 17.0. Corrections for DIM, parity, and health and activity were included in the experimental design. For correction of data see statistical calculations further down.

The statistical model ANOVA: General Linear model (GLM) was used to calculate the statistical difference between herds (Herd) and between periods (Per) for the variables DIM, ECM, MY, MF, MP, FATtot, FApr, C16:0, and C18:0, C18:1, MUFA, PUFA, SFA, UFA, CH_4 of LMD, Ch_4 of LMD per kg ECM and amount of EL (table 6). Variables that potentially could have a significant effect was included in the calculation. If there was no significant effect, the variable was withdrawn from the calculation, such a variable was Herd. Herd and Per were fixed in the model. A pairwise comparison with a confidential level of 95% was used to locate the significant difference between specific periods (table 6). Means of variables were also generated through the pairwise comparisons with confidential level of 95%. The mean values were then used to create bar graphs, in Excel, for each variable. Descriptive charts for DIM, EL and number of cows was done in Minitab or excel. No correlations lower than 0.5 are reported in this paper unless required for special reasons.

For the non-significant Herd values, a new GLM was done where Herd was erased from the model.

Correlation between LMD measurements and CH_4 from Visiolait and CH_4 from NorFor was done.

4. Results

4.1 Descriptive data

Number of cows in the testgroups (figure 5), DIM, daily DMI did not differ significantly between periods (p=0.34) or between herds (exception of herd 5 with lower daily DMI compared to the other herds) (table 6; table 3).

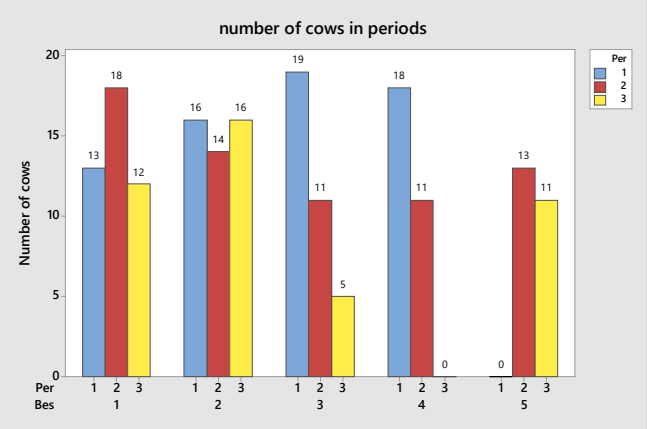

Figure 5. Number of cows at each herd and for every period in the final test group.

Ingestion of extruded linseed differed between herds. However, no statistic calculation of this was made (table 3). Results from one day feeding control are shown in tables 3 and 4.

Table 3. Average daily feed intake per cow on herd level and chemical composition of diets in the five herds. Amounts of feed and composition were provided from the herdsmen. Amount of linseed was calculated from the amount of Easylin 100/30 supplied in the diets during period 2.

	Period 1	Period 2	Period 3
Herd 1			
No. of cows in test group	13	18	12
No. of lactating cows	517	519	522
Total dry matter intake, kg DM	22.4	23.0	21.4
Roughage intake, kg DM	13.5	13.4	12.7
Concentrate intake, kg DM	8.9	9.6	8.7
Extruded linseed g feed	0	867	0
NDF, g/kg DM	350	351	357
Crude protein, g/kg DM	171	160	180
Crude fat, g/kg DM	46	47	49
Herd 2			
No. of cows in test group	16	14	16

No. of lactating cows	138	137	136
Total dry matter intake, kg DM	23.6	20.8	18.8
Roughage intake, kg DM	12.6	11.3	8.8
Concentrate intake, kg DM	11.0	9.5	10.0
Expelled linseed, g feed	0	1065	0
NDF, g/kg DM	353	340	332
Crude protein, g/kg DM	172	177	189
Crude fat, g/kg DM	46	39	48
Herd 3			
No. of cows in test group	19	11	5
No. of lactationg cows	188	185	191
Total dry matter intake, kg DM	27.4	29.6	27.8
Roughage intake, kg DM	12.1	13.6	11.5
Concentrate intake, kg DM	15.3	16.0	16.3
Expelled linseed, g feed	0	924	0
NDF, g/kg DM	389	328	360
Crude protein, g/kg DM	197	185	182
Crude fat, g/kg DM	58	55	53
Herd 4			
No. of cows in test group	18	11	-
No. of lactating cows	138	135	-
Total dry matter intake, kg DM	21.5	25.3	
Roughage intake, kg DM	14.1	11.4	
Concentrate intake, kg DM	7.4	13.9	
Expelled linseed, g feed	0	1000	0
NDF, g/kg DM	286	315	
Crude protein, g/kg DM	195	184	
Crude fat, g/kg DM	47	52	
Herd 5			
No. of cows in test group		13	11
No. of lactating cows	-	375	375
Total dry matter intake, kg DM		23.8	28.1
Roughage intake, kg DM		13.9	18.6
Concentrate intake, kg DM		9.9	9.6
Expelled linseed, g feed	0	1126	0
NDF, g/kg DM		347	343
Crude protein, g/kg DM		190	188
Crude fat, g/kg DM		44	42

Table 4. Milk yield and milk composition of all lactating cows of the five herds. Data was provided by Växa Sverige

	Period 1	Period 2	Period 3
Herd 1			
No. of cows	597	613	615
Days in milk	150	206	234
Day of sampling from previous period	-	35	14
Milk yield, kg	25.3	24.5	24.7
Energy corrected milk, kg	25	24.3	24.3

Milk fat, %	3.8	3.9	3.9
Milk protein, %	3.5	3.5	3.4
Herd 2			
No. of cows	138	138	137
Days in milk	127	223	244
Day of sampling from previous period	-	70	3
Milk yield, kg	29.9	29.6	31.2
Energy corrected milk, kg	30.5	31.0	32.1
Milk fat, %	4.1	4.2	4.1
Milk protein, %	3.6	3.7	3.6
Herd 3			
No. of cows	194	185	187
Days in milk	186	249	222
Day of sampling from previous period	-	49	7
Milk yield, kg	26.1	27.2	(26.0)
Energy corrected milk, kg	27.8	28.6	(27.7)
Milk fat, %	4.3	4.3	(4.1)
Milk protein, %	3.7	3.6	(3.6)
Herd 4			
No. of cows	135	130	-
Days in milk	228	255	-
Day of sampling from previous period	-	24	-
Milk yield, kg	30.7	32.0	-
Energy corrected milk, kg	30.8	31.7	-
Milk fat, %	4.0	4.0	-
Milk protein, %	3.4	3.4	-
Herd 5			
No. of cows	-	375	413
Days in milk	-	208	248
Day of sampling from previous period	-	28	24
Milk yield, kg	-	31.5	29.4
Energy corrected milk, kg	-	32.3	30.6
Milk fat, %	-	4.1	4.3
Milk protein, %	-	3.6	3.5

4.2 Milk yield and milk composition

4.2.1 Milk yield

Calculations based on data from cows in test groups showed that Milk Yield (P=0.55) and ECM (P=0.24) did not differ significantly between treatment period and control periods (table 6).

4.2.2 Protein

Protein content in milk, MP, increased significantly (P=0.006) from 3.4 in Per 1 to 3.5 in Per 2. Protein, FApr, did not increase significantly.

4.2.3 Unsaturated fatty acids in milk

PUFA, increased significantly (P<0.0001) from period 1, 0.13g per 100g, to period 2, 0.15g per 100g, and decreased mean numerically from period 2 to period 3, 0.13g per 100g (Figure 6; table 6).

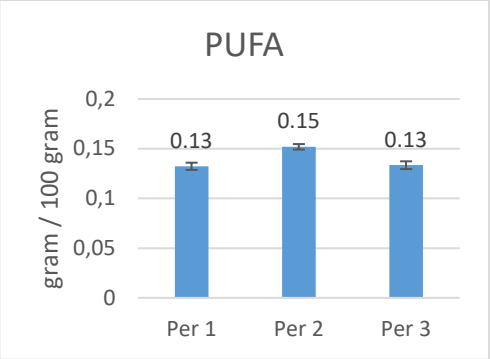

Figure 6. Mean and their SE of PUFA for period1, 2 and 3.

Herd 1 had significantly (P=0.008), higher mean of unsaturated acids compared to herd 3 and 4. There was a significant (P=0.005) decrease of mean of UFA from period 2, 1.15 g per 100g, to period 3, 1.03 g per 100g. There was a numerical increase of unsaturated acids from Per 1 to Per 2, however not significant (Figure 7; table 6).

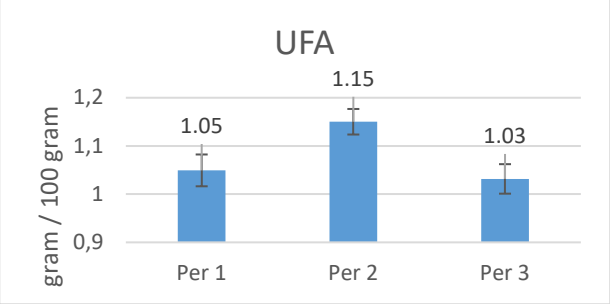

Figure 7. Mean and SE Mean of UFA for period.

Oleic acid, C 18:1 (P=0.36) and MUFA (P=0.83) did not differ significantly between periods.

4.2.4 Saturated milk fatty acids

Saturated FA did not differ significantly between periods (P=0.64) or between herds (P=0.28) (table 6). Palmitic acid, C16:0, tended to decrease (P=0.055) from period 1 to period 2. A small numerically increase was seen in period 3 (figure 8; table 3).

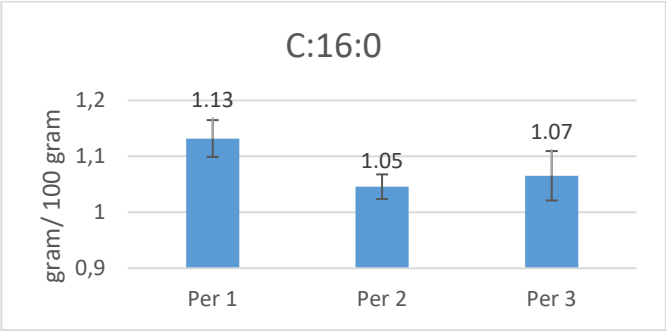

Figure 8. Mean and SE Mean for palmitic acid (C16:0) in periods.

4.3 Methane output

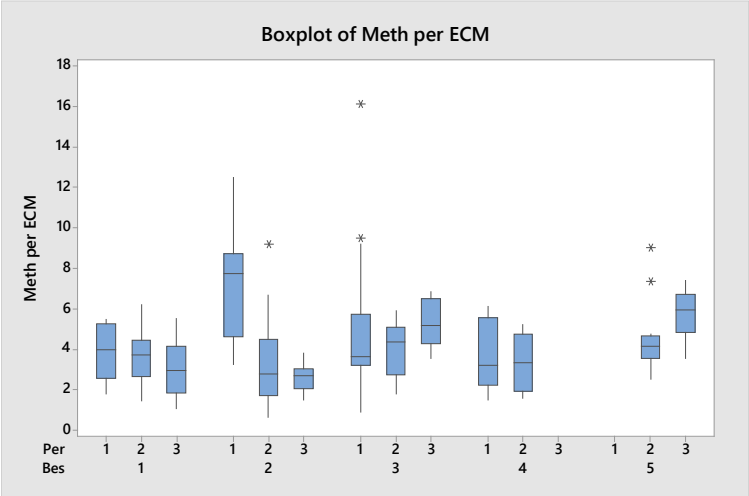

Figure 9. Boxplot of CH_4 measurements by LMD on individual cows (ppm per kg ECM) for all herds (Bes) and periods (Per). * and ** indicate significance level on Per within Bes (* P<0.05; ** P<0.01)

Mean of momentary CH_4 ppm per daily ECM yield differed significantly (P< 0.0001) between period 1 and 2, between period 1 and 3. Mean of CH_4 per ECM decreased significantly (p<0.001) from Period 1, 4.9, to period 2, 3.8 which corresponds to a reduction of 22 %. Mean of CH_4 per ECM increased, however only numerically, from Per 2 to Per 3, 3.8. (Figure 10).

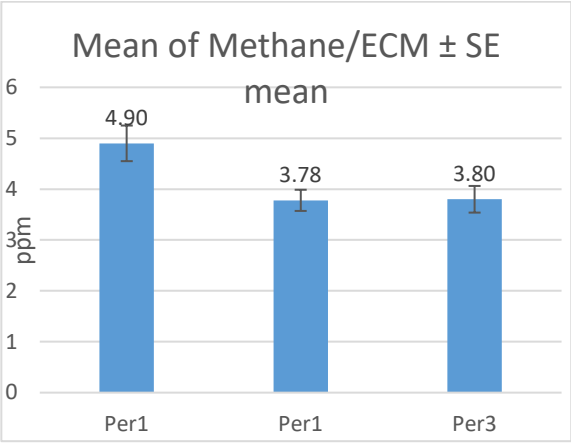

Figure 10. Mean and SE Mean of CH_4 ppm per kg ECM for periods.

4.3.1 Estimated methane value through fatty acid analyses, Visiolait

There was no significant (p=0.16) relationship (r= 0.46) between LMD measured CH_4 ppm production and Visiolait estimated CH_4 production g per litre milk. Data from Per 1 and Per 2 was used (table 5).

4.3.2 Estimated methane value through NorFor

There was no significant (p= 0.53) correlation (r= 0.53) between measured CH_4 by LMD and the estimated CH_4, production gram per kg ECM, done by NorFor one-day feed control. Data from Per 1 and Per 2 was used (Table 5).

Table 5. CH_4 data for the five herds. CH_4 measurement of laser methane detector (LMD) and CH_4 estimation by Visiolait (estimated through fatty acid composition) are based on cows in the test group, while CH_4 estimation by NorFor (calculated in a one-day feeding control) is based on all lactating cows.* shows missing values

	Per1	Per2
Herd 1		
CH_4 LMD, ppm per ECM	3.80	3.70
Visiolait, g per litre milk	*	15.0
NorFor g per kg ECM	505	520
Herd 2		
CH_4 ppm per ECM	7.17	3.41
Visiolait, g per litre milk	16.3	16.0
NorFor g per kg ECM	535	476
Herd3		
CH_4 ppm per ECM	4.82	3.99
Visiolait g per litre milk	18.3	16.1
NorFor g per kg ECM	613	674
Herd 4		
CH_4 ppm per ECM	3.69	3.30
Visiolait g per litre milk	*	15.3
NorFor g per kg ECM	487	568
Herd 5		
CH_4 ppm per ECM	*	4.53

Visiolait g per litre milk	*	16
NorFor g per kg ECM	*	549

Table 6. Effects of EL on milk yield, milk composition and CH_4 output for herd and Period (Per). Mean from Basic statistics. LSmean for Per 1, 2 and 3 is created from individual data from the same period. When no p-value under herd, the variable herd was not used in the statistical calculation due to that it was not significant.

Variable	Periods			P-value		Significance level		
	1	2	3	Herd	Per	Per 1-2	Per 2-3	Per 1-3
DIM days	103	99	100	0.000	0.993			
MY_1 kg per day	37	39	39	0.001	0.553			
MF_1, %	3.9	3.9	3.9	0.001	0.657			
MP_1, %	3.4	3.5	3.4	0.000	0.006	sig		
ECM_1 kg/d	36	39	39	0.018	0.237			
$FAtot_2$ g per 100g	3.7	3.8	3.6	-	0.363			
$FApr_2$ g per 100g	3.3	3.4	3.3	-	0.098			
$C16:0_2$ g per 100g	1.1	1.0	1.0	-	0.055			
$C18:0_2$ g per 100g	0.37	0.40	0.35	-	0.003	sig	sig	
$C18:1_2$ g per 100g	0.65	0.67	0.63	0.026	0.356			
$MUFA_2$ g per 100g	0.96	0.98	0.96	-	0.833			
$PUFA_2$ g per 100g	0.13	0.15	0.13	0.004	0.000	sig		
SFA_2 g per 100g	2.5	2.4	2.4	-	0.641			
UFA_2 g per 100g	1.0	1.2	1.0	0.008	0.005		sig	
LMD, ppm	170	140	140	0.000	0.001	sig		Sig
LMD, ppm per kg ECM	4.9	3.8	3.8	0.001	0.000	sig		Sig

Sig= significant. DIM=days in milk. MY= milk yield. MF= milk fat. MP= milk protein. ECM= energy corrected milk. FATtot= Total fatty acids in milk. C16_0= palmitic acid. C18_0= saturated fatty acid with 18 carbons. C18_1=unsaturated fatty acid with one double bound. MUFA= monounsaturated acids. PUFA= long chain polyunsaturated fatty acids. SFA=saturated fatty acids. UFA= unsaturated fatty acids. LMD= Laser Methane Detector. Measurements of methane output of concentration measured in parts of million. LMD/ECM= Mean CH_4 output per ECM. 3 Minutes= CH_4 measurement, duration of 3 minutes. 1=data from official Swedish milking recording scheme was distributed via VäxaSverige. 2= data from FTIR was distributed via Agrosom.

5. Discussion

5.1 Effect of Extruded linseed

A lowered DMI due to an intake of EL (Martin et al., 2008) was not found in this study. Due to that the quantity of EL used in this study, from 2.4 to 4.1 % of DMI per herd, to previous studies where a quantity of 7.9 % (Zachut, 2010), 12.6% (Gonthier, 2005) 15% (Chilliard, 2009) 16.6 % (Akrim, 2007) was used, the percentage of EL in this study can be considered low. The unchanged DMI in this study concludes that a quantity of EL up to 4.1 % will not affect the DMI.

5.1.1 Milk Yield

EL did not increase MY or ECM in the present study. This is in accordance with other studies Chilliard *et al*, 2009; Martin *et al*, 2008; Petit Côrtes 2010.The hypothesis that EL increases MY could not be confirmed.

Chilliard et al. (2009) observed a decrease in MY due to a high amount of EL.
The amount of fed EL in the present experiment was lower than other studies, thus comparisons with other studies must be taken with caution.

5.1.2 Milk fat

In this study EL had no significant effect on milk fat yield between periods (table 6). The result corresponds with former studies with feed linseeds (Gonthier *et al*, 2005: Beuchemin *et al* 2009). However other studies with EL in feed, showed a decreased fat yield in milk. This was thought to be a result of a high fat supplement of 5% of DMI that resulted in a lower digestibility of feed (Martin et al, 2008). This concludes that a quantity of up to 4.1 % of EL will not effect milk fat yield.

EL did increase content of PUFA in milk between Per 1 and per 2 (table 6). Due to the high deviation of measurement of UFA (Kaylegian et al., 2009), it must be emphasised that the FTIR method is not accurate to predict FA or groups of such acids with low concentrations. The concentration of PUFA in milk fat is generally less than 5%. Never the less the present result is in accordance with other studies (Petit & Côrtes, 2010; Gonthier et al, 2005; Zaucht et al., 2010) where infrared analyse was used for fat samples (Gonthier et al., 2005; Zaucht et al., 2010). Due to that both period and herd had a significant effect on PUFA, the treatment effect that is seen between periods can be due to the farm effect. EL also tended (P=0.055) to decrease saturated FA in form of C 16:0 from Per 1 to Per 2 and increased numerically from Per 2 to Per 3 (figure 8). This is not in accordance with other studies were the decrease during EL-feed ratio have been significant. Also, the increase after finished treatment of C 16:0 has been significant in other studies. The fact that sampling was done close after transition from Per 2 to per 3 with the

result that the cows only consumed the control feed for a few days is probably the reason for the results.

5.1.3 Methane output

The result of the present study showing a decreased CH_4 output from Per 1 to Per 2 indicates that EL might have a lowering effect on CH_4 production. The result is in accordance with results of other studies of Martin et al. (2008) and Beauchemin et al. (2009). The reduction is difficult to compare with other studies of CH_4 output since the CH_4 measurements in this trial were only performed during a single activity. However, methane measurements of cows during feeding was made by Changunda 2013 with a result of 284 ppm. Also, a respiration camber measurement of methane was done with the result of a mean of 356.3 ppm. Measurements of this study, 140 and 170 ppm, is comparatively low to Changundas measurements. Due to the big divergent levels in this study the data measurements cannot be considered to be accurate or be trustworthy. The reason for this difference could be environmental factors at the measuring occasion such as low light, wind draught or differences in measuring device. The small numeric increase of CH_4 from Per 2 to per 3 was not expected result and is not in accordance with other studies. The short time between the end of Per 2 and sampling in Per 3 (table 4 and 6) could explain a lingering treatment effect in Per 3. This is also in accordance with a study were the adaptation time for cows on new feed was 21 days (Gonthier, 2005). The reason for the short adaptation time in Per 3 was the upcoming grazing season. CH4 level of LMD for farm two, period one is high, compared to the other farms and periods (table 5). The reason for this high value could be miss calculations or cows muzels to close to each other during measurements.

The non-significant relationship between CH_4 measurement by LMD and Visiolait estimations, CH_4 and NorFor estimations could depend on different factors. The mean value of the CH_4 measurements, LMD, done in this study represents a “snapshot” of CH_4 output for the cows in the test group during the specific activity of eating and should not be mistaken for a value of CH_4 production per day. Visiolait is however an estimation of CH_4 g per litres of milk and NorFor is an estimation expressed in gram per kg ECM. These both estimations are made on herdlevel. Therefore, the CH_4 estimations are not directly comparable. Although despite the differences in units the CH_4 values should have a similar variation between the periods due to the treatment feed. The lack of a significant correlation could be the result of a non-correct CH_4 measurements or CH_4 estimations or both.

5.2 Deviations from experimental design

5.2.1 Herds and experimental design

Originally 7 herds had accepted to participate in the study however two herds decided to leave the project at an early stage.

5.2.2 Number of cows

The number of cows in the test groups (figure 5) was reduced due to mastitis and other illnesses. Individual cows were also excluded from the group since EL- feed was stopped for individual cows before measurement, incomplete FA analysis with some exceptions under divergent data.

The goal was to achieve similar average stage of lactation among cows representing the different herds, thus cows with too high or too low DIM was extracted from the test group. This resulted in smaller test groups than the 20 that was originally planned for. The results of this study should thus be considered with caution.

5.2.3 Sampling

Due to the specific release pattern of CH_4 from cows it is likely that the LMD- technique, with its frequent sampling every 0.5 second, can capture the levels of CH_4 output from cows.

The LMD is not the most accurate way of measuring CH_4 but is comparably cheap and is a user-friendly way to measure many cows in a herd. Due to that CH_4 measured with LMD is done once per individual cow in each period, could be considered less accurate than the Greenfeed method and tracer gas method which makes several measurements per cow and day. Also due to that LMD measurement are done in open air close to other individual cows and not within an enclosed space as with the Greenfeed method, it is likely that CH_4 output from other individual could affect the measurement. The calculated accuracies for LMD differ in studies. The risk of report wrong CH_4 measurement the individual cow is likely to be increased due to that the LMD had no ear tag number detector, compared to the Greenfeed method.

A correlation of r= 0.93 between LMD and respiration chamber was found of Ricci et al 2014 which is likely to give trustworthy results with LMD. Changunda 2013 calculated a correlation to r= 0.47. Ricci 2014 deleted background data which could be the reason for the difference between the two results. Another potential difference between the studies could be the animal used were steers and not dairy cows. In this study no background data was deleted, and dairy cows was used similar to the study of Changunda 2013. Therefore, the data and design of data of this study is more alike the study of Changunda 2013.

The fact that not all CH_4 measurements (Herd 1 Per 2) took place in high light can also have affected the result. According to the producer of the LDM measurements should preferable be made in strong sunshine and

outside (Crowcon production voucher 2013). None of the measurements was done outside and therefore this should be taken in consideration when reading the result.

Sampling of CH_4 and milk was supposed to be done within the duration of approximate one week according to CIP project. In this project, time varied between milk recording and LMD measurements from 0 to 40 days. This extra time gap could have been accompanied with a differentiated environment like changed feed ratio.

Deviation from the experimental design in form of cows not eating during the LDM measurements was a fact in this experiment. Although the number of cows that was eating was predominant, the number of cows not eating varied between periods and between herds. This is a factor of uncertainty according to Changunda et al. (2009).

CH_4 measurements was supposed to be 4 minutes long but deviations from this happened and some measurements were only 3 minutes long. However, a measurement of 3 minutes is in accordance with other scientific CH_4 measurements (Changunda et al.,2009; Changunda 2013). Therefore, this deviation did probably not affect the reliability of the data.

5.2.4 Feed

The level of feed EL for individuals in the herds could be affected by the feeding strategy of the farm. Herd 1, 2, 5 fed EL in feeding stations, the given amount of EL can be understood as consumed of the correct individual. However, no protection gates were integrated on the feeding stations and thus cows could have been disturbed and or backed out from the feeding station after given ration. Due to the human interference when EL feed are scooped into the mixer wagon it is likely that feeding stations are more accurate when distributing the EL feed/cow. Also, for herds with TMR (table 2) it is possible that some cows consumed more EL-feed compared to others due to hierarchy between cows at the feeding table.

Forage differed between the test periods at the farms. There effect of a changed forage on CH_4 and FA profile in this study is unknown.

5.3 Future

Future questions to be answered is:

If and how much EL decreases the NDF degradation in rumen.

If and how a payment for reduced CH_4 output in relation to milk yield could be introduced, and what effect it could have on a lowered CH_4 output from herds. Today the payment for milk is based on milk yield, milk fat and protein (Arla, 2018).

If EL have a long-term effect of CH_4 output.

6. Conclusion

The results suggested that EL increases unsaturated fats in the milk and decreases the saturated fat. Results further suggest that EL does not increase the milk yield. The results do not show that EL decreases methane output. However, the method to measure methane emissions might have a limited accuracy.

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