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Effects of take-off level at udder quarter level and feeding during milking on milk fat quality and milk somatic cell count in an automatic milking system

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Effekten av avtagningsnivå på juverfjärdedelsnivå och utfodring under mjölkning, på mjölkfettskvalitet och mjölkcelltal i automatiska mjölkningssystem

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Table of contents

1. Abstract

The number of dairy farms in Sweden is decreasing, while the number of cows per farm is increasing. Since milking is very labour intensive many farmers have invested in new technology like automatic milking system (AMS) in order to reduce the work load. To increase the milking efficiency, it is important to have set take-off level of the milking cluster at a milk flow rate that minimize the time the cows spend in the AMS without affecting the milk yield and quality. Previous studies have indicated that milk yield decreases with increased take-off level when the take off level is set at milk flow from whole udder level. Furthermore has it been observed that feeding during milking might affect the milking time and milk yield. The aim of the present study was to examine how the take-off level of 100 g/min, 300 g/min, and 500 g/min at udder quarter level combined with or without feed during milking influenced the milk yield, milk fat globule size, content of cholesterol and beta-hydroxybutyrate in the milk, and the milk somatic cell count when the cows were milked in AMS. In the study, 30 cows of the breeds SRB (N=21) and Swedish Holstein (N=9) were included. The study lasted for six weeks and each treatment period was seven days. The treatments were arranged in a 6x6 Latin square to include all combinations of treatments every treatment period. Milk samples were taken during five milkings during the last two days of each treatment period. Samples taken for analyses of milk composition were collected during all five milkings, samples for the fat analysis were collected one milking each treatment period and determination of residual milk and samples from the residual milk were collected during the last milking every second treatment period. Milk samples from separate udder quarters were collected for analyses of milk composition during two milkings and for fat analysis during one milking. The results showed that the amount of milk was not affected by the different take-off levels, but total milking time was shortened with 0.5 min if using a take-off level on 500 g/min compared to a take-off level on 100 g/min ($P < 0.001$). There was no difference in milk fat globule size, milk cholesterol or milk somatic cell count between the different treatments but beta-hydroxybutyrate was greater in take-off level 500 g/min compared to take-off level 100 g/min ($P < 0.05$). Feeding during milking had only effect on milking time, which gave a longer milking time compared to no feeding during milking ($P< 0.001$).

2. Sammanfattning

Antalet gårdar i Sverige minskar men de som blir kvar växer och blir allt större. Detta kräver att bönderna modernisera gården för att anpassa den till det antal kor som finns på gården. Detta har gjort att många bönder väljer att investera i automatiska mjölkningssystem. För att effektivisera mjölkningen är det viktigt att ha en korrekt avtagningsnivå och en avtagningsnivå som inte försämrar mjölkens sammansättning Tidigare studier har visat att mjölksammansättningen förändras under mjölkningen samt att mjölkmängden minskar vid ökad avtagningsnivå. Syftet med denna studie var att studera hur avtagningsnivåerna 100g/min, 300 g/min och 500 g/min kombinerat med eller utan foder under mjölkningen påverkar mjölkmängden, fettkulestorleken, kolesterol, beta-hydroxybutyrat och celltal i automatiska mjölkningssystem. I studien användes 30 stycken kor, varav 21 SRB och 9 Holstein. Studien pågick under 6 veckor och varje behandlingsperiod varade i sju dagar. Behandlingarna arrangerades i en 6x6 Latin Square design för att få med alla kombinationer av behandlingar varje vecka. Provtagningar gjordes under fem mjölkningar under de två sista dagarna varje behandlingsperiod. Provtagningarna för mjölksammansättningen gjordes under alla fem mjölkningar, prover för fettanalys togs på en mjölkning varje behandlingsperiod och provtagning på residualmjölken gjordes under den sista mjölkning varannan behandlingsperiod. Provtagning på fjärdedelsnivå gjordes på mjölksammansättningen under två mjölkningar och på fettanalysen under en mjölkning. Resultaten från denna studie var att mjölkmängden inte förändras vid de olika avtagningsnivåerna men mjölkningstiden minskade med 0.5 minuter vid användning av en avtagningsnivå på 500 g/min jämfört med en avtagning på 100 g/min ($P < 0.001$). Det var inte någon skillnad i fettkulestorlek, kolesterol eller celltal mellan de olika behandlingarna, högre beta- hydroxybutyratinnehåll upptäcktes med avtagningsnivå 500 g/min jämfört med 100 g/min ($P < 0.05$). Utfodring under mjölkning hade endast effekt på mjölkningstiden, där utfodring under mjölkning gav en längre mjölkningstid jämfört om man inte utfodrade under mjölkning ($P< 0.001$).

3. Introduction

The number of dairy farms in Sweden is decreasing, but the number of cows per farm is increasing (Växa Sverige, 2013) and the milk yield per cow has increased through the last ten years (Lukkarinen and Lannhard Öberg, 2012). Milking is very labor intensive for the farmer, therefore, other management practices have been introduced to reduce the work load. Automatic milking system (AMS) is a good way to improve the milking efficiency. The cows can be milked more frequently than two times a day, even during the time when the farmer is not in the barn. The milk yield also increases over the day when cows are milked more frequently compared to conventional twice daily milking (de Koning and Rodenburg, 2004). Therefore, AMS is an important part of the modernization of the milk production for dairy farmers. The farmers can spend time on other activities in the barn instead of milking cows.

The first AMS in Europe was introduced during 1992 in the Netherlands (de Koning *et al.* 2003; Svennersten-Sjaunja and Petterson, 2008). One AMS milking unit (MU) can serve between 50 and 70 cows in a loose housing system, but the number of cows per MU depends on how evenly the milkings are spread over a 24 h period (Hogeveen *et al.* 2001). The number of cows milked per hour was increased by automatic pre-stimulation compared to a manual pre-stimulation (Edwards *et al.* 2013b). The quality of milk was slightly reduced after introducing of AMS, e.g. the content of free fatty acids (FFA) in the milk when cows in AMS compared to when cows were milked in a conventional milking system (Klungel *et al.* 2005).
Studies have observed that the milking time decrease with a higher take-off (Stewart *et al.* 2002; Magliaro and Kensinger, 2005) and feeding during milking also affect the milking effectivity (Johansson *et al.* 1999a). Feeding during milking increase the motivation to the MU (Prescott *et al*. 1998).

4. Literature review

4.1. Automatic milking system

AMS consists of an automatic MU with a robotic arm that cleans the teats and attaches the teat cups. Management settings are used to promote around the clock activity in the barn and ensure sufficient milking interval for each cow. Cows milked in AMS are housed in loose housing systems with access to a feeding area and a resting area. The cows are free to move between the different sections in the barn. The average milking interval of 4hrs -16hrs is depending on group size, use of teaser feeding in the MU, cow traffic, system and feeding routines (Hogeveen *et al.* 2001).

4.1.1 Cow traffic

Three main types of cow traffic are used in AMS; free traffic or forced traffic. The forced cow traffic system is further divided into two types: Milk First™ or Feed First™. Concentrate can be fed during milking to motivate the cows to visit the MU (Prescott *et al*. 1998). This can be recommended to reduce the number of fetched cows for milking.

4.1.1.1. Milk First system

Milk First™ system is structured so that the cows have to pass the MU to access the feeding area. The selection gates are set so that cows with milking permission are directed to the waiting

area for the MU while cows without milking permission are directed to the feeding area. Settings for the cow traffic includes a time interval in which cows need to pass through the MU to access feed. If the time since the previous milking has not passed, the cow can access the feeding area directly without passing the MU (Melin *et al.* 2006).

4.1.1.2 Feed First system

The Feed First™ system is built up the same way as the Milk First™ system, but here the cows are directed to milking when they exit the feeding area. If the cows have milking permission, they will come to the MU. If they do not have milking permission, they can proceed straight to the resting area (DeLaval, 2011).

4.1.1.3 Free cow traffic system

In free cow traffic system the cows move freely between feeding area, resting area and milking. These systems are therefore described as giving the cows a free choice of when to be milked (Hermans *et al.* 2003) or to visit feeding area.

4.2 Pre-stimulation

Different types of pre-stimulation can be used to ensure a sufficient milk ejection, and hence, increased milk flow and milk yield (Johansson *et al.* 1999a). The pre-stimulation can, for example, be tactile stimulation of the teats (Bruckmaier, 2001) or the provision of feed (Johansson *et al.* 1999a). Stimulation of the teats elicits nerve signals to the hypothalamus and results in secretion of the hormone oxytocin, which binds to myoepithelial cells in the secretory tissue in the mammary glands. When this occurs, the myoepithelium contracts, which results in milk moving from the alveolar to the cisternal compartment and the milk is thereby available for suckling or milking. This neuroendocrine event is referred to as milk let-down or milk ejection (Bruckmaier, 2001). Pre-stimulation causes the cow to rapidly achieve a high milk flow (Figure 1) compared with no pre-stimulation (Figure 2). Therefore, it is important to stimulate the teats, e.g. by washing them before the milking starts so the milk will flow from the milk alveoli to the milk cistern (Bruckmaier, 2001). Providing feed during milking has been found to increase milking related oxytocin release, and an indication with increased milk yield and milk flow was observed (Johansson *et al.* 1999a).

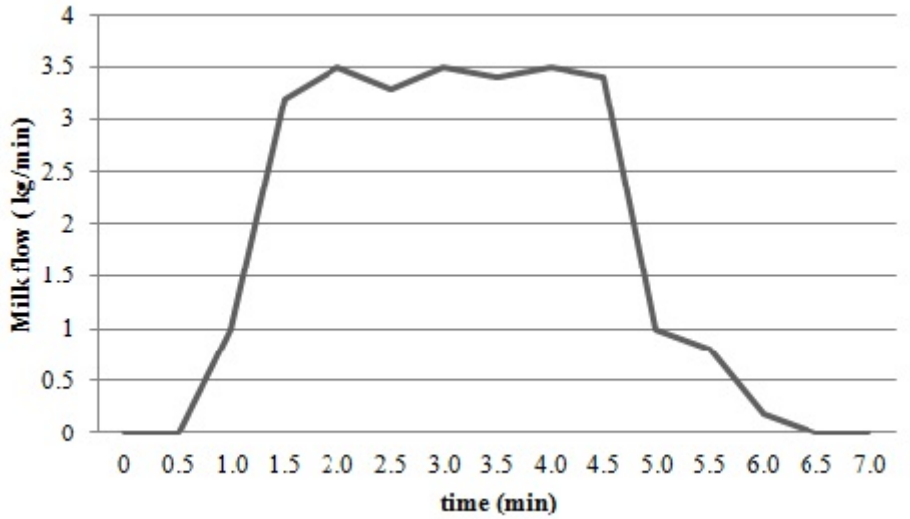

Figure 1. Schematic picture of milk flow during milking with pre-stimulation before milking. Modified from Bruckmaier *et al.* (2001). The milk flow increases sharply in the beginning of the milking and then flattens out before the flow decreases at the end of the milking.

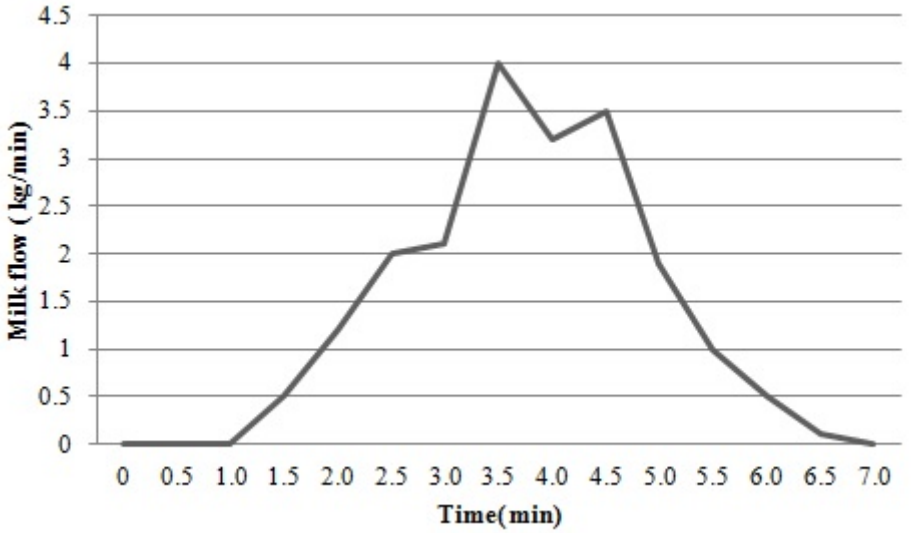

Figure 2. Schematic picture of milk flow during milking without pre-stimulation before milking. Modified from Bruckmaier *et al.* (2001). It takes longer for the cow to reach a high milk flow without pre-stimulation and the flow does not flatten out. Instead, it starts to decrease rapidly after the peak flow.

4.3 Take-off level

The efficiency of the MU, expressed as kg milk harvested during 24 h, is affected by the number of milkings per cow and the average milk flow during milking. Take-off level on 200 g/min have been a common practice (Ginsberg, 2012). Take-off level is the level of milk flow that determines when the teat cups are removed to terminate milking. The take-off level can be set on whole udder level or quarter level. The take-off level has a strong influence on the total machine-on time and can also affect the total milk yield (Magliaro and Kensinger, 2005). In the study by Magliaro and Kensinger (2005), three different take-off levels were used, measured as g/min on whole udder level. In the study, 60 Holstein were included which were milked twice a day. They found that milk yield decreased by 0.5 kg of milk/milking, if the cows had a take-off level of 800 g/min compared to 480 g/min or 600 g/min. They also observed that

the milking time was shorter at a higher take-off level. A study on take-off on whole udder level in peak lactation by Edwards *et al.* (2013a) found that there were no differences in milk yield and SCC with a take-off level of 200 g/min, 400 g/min, 600 g/min, or 800 g/min. A take-off level on 800 g/min decreased the milking time with 72-81s compared with a take-off level on 200 g/min, 400 g/min and 600 g/min (Edwards *et al.* 2013a). Milk flow was measured in Edwards *et al.* (2013a) and no differences were found between treatments for cows in peak lactation. Stewart *et al.* (2002) studied take-off levels of 500 g/min, 640 g/min, 730 g/min, or 820 g/min at whole-udder level and observed that the milking time decreased with a higher take-off level, but no difference in milk yield was shown. With an adapted take-off level on udder quarter level, over milking of individual udder quarters can be reduced. Over-milking affects the teats negatively, which increases the risk of infections in the udder (Hillerton *et al*. 2002).

4.4 Udder anatomy and milk synthesis

The udder has four compartments separated from each other by connective tissue. In each udder quarter, lobules containing lobes of secretory alveoli are found (Sjaastad *et al.* 2010). Each lobe contains 150-200 alveoli. Milk is synthesized by the secretory cells of the alveoli. The alveoli are drained by small milk ducts that join into larger ducts that lead the milk into the gland cistern and from there down to the teat. Each udder quarter has its own secretory tissue and gland cistern (Sjaastad *et al.* 2010). The blood supply to the udder consists of arteries, which are divided between the different udder quarters, (Sjaastad *et al.* 2010). Some smaller arteries go through all the udder quarter parts. The blood is then transported back to the heart through the veins. It requires 500 liters of blood transported through the udder to produce one liter of milk (Sjaastad *et al.* 2010). The udder is also provided with nerves and sensory nerves is present in the teats. Moreover the udder has a lymphatic system (Sjaastad *et al.* 2010).

The precursors for milk synthesis are transported by the blood to the udder. Lactose is only produced in the mammary glands in the udder (Sjaastad *et al.* 2010), and consists of one glucose and one galactose (Sjaastad *et al.* 2010; McDonald *et al.* 2011). Milk fat is created by three fatty acids of different length integrated with a glycerol molecule forming triglycerides. The triglycerides aggregate and form larger fat droplets inside the secretory cell. Then, they are excreted through budding off at the apical membrane and forms milk fat globules (Wiking *et al.* 2006). Milk protein is mostly caseins (Dalgleish, 1992; McDonald *et al*. 2011) but also whey proteins (Dalgleish, 1992) and they consists of amino acids.

4.5 Udder emptying

If milk removal is incomplete, milk left in the secretory tissue can cause losses in milk production. For the same reason, milk yield decreases with a lower milking frequency (Davis *et al.* 1999). Milk contains protein that inhibits milk synthesis if the udder emptying is low. The fat content in the milk will increase with a better udder emptying (Nielsen *et al*. 2005), a low fat content in residual milk demonstrates the potential effect of incomplete milking. Oxytocin stimulates the milk let down (Sjaastad *et al*. 2010) and, therefore, an injection with oxytocin after the regular milking is needed to collect the residual milk.

Researchers have found that there is a substance in milk that has an inhibitory function on the milk synthesis. During the decades of 1990, this mechanism was named Feedback Inhibitor of Lactation (FIL) (Wilde *et al.* 1995; Peaker and Wilde, 1996; Knight *et al.* 1998; Hernandez *et al.* 2008). FIL is synthesized in the mammary glands epithelial cells (Wilde *et al.* 1995; Peaker and Wilde, 1996). FIL binds in to a membrane receptor on secretory cells and inhibits protein passage to the Golgi (Peaker and Wilde, 1996; Knight *et al.* 1998). But later, researchers have begun to discuss whether it is the hormone serotonin, which is synthesized from tryptophan, that has an inhibition function of the milk synthesis (Hernandez *et al.* 2008). Therefore, seretonin has been categorized as a FIL protein.

4.6 Milk composition and changes in composition during milking

The largest fraction, about 90 %, of cow milk is water. Apart from water, cow milk also contains protein (3.1–3.7 %), fat (3.3–4.3 %), and lactose (4.5–4.8 %) (McDonald *et al*. 2011). Nielsen *et al.* (2005) reported that the milk composition changes with different milking intervals. In that study they used two different milking intervals, 6 hrs and 12 hrs. They milked two teats with 6 hrs interval and two with 12 hrs interval on each cow. They showed that it was a higher content of BHB, SCC and milk fat in the udder quarters that were milked with 12 hrs interval compared to 6 hrs and a lower content of lactose and protein with 12 hrs interval than 6 hrs. A study by Wiking *et al.* (2006) also showed a difference in milk composition with different milking intervals. MFG size was affected by milking intervals. The MFG sizes was greater in cows milked four times a day compared to when the cows were milked twice a day (Wiking *et al.* 2006).

4.6.1 Milk fat

The main part of the fat is in the form as triglycerides consisting of fatty acids and glycerol (Nielsen *et al.* 2005). Triglycerides constitute over 95 % of the milk fat (Bauman and Griinari, 2003). The remaining 5 % of the milk fat includes volatile fatty acids, e.g. acetic, butyric, propionic acid (Hanuš *et al.* 2008), monoglycerols, cholesterol, phosphlipids and diacylgrycerol (Bauman and Griinari, 2001). Fatty acids with C4 up to C16 are synthesized in the *de novo* synthesis in the udder. It is only a small part of the content of C16 fatty acids, which are synthesized in the udder (Akers, 2002). The remaining, medium and long fatty acids (C16-C18) are transported to the udder by the blood and originate from the digestive tract or from adipose tissue (Bauman and Griinari, 2001).

The content of fat in milk is dependent on breed Graves *et al.* 2007), days in milk (DIM) (Bauman and Griinari, 2003; Kay *et al.* 2005), feed structure (Avramis *et al.* 2003; Bauman and Griinari, 2003; Wiking, 2005; Carroll *et al.* 2006; Couvreur *et al.* 2007) and milk yield (Forsbäck *et al*. 2010). The front teats have a higher fat content then the rear teats (Berglund *et al*. 2007), which could be due to a dilution effect for the rear teats. Nielsen *et al.* (2005) found in a study with 11 Danish Holstein cows, that milk fat increased throughout milking. Samples were taken on quarter level every 45 second. The milk from the alveoli had a higher content of fat compared to the milk in the cistern (Abeni *et al.* 2003), which could explain why milk has a higher fat content at the end of milking than in the beginning.

4.6.1.1 Milk fat globule size

Milk fat globule size (MFG size) is dependent on milk fat percentage and stage of lactation, with larger MFG in high fat milk (Wiking *et al.* 2004; Wiking, 2005), compared to low fat milk. A membrane protects the triglycerides, which are located inside the membrane, against lipolysis and oxidation (Wiking *et al.* 2004). MFG size is measured in µm, usually around 1.0-10µm depending on breed. Analysis of MFG size is done with integrated light scattering (Wiking *et al.* 2004).

Feeding can affect the size of MFG (Avramis *et al.* 2003; Wiking, 2005; Couvreur *et al.* 2007). Avramis *et al.* (2003) found that inclusion of fishmeal in feed to lactating dairy cows decreased MFG size. Couvreur *et al.* (2007) reported the MFG size decreased for cows grazing and supplemented with concentrate compared to cows fed corn silage and soymeal. There is also a breed difference for the size of MFG (Graves *et al.* 2007). The MFG membrane consists of phospholipids, lipoprotein, glycolipids, cholesterol and enzyme, As cholesterol is part of the membrane, analyzing cholesterol in milk, will give an indirect measure of the membrane stability (Larsen, 2011).

4.6.1.2 Free fatty acids in milk

Free fatty acids (FFA) in milk are fatty acids not bound to glycerol (Svennersten-Sjaunja and Wiktorsson, 2002). FFA is a non-desirable component in milk as it decreases milk quality and shelf-life (Hanuš *et al.* 2008*)* and affects the taste of the milk negatively (Andersson and Gyllenswärd, 2004). The proportion of FFA in milk is affected by the rate of lipolysis; the enzymatic breakdown of triglycerides by lipase. The risk for lipolysis increases when the MFG are damaged (Svennersten-Sjaunja and Wiktorsson, 2002; Andersson and Gyllenswärd, 2004), allowing the enzyme lipase to get through the MFG membrane (Svennersten-Sjaunja and Wiktorsson,2002). FFA may increase when milk is heated as heat also damages the membrane (Wiking, 2005). The larger MFG, the higher risk for lipolysis, due to a less stable MFG membrane (Wiking, 2005). Therefore, small MFG is preferred for high quality milk.

According to a study by Hanuš *et al.* (2008), more than 1.2 mmol FFA/100g milk fat decreased milk quality and deteriorated the taste of the milk. In a study with different take-off levels on whole udder level with or without inclusion of feed during milking, it was demonstrated that cows fed during milking had a lower FFA content in the milk compared to the cows without feeding during milking (Stadtmüller, 2014). de Koning *et al*. (2003) observed that the FFA content was higher in milk from cows who were milked three times a day compared to cows that were milked twice a day. Another study by Klungel *et al*. (2005) observed that there were higher levels of FFA in milk from cows milked three times daily in AMS compared to milk from farms with conventional milking two times daily.

4.6.2 Beta-hydroxybutyrate

Beta-hyroxybuturate (BHB) is a ketone body of the volatile fatty acid butyrate, formed in the rumen when carbohydrates are fermented. Butyrate is converted to BHB either when during transport through the rumen epithelium or in the liver (Bergman, 1971). A high concentration of BHB indicates a high milk fat concentration in milk (Duffield *et al. 2009;* Melendez *et al.*

2016). The short chained fatty acids in milk fat with 4-14 carbon units are synthesized from BHB and acetate (Wiking, 2005). Cows create ketone bodies to provide energy to the body. High yielding dairy cows sometimes have difficulties meeting the increased energy requirement in early lactation. If this requirement is not met, the energy balance for the cow will be negative, with an increased risk of the metabolic disease ketosis as a result (Bergman, 1971; Enjalbert *et al.* 2001). When in negative energy balance, the cow forms ketone bodies from FFA in the liver rather than in the rumen using butyrate, acetate, and acetoacetate as substrate (Enjalbert *et al.* 2001). Ketone bodies can be excreted in milk and urine (Bergman, 1971), and a high content of BHB in the milk indicates ketosis (Jorritsma *et al.* 1998).

A study by Nielsen *et al.* (2005), demonstrated that BHB content in the milk was constant during milking if the cows were milked with a 6hrs interval compared to a 12 hrs interval. If the cows were milked in a 12 hrs interval, the BHB content was low in the beginning of the milking and increased throughout the whole milking.

4.6.3 Milk Protein

The milk contains around 3.1 to 3.7 % protein depending on breed, age and the protein content increase with increasing stage of lactation (McDonald *et al*. 2011). Nielsen *et al.* (2005) observed that the protein concentration dropped rapidly at the end of the milking, but the content was constant until then.

Two main groups of protein are present in milk; caseins and whey proteins. Casein is the major protein group in the milk. The casein fraction includes four different types of caseins; α_{s1}-, α_{s2}-, β- and κ-caseins. The whey protein constitutes a smaller amount in the milk than the caseins. Whey proteins include β-lactoglobulin, α-lactalbumin and immunoglobulin (Dalgleish, 1992). Caseins are important components for the food industry as they are needed to produce cheese (Aleandri *et al.* 1990).

4.6.4 Lactose

Lactose is a disaccharide made up of one glucose and one galactose molecule. Lactose is synthesized in the Golgi apparatus located in the secretory cells in the mammary gland (Sjaastad *et al.* 2010; McDonald *et al*. 2011). Lactose has a high osmotic function in the milk (Auldist and Hubble, 1998;Sjaastad *et al*. 2010), which means that lactose is a major source of milk volume regulation. The more lactose in the milk, the more water draws into the milk from the cells (Auldist and Hubble, 1998). Milk is composed of about 4.5 to 4.8 % lactose, depending on breed (McDonald *et al*. 2011). Lactose content is negatively correlated to fat during milking. The more fat present in milk, the less lactose (Nielsen *et al.* 2005). Nielsen *et al.* (2005) found that milk lactose was approximately constant until the last portion of milking when the lactose content dropped quickly. The lactose content in milk will decrease if the cow have mastitis (Forsbäck *et al*. 2010) and the content of lactose is reduced because of the bacteria growth in the udder causing udder inflammation (Leitner *et al*. 2006)

4.7 Udder health

An indicator of udder health and milk quality widely used in the world is milk somatic cell count (SCC). A commonly used threshold for good udder health and good milk quality is SCC below 200.000 cells/ml (Eldeen Idriss *et al.* 2013). High SCC in the milk indicates an inflammation in the udder, which can be caused by pathogenic bacteria. This reduces the milk quality and udder health and also the cow's general condition. In order to ensure good udder health, it is important that the teats are clean before milking, so pathogenic bacteria cannot get into the teat canal. The cows are at a higher risk of infection during the first 30 minutes after milking, since the teat canal does not close immediately after milking finishes (Johansson *et al.* 1999b).

Nielsen *et al.* (2005) determined that SCC was higher when cows were milked with 6 h interval than 12 h, and that the SCC increased during milking. Fernandes *et al.* (2007) showed that milk with > 400 000 cells/ml, had an undesired increase in FFA concentration, which can shorten the shelf life of the milk.

5. Aim and hypotheses

The aim of this study was to investigate how milk yield and milk quality is affected by different take-off levels at udder quarter level with or without the provision of feed during milking in an AMS. Milk quality in this study was measured as MFG size, SCC, cholesterol and BHB.

The hypotheses were:

1) Milk yield will be greater with take-off level 100 g/min than with 300 g/min or 500 g/min.
2) The MFG will have a larger diameter in take-off level 100 g/min than 500 g/min.
3) The content of cholesterol will be lower with take-off level 500 g/min than in 300 g/min, or 100 g/min.
4) SCC will be higher with take-off level 100 g/min, or 500 g/min compared to 300 g/min.
5) The milking efficiency will be increased at a take-off level 500 g/min compared to 100 g/min, or 300 g/min.
6) The milking efficient will be increased with feeding during milking compared to no feeding during milking.

6. Material and Method

6.1 Animals and housing

All animal handling was approved by the Uppsala animal ethics committee (Ref C6/14).
The study included 30 mid-lactation cows, 17 pregnant and 13 non-pregnant of the Swedish Red breed (SRB; N=21) and Swedish Holstein (SH; N=9) from Swedish Livestock Research Centre, SLU Uppsala, Sweden. The cows were housed in a loose housing system with a Feed First™ automatic milking system (Voluntary Milking System™, DeLaval, Tumba, Sweden) and had access to one milking robot. The cows had *ad libitum* access to grass silage (11 MJ and 158 g CP) and were fed with concentrate individually adjusted according to milk production.

6.2 Experimental design

Six treatments with the take-off levels 100 g/min, 300 g/min, and 500 g/min on quarter level with or without feeding during milking were tested. Treatments were implemented using a 6 x 6 Latin Square Design (Beob and Stein, 2009) (Table 1). The study lasted for 6 weeks. Each treatment period was seven days. The cows were divided into treatment groups after lactation number, lactose content, and SCC. Based on these criteria, the selected cows had an average SCC of 92 000 ± 37 000 cells/ml milk, lactation number was 3 ± 2, were 166 ± 56 DIM and a mean lactose content of 4.84 ± 0.2 %. The mean milking interval in this study was 9 h (including the milking interval after residual milk sampling). The milking interval without the interval after residual milk sampling was 8h and 43 min.

Table 1. Treatments in a 6 x 6 Latin square design with different take-off levels (100 g/min, 300 g/min and 500 g/min) and with feeding (f) or without feeding (nf) during milking.

Group	Study week					
	1	**2**	**3**	**4**	**5**	**6**
A	500f	100f	100nf	300f	300nf	500nf
B	100f	300f	500f	500nf	100nf	300nf
C	300f	500nf	100f	300nf	500f	100nf
D	500nf	300nf	300f	100nf	100f	500f
E	300nf	100nf	500nf	500f	300f	100f
F	100nf	500f	300nf	100f	500nf	300f

6.3 Milk sampling

The milk yield was measured by DelPro™ (DeLaval, Tumba Sweden). Milk samples were collected using an automatic sampler (Automatic Milk Sampler, DeLaval, Tumba, Sweden). A representative 50 mL subsample was collected by the automatic sampler for analysis of milk composition (fat, protein, lactose and SCC), chilled after sampling and preserved using Bronopol ($C_3H_6BrNO_4$). Samples were turned three times to ensure distribution of the preservative. Milk samples for analysis of FFA, MFG size and distribution and MFG membrane stability were collected using milk from the larger sampling vessel in the automatic milk sampler, and chilled after sampling. All milk samples for the quarter level testing were taken by hand before attachment of teat cups. All samples were kept on icepack during sampling, after milking they were transferred to a +4ºC refrigerator and stored for 24-48 hrs. The samples

collected for FFA analysis were heat treated in 68°C water bath, 2 x 5 minutes, 24 hrs after sampling. The samples were stored at -18 ºC until shipped for analysis.

6.3.1 Milk composition on udder level

Samples for milk composition were collected during five consecutive milkings every week; morning around 05:00am-02:00pm, evening 02:00pm-11:00pm and night 11:00pm-05:00am.

6.3.2 Fat samples on udder level

Samples for fat analysis (FFA, MFG size and MFG stability, BHB and cholesterol) were collected during the morning milking on day six every week. No preservatives were added in these samples as analysis was conducted on fresh milk.

6.3.3 Residual milk sampling

The residual milk was harvested during treatment period one, three and five. The teat cups were attached manually for the main milking during these sessions. Before attachment each teat was cleaned by hand using a wet cloth until any debris was removed, and milk was checked for abnormalities. Cleaning of teats was always performed in the following order; right front, right rear, left front and left rear. Each teat was stripped in four strokes down in a collection vessel before the milking machine was attached by hand. When milking was finished, the cow was injected intramuscularly with 5-6 ml oxytocin (Partoxin® vet. 17µg (10 IU)/ml) depending on the cow's weight, in the left thigh muscle. Three minutes after the injection, the residual milk was harvested in a separate bucket using a manual milking machine with cluster milking. The milking machine was removed when the flow of milk could no longer be visually detected. The residual milk was transferred to a bucket and weighed on a digital scale.

6.3.4 Milk sampling at udder quarter level

Milk samples at udder quarter level were collected during treatment period number four. Samples for fat content were taken during the morning milking on day six and samples for milk composition were taken during the afternoon milking on day six and during the morning milking on day seven. The quarter level samples were collected manually before the teat cups were attached according to the following procedure. First all teats were cleaned manually in five seconds and thereafter strip milk yield was collected by four to five strokes in a collection vessel before sampling. The cleaning and sampling was performed in the same order as the residual milk sampling. Milk samples were collected in four separate small tubes for each teat.

6.4 Milk analysis

Milk composition (protein, lactose and fat content) and SCC for both main milk and residual milk, was analyzed at the laboratory at the Department of Animal Nutrition and Management, SLU Uppsala, Sweden. For samples collected during study week 1 and 2, a MilkoScan FT120 (FOSS Electric A/S, Hillerød, Denmark) was used to determine milk composition and a Fossomatic 5000 (FOSS Electric A/S, Hillerød, Denmark) was used to determine SCC. For samples collected during the remaining study weeks, a LactoScope FTIR (Delta instruments, Drachten, Netherlands) was used to determine milk composition and a Somascope (Delta instruments, Drachten, Netherlands) was used to determine SCC. The analysis for fat globule size, membrane stability, FFA and BHB were conducted at Aarhus University, Folum research station, Tjele, Denmark. An integrated light scattering (Mastersizer 2000, Malvern, United

Kingdom) was used to determine the MFG size (Wiking *et al.* 2004). A fluorometric detection was used to determinate BHB according to Larsen and Nielsen (2005). FFA content was determined using a gas chromatography-mass spectrometry as described by Amer *et al.* (2013), and an enzymatic-fluorometric method was used to quantify cholesterol (Larsen, 2011).

6.5 Statistical analyses of data

For all analyses, the individual cow served as the experimental and observational unit. Harvested milk samples and residual milk samples were analysed separately. The data were analysed by ANOVA for a 6 × 6 Latin square with a 2 × 3 factorial arrangement of treatments in a linear mixed-effects model using repeated measures in the statistical software SAS (SAS Institute Inc., Cary, NC, USA). The model included the fixed effects of period, lactation number, DIM, take-off level, feeding and their interaction and the random effect of cow within group. Cow within group was included as a repeated measure. Data on SCC were log transformed prior to analysis due to non-normal distribution. Differences in milk composition between harvested milk samples and residual milk samples were evaluated with a Student's T-Test.

Values presented are LSmean unless otherwise stated. Treatment effects were declared significant at $p \leq 0.05$, while a trend was assumed for probabilities $p < 0.1$ and $p > 0.05$. Posthoc means separation for significant main effects was done using a Tukey's.

7. Results

Some cows were removed from the data during different treatment periods due to various reasons. One cow was removed on study week three and four due to lameness. During the fourth study week, the treatment settings did not come through on the set date, which resulted in a 4-day shorter treatment period. Two cows were excluded as they had high SCC during the whole study. One cow in heat was excluded during one study week due to milk let down failure.

7.1 Udder level

7.1.1 Milking time

Milking time decreased with increased take-off level ($P < 0.001$; Table 2). A take-off level on 100 g/min had an average on 7.9 ± 0.3 min/milking, 500 g/min averaged on 6.8 ± 0.35 min/milking. Feeding during milking increased the milking time compared to no feeding during milking ($P < 0.001$).

7.1.2 Milk yield and SCC

There was no significant effect of take-off level ($P = 0.87$), feed inclusion ($P = 0.54$) or the interaction of them ($P = 0.58$) on milk yield. Holstein cows had higher milk yield than SRB cows ($P < 0.01$). No significant difference between treatments was found for SCC ($P = 0.97$). There was a breed difference for the SCC, with SRB having higher SCC than Holstein ($P < 0.001$; Table 2).

Table 2. Milking time, milk yield and somatic cell count (SCC) from whole udder milk samples taken at treatments with different take off levels (TO) and feeding (f) versus no feeding (nf) during milking

	Treatment	**P- values for fixed effects**

	100f	300f	500f	100nf	300nf	500nf	TO	Feed	TO x Feed	Breed
Milking time (min)	8.2	7.7	7.0	7.6	7.0	6.7	< 0.001	< 0.001	0.58	0.29
Milk yield/ milking (kg)	12.8	13.1	12.8	12.9	12.6	12.6	0.87	0.54	0.70	< 0.001
SCC (x1000 cells/ml)	24	25	24	27	23	23	0.64	0.90	0.97	0.0001

7.1.3 Milk fat globule size and membrane stability

MFG size and distribution was not affected by take-off level (P=0.32), the inclusion of feed (P=0.36), or the interaction (P=0.65). MFG size was larger for Holstein breed (4.2 µm) than for the Swedish Red breed 4.0 µm; $P < 0.05$; Table 3).

No significant difference was found for cholesterol content between take-off level (P=0.85), the inclusion of feed (P=0.65), or the interaction (P=0.69; Table 3).

7.1.4 Beta- hydroxybutyrate

No significant difference was found for take-off level (P=0.59), the inclusion of feed (P=0.0784), or the interaction (P=0.46; Table 3).

Table 3. Milk fat globule size (MFG size) (d[4.3]), cholesterol and beta-hydroxybutyrate (BHB) from whole udder milk samples taken at treatments with different take off levels (TO) and feeding (f) versus no feeding (nf) during milking.

	Treatment						P- values for fixed effects			
	100f	300f	500f	100nf	300nf	500nf	TO	Feed	Feed × TO	Breed
MFG size d[4.3], (µm)	4.17	4.12	4.11	4.11	4.13	4.04	0.32	0.36	0.65	0.04
Cholesterol (µm/l)	232	212	217	212	215	217	0.85	0.65	0.69	0.15
BHB (µm/l)	128	124	129	123	118	106	0.59	0.08	0.46	0.48

7.2 Udder quarter level

7.2.1 Milk yield and somatic cell count

The milk yield was higher in the rear teats than in the front teats ($P < 0.0001$). Milk yield in separate udder quarters was not affected by take-off level (P=0.43), inclusion of feed (P=0.65), or the interaction of take-off level x feed (P=0.60).

No effect on SCC was found for take-off level (P=0.73), inclusion of feed (P=0.61) or the interaction take-off level x feed (P=0.53). Milk in the front quarters had a higher SCC than milk in the rear quarters ($P < 0.0001$; Table 4).

7.2.2 Milk fat globule size and membrane stability

MFG size was not affected by take-off level (P=0.14), inclusion of feed (P=0.68), or the interaction of them (P=0.68; Table 4).

7.2.3 Cholesterol content

No effect on cholesterol content in was found for take-off level (P=0.13), the inclusion of feed, or the interaction of them. Left quarters had greater concentrations of cholesterol compared to right quarters (P <0.01) and front quarters had greater concentrations of cholesterol compared to the rear quarters; Table 4).

7.2.4 Beta-hydroxybutyrate

The take-off level at 100 g/min resulted in a higher average content of BHB (124 µm/l) compared to take-off level at 300 g/min (86 µm/l) or take-off level 500 g/min (81 µm/l; P <0.05). There were no significant differences in BHB content between separate udder quarters, inclusion of feed, or the interaction of take-off level and inclusion of feed (Table 4).

Table 4: Somatic cell count (SCC), cholesterol, beta-hydroxybutyrate (BHB) and milk fat globule size (MFG size) (d[4.3]) from udder quarter samples taken at treatments with different take off levels (TO) and feeding (f) versus no feeding (nf) during milking.

	Udder quarters				**P- values for fixed effects**			
	Left front	**Left rear**	**Right front**	**Right rear**	**TO**	**Feed**	**TO × Feed**	**Quarter**
Milk yield (kg)	2.55	3.81	2.63	3.86	0.43	0.65	0.60	<0.0001
SCC (cells/ml)	14067	13580	24877	14743	0.73	0.61	0.53	<0.0001
Cholesterol (µm/l)	137.58	121.74	134.23	114.34	0.13	0.42	0.49	0.0031
BHB (µm/l)	98.63	109.22	83.28	97.63	0.03	0.39	0.23	0.32
MFG size, (µm)	4.04	4.03	4.04	3.98	0.14	0.60	0.68	0.36

7.3 Residual milk

7.3.1 Milk yield and SCC

Residual milk yield was not affected by take-off level, the inclusion of feed, or the interaction of the two (Table 5). SCC in residual milk was not affected by take-off level, the inclusion of feed, or the interaction of the two (Table 5). The SCC was greater in the residual milk (74500 ± 35500 cells/ml) compared to the main milk (24333 ± 2670 cells/ml).

Table 5. Milk yield, somatic cell count (SCC), cholesterol and beta-hydroxybutyrate (BHB) from residual milk samples taken at treatments with different take off levels (TO) and feeding (f) versus no feeding (nf) during milking.

	Treatment						**P- values for fixed effects**			
	100f	**300f**	**500f**	**100nf**	**300nf**	**500nf**	**TO**	**Feed**	**Feed × TO**	**Breed**
Milk yield/ milking (kg)	0.61	1.38	1.20	0.99	0.71	1.14	0.52	0.67	0.28	0.37
SCC (x1000 cells/ml)	83	53	59	110	65	77	0.37	0.27	0.99	0.21

8. Discussion

The reduced milking time of 0.5 min per cow at a take-off level on 500 g/min compared to take-off level 100 g/min suggests increased milking efficiency because more cows can be milked per hour. Assume that the cows in this study are milked during 7 minutes with take-off level 100 g/min, it should take 30 x 7 = 210 minutes to milk them. If the cows are milked with a take-off level on 500 g/min, then the time should decrease to 30 x 6.5 = 195 min. If the cows are milked 2.6 times a day, (210-195) x 2.6 = 39 minutes will be saved each day. In this study, with a small number of cows, the total minutes saved was relatively small, but herds with a high number of cows should benefit to a greater extent. This result is consistent with the study by Stewart *et al.* (2002), who reported that milking time decreased by 10.2s to 15.6s per cow with a higher take-off level.

Milk yields did not differ among treatments, which was consistent with previous studies by Edwards *et al.* (2013a), Edwards *et al.* (2013b) and Stewart *et al.* (2002). In the study by Edwards *et al.* (2013b), a take-off level on udder level of 820 g/min and a take-off level on 200 g/min did not affected milk yield.

Surprisingly, inclusion of feed during milking did not affect milk yield in the present study. It has been indicated earlier that inclusion of feeding during milking might enhance milk ejection and thereby milk yields (Johansson *et al*. 1999a).

The reason why there was no significant effect of take-off levels or the inclusion of feed of the milk yield may be because the milk flow possibly dropped quickly after 500 g/min. As we did not see a difference in yield we did not expect that the different take-off levels would affect milk composition. Since several studies (Edwards *et al.* 2013a; Edwards *et al.* 2013b; Stewart *et al.* 2002) obtained similar results, the result in this study would probably not change if other cows had been used. Both cows in peak lactation (Edwards *et al.* 2013a) and cows in late lactation (Edwards *et al.* 2013b) have been studied in previous research on take-off levels. The earlier studies had treatment periods on seven days (Stewart *et al.* 2002), fourteen days (Edwards *et al.* 2013a) and twenty-one days (Edwards *et al.* 2013b). Therefore, we believe that the results would probably not have changed even if the treatments periods were increased.

There were no significant differences in SCC content between the different treatments. It was only a difference between breeds. The results in the present study differ from Nielsen *et al.* (2005) who observed that higher cell count were evident at the end of the milking. In our study,

SCC was lowest in take-off level 100 g/min with feeding, but highest in take-off level 100 g/min without feeding. So in our study we cannot say that it is higher SCC in the end of milking.

In the present study, Holstein cows had higher content of larger MFG than SRB. Previous studies reported that a greater fat content increased the MFG size (Wiking *et al.* 2004;Wiking, 2005). Holstein had a tendency for greater fat content than SRB in our study**,** but it is not statistically established (P = 0.09). That could explain the larger MFG size in Holstein. Larger MFG size should be present at the end of milking, because the fat content is greater then and fat content drives MFG size (Wiking *et al.* 2004; Nielsen *et al.* 2005; Wiking, 2005). Therefore, it was expected that larger MFG size would occur at take-off level 100 g/min. However, no significant differences between take-off levels were evident. Inclusion of feed did not affect the MFG size. This was expected since we did not see an effect on either milk yield or milk composition.

In present study the BHB in milk decrease with a high take-off level. This agrees with Nielsen *et al.* (2005) where they observed that there was a greater content of BHB in the end of milking than in the beginning. It was strange that BHB changed between take-off level, because there was no difference in milk yield between the take-off levels. As a result, the mechanism for this difference is not clear.

There were technical problems with the automatic feed wagon during week two, which compromised *ad libitum* access to silage. Barn staff were distributing silage manually during this period until the feed wagon was mended. It is possible that this created a shortage of feed and that feed intake was affected, which in turn may have affected MFG size (Avramis *et al.* 2003; Wiking, 2005; Couvreur *et al.* 2007). The change in roughage in the first week can explain why the BHB content was much higher in week one than in the other weeks. BHB is a ketone body and would increase if the cows eats a smaller amount than they should (Bergman,1971).

8.1 Method reflections

In this study, it was important to have regular milking intervals. The planned milking interval was 8 h, but the actual average milking interval was 9 hrs (range 6-16 hrs) and without the residual milk sampling, 8 h and 43min (also a range on 6-16 hrs), which could have changed if the cows were fetched for milking even earlier, but that would have been difficult to carry out since there is no staff in the barn between 23 and 05 every night. The cow who had a milking interval at 16 hrs in the first sampling day without influence of residual milk sampling. Without that the milking interval should have a range on 6-13 hrs. Studies have shown that the milk composition changes with different milking intervals (Nielsen *et al*. 2005; Wiking *et al*. 2006). Therefore, 8 hrs milking intervals were chosen to get an even spread of milkings over the day, so there would be a small daily influence as possible on milk composition. Since we did not get a milking interval with an average of 8 hrs, it could have affected the results of milk composition. The interval in this study was slightly displaced when the residual milk sampling was carried out because it took a very long time to collect these samples. That is why the milking interval could be longer during the afternoon with residual milk sampling. However, since no period effect was observed for the study, it is believed that the longer milking intervals during

residual milk sampling did not affect the results. In general in farms, the take-off level is between 500 g/min and 700 g/min set on udder level (Nyman, 2010). In this study, 100 g/min was chosen as an extreme low value, 300 g/min as a normal value and 500 g/min as a high value. It was planned to have 700 g/min as the highest take-off level, but the peak flow for some cows did just barely reach 700 g/min on udder quarter level, and it was therefore considered too high, why 500 g/min was used instead.

The results of our study would have been different if the quarter sampling would have been taken during the whole milking instead in the beginning. This because the concentration of milk components change during the milking. Syringes in the milk tube pulling milk during the whole milking were tested as a continuous sampling method for quarter level sampling. However, this was not possible, and it was therefore decided to take the samples by hand before the milking machine was attached. This likely means the quarter samples were not representative of the whole milking. The change of equipment for milk analyses at the lab may have altered the results the milk composition. The lab staff calibrated the machines and checked correlations, hence the risk for this was considered rather small.

9. Conclusion

Results from this study demonstrated that using a high take-off level of 500 g/min on udder quarter level will decrease milking time without compromising milk composition or quality, compared to the take-off levels 300 g/min or 100 g/min, except BHB; less BHB with higher take-off level. The inclusion of feed and the possible effect on milk flow during milking needs to be further investigated.

10. References

Abeni, F., Degano, L., Giangiacomo, R., Speroni, M., Pirlo, G. (2003). Robotic milking and milk quality: effects on the cheese - making properties of milk. *Italian Journal of Animal Science*. vol. 2. pp.301-312.

Aleandri, R,. Buttazzoni, L.G., Schneider, J.C., Caroli, A., Davoli, R. (1990). The Effects of Milk Protein Polymorphisms on Milk Components and Cheese-Producing Ability. *Journal of Dairy Science*. vol. 73. pp. 241 -255. [Abstract]

Akers, M, R. (2002). Lactation and the Mammary gland. Iowa: Iowa state press

Amer, B., Nebel, C., Bertram, H.C., Mortensen, G., Hermansen, K., Dalsgaard., T.K. (2013). Novel method for quantification of individual free fatty acids in milk using an in-solution derivatisation approach and gas chromatography-mass spectrometry. *International Dairy Journal*. vol. 32. pp. 199-203.

Andersson, I., Gyllenswärd, M. (2004). *Fältmässig undersökning av gårdar med höga respektive normala halter av fria fettsyror i mjölken.* Svensk mjölk. (Rapport: 7072-I)

Auldist, M.J, Hubble, I.B. (1998). Effects of mastitis on raw milk and dairy products. *The Australian Journal of Dairy Technology*. vol. 53. pp. 28- 36

Avramis, C.A., Wang, H., McBride, B.W., Wright, T.C., Hill., A.R. (2003). Physical and Processing Properties of Milk, Butter, and Chedar Cheese from Cows Fed Supplemental Fish Meal. *Journal of Dairy Science*. vol. 86. pp: 2568-2576.

Bauman, D.E., Griinari,J.M. (2001). Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. *Livestock Production Science*. vol. 70 pp. 15-29.

Bauman, D.E., Griinari, J.M. (2003). Nutritional Regulation of Milk Fat Synthesis. *Annual Reviews Nutrition.* vol. 23. pp. 203-227.

Beob,K.G., Stein, H. (2009). *A spreadsheet program for making a balanced Latin Square design* . http://www.scielo.org.co/scielo.php?pid=S0120-06902009000400002&script=sci_arttext [available: 2015-10-25]

Berglund, I., Petterson, G., Östensson, K., Svennersten-Sjaunja, K. (2007). Quarter Milking for Improved Detection of Increased SCC. *Reproduction in Domestic Animals*. vol. 42. pp. 427 - 432.

Bergman,E.N. (1971). Hyperketonemia - Ketogenesis and Ketone Body Metabolism. *Journal of Dairy Science.* vol. 54. pp. 936 - 948.

Bruckmaier, R.M. (2001). Milk ejection during machine milking in dairy cows. *Livestock Production Science*. vol. 70. pp.121-124.

Bruckmaier, R.M., Macuhova, J., Meyer, H.H.D. (2001). Specific aspects of milk ejection in robot milking: a review. *Livestock Production Science*. vol 72. pp. 169-176.

Carroll, S.M., DePeters, E.J., Taylor, S.J., Rosenberg, M., Perez - Monti, H., Capps, V.A. (2006). Milk composition of Holstein, Jersey, and Brown Swiss cows in response to increasing levels of dietary fat. *Animal Feed Science and Technology*. vol. 131. pp. 451-473.

Couvreur, S., Hurtaud, C., Marnet, P.G., Faverdin, P., Peyraud, J.L. (2007). Composition of Milk Fat from Cows Selected for Milk Fat Globule Size and Offered Either Fresh Pasture or a Corn Silage-base Diet.*American Dairy Science Association*. vol. 90. pp. 392-403.

Dalgleish, D.G. (1992). Bovine milk protein properties and the manufacturing quality of milk. *Livestock Production Science*. vol. 35. pp. 75-93.

Davis, S.R., Farr, V.C., Stelwagen, K. (1999). Regulation of yield loss and milk composition during once-daliy milking: a review. *Livestock Production science*.vol 59. pp.77-94.

de Koning, K., Slaghuis, B., van der Vorst, Y. (2003). Robotic milking and milk quality: effect on bacterial counts, somatic cell counts, freezing point and free fatty acids. *Italian journal of Animal Science.* Vol 2. 291-299.

de Koning, K., Rodenburg, J. (2004). Automatic Milking: State of Art in Europe and North America. In: Meijering, A., Hogeveen, H., de Koning, C.J.A.M. Automatic milking - A better understanding. Wageningen, Netherlands: Wageningen Academic Publishers. pp. 27 - 37.

DeLaval. (2011). *Koncept för kotrafik*. Tillgänglig: http://www.delaval.se/-/Produkt-Information/Mjolkning/Systems/Automatic/Cow-traffic-concepts/ [2016-11-21]

Duffield, T.F., Lissemore, K.D., McBride, B.W., Leslie, K.E. (2009). Impact of hyperketonemia in early lactation dairy cows on health and production. *Journal of Dairy Science*. vol. 92. pp. 571-580.

Edwards, J.P., Jago, J.G., Lopez-Villalobos, N. (2013a). Short-term application of prestimulation and increased automatic cluster remover threshold affect milking characteristics of grazing dairy cows in late lactation. *Journal of Dairy Science*. vol. 96. pp.1886-1893.

Edwards, J.P., Jago, J.G., Lopez-Villalobos, N. (2013b). Milking efficiency for grazing cows can be improved by increasing automatic cluster remover thresholds without applying premilking stimulation. *Journal of Dairy Science*. vol. 96. pp. 3766-3773.

Eldeen Idriss, S., Tančin, V., Foltys, V., Kirchnerová, K., Tančinová, D., Vršková, M. (2013). Relationship between mastitis causative patogens and somatic cell counts in milk of dairy cows. Potravinarstvo. vol. 7 pp. 207-212.

Enjalbert, F., Nicot, M.C., Bayourthe, C., Moncoulon, R. (2001). Ketone Bodies in Milk and Blood of Dairy Cows: Relationship between Concentrations and Utilization for Detection of Subclinical ketosis. *Journal of Dairy Science*. vol. 84. pp. 583-589.

Fernandez, A.M., Oliveria, C.A.F., Lima, C.G. (2007). Effects of somatic cell counts in milk on physical and chemical characteristics of yoghurt. *International Dairy Journal*. vol.17. pp. 111-115.

Forsbäck, L., Lindmark-Månsson, H., Andrén, A., Åkerstedt, M., Andrée, L., Svennersten-Sjaunja, K. (2010). Day-to-day variation in milk yield and milk composition at the udder-quarter level. *Journal of Dairy Science*. vol. 93. pp. 3569-3577.

Ginsberg, R. (2012). Influence of milk yield and take-off settings on milking parlour performance and udder health. I:Hogeveen, H., Lam, T.J.G.M (red). *Udder health and communication*. Wageningen Academic Publisher. pp. 407-414

Graves, E.L.F., Beaulieu, A.D., Drackley, J.K. (2007). Factors Affecting the Concentration of Sphingomyelin in Bovine Milk. Journal of Dairy Science. vol. 90. pp. 706-715.

Hanuš, O., Vergricht, J., Ferlich, J., Macek, A., Bjelka, M., Louda, F., Janů, L. (2008). Analysis of raw cow milk quality according to free fatty acid contents in Czech Republic. *Czech J. Anim. Sci.* vol. 53. pp. 17-30.

Hermans, G.G.N., Ipema, A.H., Stefanowska, J., Metz, J.H.M. (2003). The Effect of Two Traffic Situations on the Behaviour and Performance of Cows in an Automatic Milking System. *American Dairy Science Association.* vol. 86. pp. 1997-2004.

Hernandez, L.L., Stiening, C.M., Wheelock, J.B., Baumgard, L.H., Parkhurst, A.M., Collier, R.J. (2008). Evalution of Serotonin as a Feedback Inhibitor of Lactation in the Bovine. *Journal of Dairy Science*. vol 91. pp. 1834-1844.

Hillerton, J.E., Pankey, J.W., Pankey, P. (2002). Effect of over-milking on teat condition. *Journal of Dairy Research*. vol. 69. pp. 81- 84.

Hogeveen, H., Ouweltjes, W., de Koning, C.J.A.M., Stelwgen,K. (2001). Milking interval, milk production and milk flow-rate in an automatic milking system. *Livestock Production Science.* vol.72. pp. 157-167.

Johansson, B., Uvnäs-Moberg, K., Knight, C.H., Svennersten-Sjaunja, K. (1999a). Effect of feeding before, during and after milking on milk production and the hormones oxytocin, prolactin, gastrin and somatostatin. *Journal of Dairy Research*. vol. 66 pp. 151-163

Johansson, B., Svennersten-Sjaunja, K., Uvnäs-Moberg, K. (1999b). Utfodring under mjölkning - kan påverka kons fysiologi, mjölkavkastning och beteende. *Fakta Jordbruk*. vol. 7.

Jorritsma, R., Baldée, S.J.C, Schukken, Y.H., Wensing, Th., Wentink, G.H. (1998). Evalution of a milk test for detection subclinical ketosis. *The Veterinary Quarterly*. vol 20 pp. 108-110.

Kay, J.K., Weber, W.J., Moore, C.E., Bauman, D.E., Hansen, L.B., Chester-Jones, H., Crooker, B.A., Baumgard, L.H. (2005). Effects of Week of lactation and genetic selection for Milk Yield on Milk Fatty Acid Composition in Holstein Cows. *Journal of Dairy Science*. vol. 88. pp. 3886-3893.

Klungel, G.H., Slaghuis, B.A., Hogeveen, H. (2005). The Effect of the introduction of Automatic Milking System on Milk Quality. *Journal of Dairy Science*. vol. 83. pp. 1998-2003.

Knight, C.H., Peaker, M., Wilde, C.J. (1998). Local control of mammary development and funktion. *Reviews of Reproduction*. vol 3. pp. 104-112.

Larsen, T., Nielsen, N.L. (2005). Fluorometric Determination of β - Hydroxybutyrate in Milk and Blood Plasma. *Journal of Dairy Science*. Vol.88. pp. 2004-2009.

Larsen, T. (2011). Enzymatic-flourometric quantification of cholesterol in bovine milk. *Food Chemistry*. vol. 135. pp. 1261-1267.

Leitner, G., Krifucks, O., Merin, U., Lavi, Y., Silanikove, N. (2006). Interactions between bacteria type, proteolysis of casein and physico-chemical properties of bovine milk. *International Dairy Journal*. vol. 16. pp 648-654.

Lukkarinen, J., Lannhard Öberg, Å. (2012). *Marknadsöversikt - Mjölk och mejeriprodukter*. Jönköping: Jordbruksverket. (Rapport, 2012:7) Available: http://www2.jordbruksverket.se/webdav/files/SJV/trycksaker/Pdf_rapporter/ra12_7.pdf (2017-04-15).

Magliaro, A.L., Kensinger, R.S., (2005). Automatic Cluster Remover Setting Affects Milk Yield and Machine-on Time in Dairy Cows. *Journal of Dairy Science.* vol. 88. pp. 148-153.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A., Wilkinson, R.G. (2011). *Animal nutrition*. 7.ed. Harlow: Pearson Education Limited.

Melendez, P., Pinedo, P., Bastias, J., Pas Marin, M., Rios, C., Bustarmante, C., Adaro, N., Duchens, M. (2016). The association between serum ß-hydroxybutyrate and milk fatty acid profile with special emphasis on conjugate linoleic acid in postpartum Holstein cows. *BMC veterinary research*. vol. 12. pp. 1-5.

Melin, M., Hermans, G.G.N., Pettersson, G., Wiktorsson, H. (2006). Cow traffic in relatio to social rank and motivation of cows in an automatic milking system with control gates and an open waiting area. *Applied Animal Behaviour Science.* vol.96. pp. 201-214.

Nielsen, N.I., Larsen, T., Bjerring, M., Ingvartsen, K.L. (2005). Quarter Health, Milking Interval, and Sampling Time During Milking Affect the Concentration of Milk Constituents. *Journal of Dairy Science*. vol.88. pp. 3186-3200.

Peaker, M., Wilde, C.J. (1996). Feedback Control of Milk Secretion from Milk. *Journal of Mammary Gland Biology an Neoplasia*. vol. 3. pp. 307-314.

Prescott, N.B., Mottram, T.T., Webster, A.J.F. (1998). Relative motivations of dairy cows to be milked or fed in a Y-maze and an automatic milking system. *Applied Animal Behaviour Science*. vol. 57. pp 23-33.

Sjaastad, V. Ø., Sand, O., Hove, K. (2010). *Physiology of Domestic Animals*. 2. ed. Oslo: Scandinavian Veterinary Press.

Stadtmüller, L. (2014).The Importance of feeding during milking and take-off level for milking efficiency and milk production. Swedish University of Agricultural Sciences. Department of Animal Nutrition and Management (Fördjupningsarbete 2014:502)

Stewart, S., Godden, S., Rapnicki, P., Reid, D., Johnson, A., Eicker, S. (2002). Effects of Automatic Cluster Remover Settings on Average milking Duration, Milk Flow, and Milk Yield. *Journal of Dairy Science.* vol. 85. pp. 818-823.

Svennersten-Sjaunja, K.M., Pettersson, G. (2008). Pros and cons of automatic milking in Europe. *Journal of Animal Science*. vol. 86. pp. 37-46.

Svennersten-Sjaunja, K., Wiktorsson, H. (2002). Mer fria fettsyror i mjölken vid korta och oregelbundna mjölkningsintervall. *Fakta Jordbruk.* vol 19. pp. 1-4.

Växa Sverige. (2013). *Mjölkkobesättningarna blir större, men antalet gårdar minskar.* http://www.vxa.se/Om-oss/Press/Nyheter/2013/Mjolkkobesattningarna-blir-storre-men-antalet-gardar-minskar/ [available : 2016-05-14]

Wiking, L., Stagsted, J., Björck, L., Nielsen, J.H. (2004). Milk fat globule size is affected by fat production in dairy cows. *International Dairy Journal*. vol. 14. pp. 909 - 913.

Wiking, L., (2005). *Milk Fat globule stability- Lipolysis with Special Reference to automatic milking systems.* Diss. Uppsala: Sveriges Lantbruksuniversitetet

Wiking, L., Nielsen, J.H., Båvious,A-k., Edvardsson, A., Svennersten-Sjaunja, K. (2006). Impact of Milking Frequencies on the Level of Free Fatty Acids in Milk, Fat Globule Size, and Fatty Acid Cmposition. *Journal of Dairy Science*. vol. 89. pp. 1004-1009.

Wilde, C.J., Addey, C.V.P., Boddy, L.M., Peaker, M. (1995). Autocrine regulation of milk secretion by a protein in milk. *Biochem. J.*vol. 305. pp. 51-58.