Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Sciences

Compact total mixed ration to dairy cows

– effects on feed hygiene, feed intake, rumen environment and milk production

Kompakt fullfoder till mjölkkor

– effekter på foderhygien, foderintag, våmmiljö och mjölkproduktion

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Abstract

Total mixed rations have been used as a feeding strategy in Swedish dairy farms since the 1990’s. One risk that has been associated with total mixed ration is sorting of the ration, which can lead to a diet being consumed by the cows other than the originally intended. Sorting of the feed ration has been linked to several health and production problems in dairy cows. A development of total mixed ration, called compact total mixed ration, has recently been suggested as a way of reducing sorting of the ration. The feed ingredients of the compact total mixed ration is manipulated by soaking of all concentrates in advance and then mixed to decrease forage particle size. The compact total mixed ration has a dry matter target of 37 % and the feed components should be close to indistinguishable from each other, due to the prolonged mixing. In the present study, the effects of compact total mixed ration on feed intake, milk production and components, rumen environment, digestibility and hygienic quality was evaluated compared to a conventional total mixed ration. When compact total mixed ration was fed, dry matter intake decreased whereas total water intake increased. Milk production, milk composition and rumen environment were not affected by treatment. The effect of dietary treatments on digestibility could not be fully established. There was a tendency for milk fat yield to decrease when compact total mixed ration was fed. The hygienic quality of the diets were similar. More research is needed to evaluate the long term effects of compact total mixed ration as well as to investigate the effect of different forage proportions in the diet. Further, it was concluded that more research is needed to evaluate the effects of compact total mixed ration during summer conditions.

Sammanfattning

Fullfoder har använts som utfodringsstrategi inom svensk mjölkproduktion sedan 90-talet. En risk med fullfoder är att fodret sorteras, vilket kan leda till att en annan foderstat än den ämnade konsumeras. Sortering av fullfoder har tidigare kopplats till flertalet hälso- och produktionsproblem hos mjölkkor. En vidareutveckling av fullfoder, kallad kompakt fullfoder, har nyligen föreslagits som ett sätt att minska sorteringen av foderstaten. Ingredienserna i kompakt fullfoder blötläggs i förväg för att sedan mixas tillsammans med grovfodret. Fodret ska mixas så pass mycket att grovfodrets partikelstorlek minskas. Det kompakta fullfodret ska ha 37 % torrsubstans som mål, och foderkomponenterna ska vara svåra att särskilja på grund av den utökade mixningen. I denna studie utvärderades effekterna av kompakt fullfoder på foderintag, mjölkproduktion, mjölksammansättning, våmmiljö, smältbarhet och hygienisk kvalitet jämfört med ett konventionellt fullfoder. När kompakt fullfoder utfodrades minskade foderintaget i kg torrsubstans medan det totala vattenintaget ökade. Mjölkproduktion, mjölksammansättning och våmmiljö påverkades inte. Effekten av foder på smältbarhet gick ej att fastställa helt. Det fanns en tendens till att mjölkfettsavkastningen minskade när kompakt fullfoder utfodrades. Mer forskning behövs för att se effekterna av kompakt fullfoder över tid, samt för att undersöka effekten kompakt fullfoder vid andra proportioner av grovfoder. Vidare drogs slutsatsen att ytterligare forskning behövs för att bestämma effekten av kompakt fullfoder på hygienisk kvalitet under sommarförhållanden.

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1. Introduction

Total mixed ration (TMR) is an established feeding strategy for dairy cows in Sweden. A TMR feeding system is when all feed components, forage as well as grain and other concentrates, are mixed together before feedout and placed on the feeding table as one feedstuff when offered to the cows. It is for instance an easy way of incorporating home grown protein sources in the feed ration, which is of particular interest to organic dairy farmers. One of the challenges of practicing TMR as the feed strategy on a farm is to prevent sorting of the feed ration (Leonardi & Armentano, 2003), especially for the high yielding dairy cows that receive rations with large amounts of concentrates. Sorting of the ration has been linked to health conditions such as sub-acute ruminal acidosis (SARA; Keunen *et al.*, 2002; Shaver, 2002).

In recent years, a development of TMR has been suggested. It is called "compact total mixed ration" (CTMR). The CTMR is wetter and cut into finer particles, compared with conventional TMR. The dry matter target is around 37 % and the feed components should be close to indistinguishable from each other (Kristensen, 2015). The technique was developed in Denmark a few years ago with the primary goal that every cow in the group should have access to *ad libitum* amounts of a TMR that cannot be sorted (Kristensen, 2015). The founders of this concept states that CTMR will reduce sorting of the ration, maintain a high nutritive quality throughout the day and generate a calmer herd by eliminating competitive behaviour at the feed bunk (Kristensen, 2015).

According to Kristensen (2015), there are two major factors to be able to succeed. First, it must be assured that cows really have *ad libitum* access to the feed by supplying enough in the feed bunk to ensure at least two percent orts. Secondly, the feedstuffs must be physically manipulated according to the protocol of CTMR. There are three steps to follow; soaking, first mixing and final mixing. In the soaking phase, all concentrates in form of pellets and other dry feedstuffs are soaked in the mixer for a minimum of one hour, depending on feedstuff. In the second phase, grass silage is added to the mixer and mixed for 15-20 minutes to decrease forage particle size (FPS) and to make the concentrates stick to the fibrous fraction of the feed ration. The final phase of mixing is when corn silage is added to the CTMR. This procedure put demands on the mixers in such terms as that they can adequately reduce the FPS and mix the ration sufficiently. Many mixers, and especially the vertical auger mixers, need modifications to be able to handle this type of feeding regime (Kristensen, 2015). According to Kristensen (2015), the mixer type that makes the most compact CTMR is the horizontal auger mixer.

Practical results from farms in Denmark have shown that feeding CTMR can result in *e.g.* higher milk production, fewer overweight cows because of the decreased sorting, improved feed efficiency and an overall better health situation for the cows (Kristensen, 2015). However, reported results have so far been from commercial farms, and no previous controlled experiments has been done on this procedure.

The objectives of this study were therefore to, under controlled conditions, investigate the effect of compact total mixed ration on dairy cow physiological factors such as feed intake, milk production, milk composition, digestibility and rumen environment as well as on hygienic quality of the feed ration compared to a conventional total mixed ration. The effect of feeding compact total mixed ration to dairy cows on sorting of the feed ration, time budget and social interactions at the feeding table will be presented in a thesis yet to be published (Robertsson, 2018).

The hypotheses were that compact total mixed ration will increase feed intake and milk yield without affecting milk composition, ruminal pH or digestibility. It was also hypothesised that the hygienic quality of the feed will be negatively affected by soaking and prolonged mixing.

2. Literature review

2.1 Total mixed ration

Total mixed ration has been performed in the US since the 1960's and the feeding practice was first introduced to Swedish farmers in the 1970's (Pehrsson & Spörndly, 1994). However, it did not start to gain ground in Sweden until in the 1990's, mostly due to the small herds and the cost to invest in the new feeding equipment that was required (Pehrsson & Spörndly, 1994). The idea with TMR is to provide a feed consistent in nutritional composition all the time (Coppock *et al*., 1981), which can be beneficial in several ways.

One of the advantages of serving the feed ration as a homogenous feedstuff is that it reduces competition at the feed bunk (Coppock *et al.*, 1981). The usage of TMR also means that feeding large portions of grain or other concentrates separate can be avoided, which decreases the risk of acidosis or SARA (Hulsen & Aerden, 2015) because of a more stable pH in the rumen (Gill, 1979). When TMR was fed in early lactation stages the risk of other metabolic disorders such as enteritis and ketosis was reduced, compared with when the ration components was separately fed (Ostergaard & Grohn, 2000). Other advantages with TMR is the possibility to easily use by-products from the food industry (Gill, 1979) and home grown protein sources, as well as that less palatable ingredients can be incorporated into the mix (Coppock *et al.*, 1981). Providing TMR to the cows has also shown to increase daily total dry matter intake (DMI) compared to feeding roughage and concentrates separately (Nocek *et al.*, 1986; Spörndly, 1987). When TMR is fed in early lactation, the increased DMI can lead to that the negative energy balance after parturition is reversed sooner than when feed ingredients are fed separately (Spörndly, 1987).

When feeding a TMR, it is common to provide the feed *ad libitum* to the animals (Martinsson, 1994). Feeding *ad libitum* means giving the animal free access to the feed. All animals can receive the same mix or the herd can be divided into smaller groups and the energy content of the mix adjusted thereafter (Martinsson, 1994). However, there is a risk that individual animals will consume more TMR than estimated for, some up to 40 % more (Pehrsson & Spörndly, 1994), which can result in over conditioned cows and use of excessive amounts of feed. Another challenge related to feeding TMR is to make the feed sufficiently homogenous when mixing the feedstuffs, to prevent the animals from sorting out the more palatable parts of the ration. In order to get a homogenous mix, a lot of technical aspects are of importance. For instance, to be precise when weighing in the ingredients into the mixer and to account for DM fluctuations in the roughage. If no sorting is done, the nutritional composition of the feed ingested will be the same all the time, for all cows.

2.2 Feeding of starch

2.2.1 Feeding starch and forages separately

One of the advantages emphasised with feeding TMR is the ability to achieve an even flow of starch to the rumen, avoiding sudden elevations of starch levels (NRC, 2001). When large portions of easily degradable carbohydrates such as starch enter the rumen, a rapid accumulation of volatile fatty acids occurs due to microbial fermentation. The increased acid production result in a lowered ruminal pH. Bicarbonate is one of the ruminal buffers (Owens, 1998). Salivary bicarbonate compose approximately half of bicarbonate that enter the rumen (Owens, 1998), thus an important contributor of the buffer. Including starch rich ingredients, such as grains, in the diet has been considered to reduce chewing time and therefore decrease the flow of bicarbonate from saliva to the rumen (Owens, 1998). The largest effect:

Feeding concentrates separately from forages in early lactation increases the risk of metabolic disorders, such as ketosis and enteritis, more than the forage to concentrate (F:C) ratio in a TMR (Ostergaard & Grohn, 2000). Another risk with separate feeding, is that the cow finishes all the concentrates allocated to her although not the all the forage, leading to the cow consuming a different feed ration than intended (Maekawa *et al.*, 2002). In fact, an actual F:C ratio of 43:57 was found when forages and concentrates was separately offered, even though the intended ratio was 50:50 (Maekawa *et al.*, 2002). If the actual ratio of the separate feeding was compared to when a TMR with about the same ratio (40:60) was fed, no evidence of differences in DMI, milk production or ruminal pH could be detected. However, if the separate feeding was compared to a TMR with the same F:C as originally intended (50:50), a lower minimum pH value could be shown as well as a tendency towards lower mean ruminal pH.

2.2.2 Forage to concentrate ratio of a TMR

Forage to concentrate ratio can vary considerably depending on measures, such as production strategy. For instance, organic certification labels can require that a lower amount of grains is used. The regulations of the Swedish organic certification label KRAV demands that the maximum inclusion of concentrate can be 40 % to dairy cows, except for the first three months of lactation when it can be increased to 50 % of feed ration DM (KRAV, 2017). The percentage of forage in a TMR diet can affect measures such as feed intake, milk production and milk constituents (Yang & Beauchemin, 2007; Nasrollahi *et al.*, 2015) as well as rumen environment (Kennelly *et al.*, 1999; Nocek *et al.*, 2002; Yang & Beauchemin, 2007) and digestibility (Yang *et al.*, 2001b).

Cows that receive diets with high inclusion of grains maintain a lower pH for a longer duration of time (Nocek *et al*., 2002; Yang & Beauchemin, 2007). For instance, when cows were fed TMR's with F:C ratios of 30:70 and 40:60, the time ruminal pH remained below 6.00 was longer, compared with when fed a F:C ratio of 50:50 (Nocek *et al*., 2002). When a feed ration with a F:C ratio of 35:65 was fed, both a lower diurnal ruminal pH and a longer duration of time below pH 5.80 was found compared to when a feed ration with F:C ratio of 60:40 was fed (Yang & Beauchemin, 2007). In the study by Yang & Beauchemin (2007), it was also seen that the low forage proportion increased the milk production but lowered the percentage of milk fat content. This is consistent with findings by Olsson *et al*. (1998) who lowered the F:C ratio from 60:40 to 40:60 in a ration to dairy cows in early lactation, which resulted in decreased milk fat content. In agreement with this, Maekawa *et al*. (2002) reported tendencies toward a reduction in milk fat percentage when forage content was lowered. A high inclusion of forage (55 % DM basis) in the TMR has further been linked to decreased digestibility of the diet, compared to when forage constituted 35 % on DM basis (Yang *et al.*, 2001b).

2.3 Sub-acute ruminal acidosis

The development of sub-acute ruminal acidosis in cattle is a response to a diet with little structure and high energy density, in combination with a rumen that has not been adapted to a diet with such properties (Kleen *et al.*, 2003). Whilst there is a high risk for SARA in early lactation when cows changes from a diet high in fiber to an energy dense diet adjusted to a high milk yield (Nocek, 1997), there is also a risk for SARA in mid-lactation (Kleen *et al.*, 2003). Since mid-lactation cows are probably already adapted to an energy dense diet, the problem during this stage is most likely related to feeding management errors or sorting of the feed ration (Kleen *et al.*, 2003). Signs of SARA includes reduced DMI (Krajcarski-Hunt *et al.*, 2002), episodes of laminitis (Nocek, 1997), milk fat depression and diarrhoea (Kleen *et al.*, 2003). It has also been concluded that when cows were suffering from SARA they were more likely to select a feed with larger particle size, when offered both alfalfa hay and alfalfa in the form of pellets (Keunen *et al.*, 2002). Induction of SARA has also been proven to reduce *in situ* digestibility of netural detergent fiber (NDF) in grass and alfalfa hays as well as corn silage (Krajcarski-Hunt *et al.*, 2002).

When the ruminal pH decreases to below 5.80, the ability to digest fiber is depressed, while a pH over 6.20 improves fiber degradation (Shriver *et al.*, 1986). There have been several suggestions for ruminal pH cut-off points to define SARA. One threshold that has been used is when pH is 5.50 or below when sampling through rumenocentesis (Garrett *et al.*, 1999). However, Duffield *et al.* (2004) concluded that pH sampling through a ruminal cannula gave a 0.30 pH units higher result than sampling with rumenocentesis. It was therefore proposed that the threshold value should be adjusted thereafter to 5.80, when evaluating samples from a ruminal cannula. Krause *et al.* (2002b), Krause & Combs (2003) and Beauchemin (2003) all studied the area or time pH was beneath 5.80. Another definition that has been used to define SARA is when ruminal pH was between 5.20 and 5.60 (Owens *et al.*, 1998; Stone, 2004), also used by Gozho *et al.* (2005) although with the addition of a duration, for at least three hours per day. Several studies have been able to show the usefulness in not solely looking at the daily average rumen pH but also monitoring the fluctuations over the day when evaluating ruminal health (Kennelly *et al.*, 1999; Krause *et al.*, 2002b; Krause & Combs, 2003). In these studies, it was demonstrated that diets tested gave very similar daily mean ruminal pH but provided considerable variations in during a 24 hour period.

2.4 Physically effective neutral detergent fiber

An energy dense feed ration is important to support a high yielding dairy cow's milk production. However, dairy cows are adapted to digesting fiber. It has long been a common routine to measure the fibrous content of feed rations to dairy cows, to be able to ensure that both animal health and milk production are maintained. An excessive fiber content in the ration can be limiting to DMI and thereby cows might fail to satisfy their energy demands (Mertens, 1994). An inadequate fiber content can on the other hand, lead to depressed ruminal pH and milk fat depression (NRC, 2001). However, the way fiber content has been measured or estimated has differed over the years. Twenty years ago, Mertens *et al*. (1997) created a systematic definition of a factor called physically effective neutral detergent fiber (peNDF).

Physically effective neutral detergent fiber is the fibrous part of the feed that stimulates chewing, rumination and contributes to the ruminal mat (Mertens, 1997). It has also been considered to be of importance for prevention of rumen disorders (Zebeli *et al.*, 2011; Zebeli *et al.*, 2010). The major physical trait associated with peNDF is the particle size of the feedstuff (Mertens, 1997). The factor predicts how much chewing is required in relation to particle size. Furthermore, peNDF is supposed to give an indication of what amount of the feed will flow out of the rumen directly and how much that will remain in the rumen for a longer period of time (Mertens, 1997). The value is based on NDF-content of the feed and how effectively the particular NDF-source encourages chewing behaviour, called the physical effectiveness factor (Mertens, 1997). To simplify the use of the physical effectiveness factor, the authors suggested that it can be equated with the amount of particles larger than 1.18 mm. The recommended amount of peNDF in the feed ration in order to keep the daily average rumen pH above 6.00 was set to 20-22 % by Mertens (1997). In order to prevent SARA, Zebeli *et al*. (2008) recommended an amount of 30-33% in the feed ration.

To measure particle size distribution of forage or TMR, a Penn state particle separator (PSPS) can be used. The PSPS in its original appearance consisted of two sieves and a bottom pan (Lammers *et al.*, 1996). The sieves are fitted with holes, where particles smaller than the diameter of the hole can pass through. The holes of the upper sieve has a diameter of 19 mm, the holes of the lower a diameter of 8 mm and the rest of the particles end up in the pan (Lammers *et al.*, 1996). To be able to measure amount of particles smaller than 1.18 mm an adjustment of the PSPS, and thus a third sieve, was proposed by Kononoff *et al.* (2003a). However, if a PSPS is used in its original appearance it has been suggested that the sum of the particle fractions of the two sieves can be used to calculate peNDF (Beauchemin & Yang, 2005). The authors emphasised, though, that the results should only be compared to other results obtained by the same method.

The distribution of particles on the sieves of the PSPS has been shown to be affected by DM content of the TMR (Felton & DeVries, 2010; Miller-Cushon & DeVries, 2009). A wetter TMR resulted in a larger fraction of feed DM retained on the 8 mm sieve at expense of the pan, and was thought to be a result from smaller particles sticking to larger particles because of the added water (Felton & DeVries, 2010; Miller-Cushon & DeVries, 2009).

2.5 Particle size of the feed ration

The feed ingredient in a feed ration that can vary the most in particle size is roughage. The particle size of a roughage can be controlled by measures such as forage cut length and mixing time before feedout. The greatest part of dietary NDF in a feed ration comes from forage sources that has a structure which encourage chewing activities (NRC, 2001). It has been suggested that it is more likely the particle size of the forages included in the diet that stimulates chewing activity, rather than the particle size distribution of the whole TMR (Yang *et al.*, 2001a). Even so, if FPS is altered in a TMR it will as a consequence affect the TMR particle size distribution as well.

Diets with high proportions of forage can therefore limit high yielding dairy cows' possibilities to fulfil their nutrient requirements because of their ruminal filling effect (Allen, 2000), which has been associated with decreased DMI (Kononoff & Heinrichs, 2003). In order to prevent forage from giving an excessive ruminal fill, it is many times chopped to smaller pieces before being preserved. The recommended mean FPS to maintain normal rumen function is at least 3 mm, according to NRC (2001). The NDF content in the feed ration should be at least 25 % with a minimum of 19 % (of total NDF content in ration) coming from forage sources (NRC, 2001). Further, it is stated that if mean FPS is smaller than 3 mm, dietary NDF need to be increased (NRC, 2001). On the other hand, a shorter forage particle length contributes to the uniformity of a TMR. There have been studies suggesting that forage feed processed into finer particles actually does give a lower ruminal fill value compared to the same feed provided in coarser particles (Nasrollahi *et al*., 2015), leading to a higher potential DMI. Some argue though that larger particles are necessary to a certain extent, to promote saliva production by giving a longer chewing time and increased rumination and thereby help buffering ruminal pH (Mertens, 1997).

2.5.1 Feed intake

Whether or not FPS has an effect on DMI has been debated and the results has differed. Forage particle size did not affect DMI when two alfalfa silage TMR's with a F:C ratio of 39:61, similar in chemical composition but differing in FPS, were compared (Table 1; Krause *et al*., 2002a). This is in compliance with a study that used TMR with a F:C ratio of 42:58 with corn silage in different chop lengths to alter FPS distribution (Table 1; Beauchemin & Yang, 2005). Also, a study comparing two different grass hay FPS in a TMR, could not prove an effect of FPS on DMI (Storm & Kristensen, 2010). Just like previously mentioned authors, Beauchemin *et al.* (2003) did not find an effect on DMI when TMR's with alfalfa silage, alfalfa hay and concentrate was provided cows and the FPS was varied by different particle lengths of the hay

(Table 1). They suggested that forage proportion (40 % of DM) in the feed ration was not enough to give an effect of decreased FPS on DMI.

Table 1. Particle size distribution of TMR, forage to concentrate ratio (F:C) on dry matter (DM) basis, and effects of altering FPS in a TMR. Dry matter intake (DMI) and milk yield expressed as kg day^{-1}, pH as daily mean ruminal pH and digestibility as DM digestibility. "NS" = non-significant, "-" = not measured

Reference		TMR particle size distribution (%)				Effects of FPS			
	F:C		>19 mm	8 mm	<8 mm	DMI	Milk yield	pH	Digestibility
Krause *et al*. (2002ab)	39:61	Diet 1	24	22	55	NS	NS	Decreased with decreased PS	NS
		Diet 2	22	24	54				
		Diet 3	0	15	85				
		Diet 4	0	11	89				
Beauchemin & Yang (2005)	42:58	Diet 1	9	26	65	NS	-	NS	-
		Diet 2	8	24	68				
		Diet 3	7	21	72				
Kononoff & Heinrichs (2003)	50:50	Diet 1	31	18	51	Increased when PS decreased	NS	Higher on Diet 2 and 3	Increased when PS decreased
		Diet 2	22	21	57				
		Diet 3	12	25	63				
		Diet 4	3	28	69				
Maulfair *et al*. (2010)	59:41	Diet 1	15	19	66	Increased when PS decreased	NS	NS	-
		Diet 2	13	20	67				
		Diet 3	11	22	67				
		Diet 4	6	24	70				
Beauchemin *et al*. (2003)	40:60	Diet 1	12	26	62	NS	NS	Decreased with decreased PS	-
		Diet 2	9	32	59				
		Diet 3	1	30	69				
		Diet 4	0	22	78				
Alamouti *et al*. (2009)	37:63	Diet 1	16	21	63	NS	NS	NS	NS
		Diet 2	14	20	66				
		Diet 3	11	21	68				
		Diet 4	11	20	69				
Yang *et al*. (2001b)	35:65 or 55:45	Diet 1	5	34	61	NS	NS	NS	NS
		Diet 2	0.5	32	67.5				

Yet, there has also been indications that a decreased FPS can lead to an increased DMI. This matter was addressed by Nasrollahi *et al.* (2015), who concluded that it was when the forage proportion in a TMR exceeded 50 % DM of the feed ration that measures such as particle size of forage played a larger role. As a result of that, reduction of the FPS when TMR forage level was above 50 % increased DMI by more than 0.5 kg per day (Nasrollahi *et al*., 2015). Leonardi *et al*. (2005) reported a linear relationship between decreasing mean FPS and increasing DMI when F:C ratio was 50:50 (DM basis) and results pointed towards that a larger FPS would be limiting for daily DMI. Kononoff & Heinrichs (2003) found a linear relationship between decreasing FPS and increasing DMI when haylage based (50 % DM) TMR's varying in FPS (Table 1) was compared. They could also see that decreasing FPS linearly reduced the eating time as well.

That decreasing FPS linearly increase DMI was further endorsed by Maulfair *et al*. (2010) where a forage based (59 %) TMR with corn silage, haylage and grass hay (29.4, 17.6 and 11.8 % on a DM basis) was fed and the FPS (Table 1) was altered by varying the chop length of hay. Forage particle size's effect on DMI was studied on cows in early lactation, 19±4 days in milk (DIM) in the study by Kononoff & Heinrichs (2003). The authors discussed that the lack of effect of decreased FPS on DMI in other studies could be due to that cows used were later in lactation and could more easily cover their energy demands.

2.5.2 Milk production and composition

It has long been a recommendation to be careful with decreasing FPS to not disturb milk fat production. Earlier literature concluded that a mean particle size of 6.4 mm was required to avoid milk fat depression (Woodford *et al*., 1986). Shaver (1986) found that when the forage in a feed ration was offered in the form of a pelleted alfalfa hay in instead of chopped or long alfalfa hay, milk fat percentages dropped by more than 0.5 percentage units. That decreased FPS can depress milk fat production was further endorsed when silage with mean geometrical lengths of 2 mm and 3.1 mm was fed in TMR's and a decrease in milk fat percentage by 0.8 percentage units was observed for the finer silage (Grant *et al.*, 1990). In more recent years, this has been supported by additional research that has been able to prove a relationship between declining milk fat percentage and reduced FPS (Nasrollahi *et al.*, 2015; Krause & Combs, 2003).

However, there are other milk production parameters besides milk fat percentage that could be influenced by FPS. Reduced FPS has been reported to linearly increase both milk yield and milk protein yield (Leonardi *et al.*, 2005b). This is supported by the work of Nasrollahi *et al*. (2015) who it was concluded that reduction of FPS also led to an increase in milk yield and milk protein yield, in addition to reduced milk fat percentage. When alfalfa hay in a TMR was altered in FPS (Table 1), a reduction in milk lactose content was observed (Alamouti *et al.*, 2009). The authors could not explain the reason for the increase in lactose yield, but speculated that the reduction in FPS could have led to an increased passage rate from the reticulorumen and therefore improved postruminal digestibility.

In contrast, there are several examples of studies that has not been able to prove that a reduction in FPS has any effect on either the milk yield or the milk composition (Krause *et al.*, 2002a; Beauchemin *et al.*, 2003; Maulfair *et al.*, 2010). These studies seem to agree on that FPS does not affect either milk yield or milk constituents. For example, when different FPS (Table 1) was fed together with moist or dry cracked corn in a TMR, no effect of FPS on milk fat production could be seen (Krause *et al*., 2002a). The studies by Beauchemin *et al.* (2003) and Krause *et al*. (2002a) lacked an effect of FPS on DMI, thus the absence of effect for FPS on milk production was not surprising. However, Maulfair *et al*. (2010) did see an increase in DMI without a subsequent increase in milk production.

It has been suggested that studies that has resulted in an increase in DMI but lacked a corresponding increase in milk yield and milk constituents could have been due to too short experimental periods or that animals are in later stages of lactation (Zebeli *et al.*, 2012). It was further suggested that when cows are late in lactation, the extra energy from feed could be used for body fat synthesis rather than milk synthesis (Zebeli *et al*., 2012). It has also been argued that if an increase in DMI can be achieved, it would explain why there would be a consequent increase in milk yield (Beauchemin *et al.*, 2003), suggesting that FPS might have a stronger link to DMI rather than directly to milk yield.

2.5.3 Rumen environment

A drop in ruminal pH promotes non-cellulolytic microbial activity and restrain microbes that utilize cellulose as their substrate (Mould *et al.*, 1983; Shriver *et al.*, 1986). Forage particle size is considered to have an effect on ruminal pH because of its role in rumination and chewing. Rumination and chewing elevates the levels of saliva in the rumen, which in its turn has a buffering effect on ruminal digesta (Allen, 1997). Ruminal pH can, apart from FPS, be affected by factors such as the amount and processing of concentrates and grains in the TMR, thus the inclusion of readily fermentable carbohydrates (Yang *et al.*, 2001b; Krause *et al.*, 2002b; Krause & Combs, 2003).

The effect of FPS on rumen environment with emphasis on ruminal pH can depend on what variable that is considered. The literature presented in the current study has indicated that studies which used a daily average of pH or only one single sampling of pH were less prominent to show significant differences (Kononoff *et al.*, 2003b; Alamouti *et al.*, 2009; Maulfair *et al.*, 2010), compared to studies which also included time or area of ruminal pH below a certain threshold during a day (Beauchemin *et al.*, 2003; Krause *et al.*, 2002b; Yang *et al.*, 2001b). Multiple studies found that when TMR's with different particle size distributions was compared, average pH between the feed rations was indeed similar (Table 1; Yang *et al.*, 2001b; Krause *et al.*, 2002b; Beauchemin *et al.*, 2003). In these studies, what really distinguished the treatment diets from each other was the time ruminal pH was below 5.80. Beauchemin *et al.* (2003) could show that a decreased FPS had no effect on mean ruminal pH, however, the decreased FPS almost doubled the time spent below pH 5.80. Even so, Krause *et al*. (2002b) found that both daily mean ruminal pH and time below pH 5.80 was decreased by decreased FPS when diets with different FPS distributions (Table 1) were compared. Although, there was a more pronounced effect of decreased FPS on time spent below 5.80 compared with mean ruminal pH.

Beauchemin & Yang (2005) could not find any differences in either mean rumen pH or time spent below pH 5.80 when comparing different FPS distributions (Table 1). This is in accordance with Maulfair *et al.* (2010) and Alamouti *et al.* (2009) who could not find that average or minimum daily ruminal pH was affected by FPS in the diet. However, Maulfair *et al*. (2010) altered the FPS of the ration by using different chop lengths of hay, which only constituted 20 % (DM basis) of the forage proportion in the TMR. The inconsistency in research on FPS's effect on ruminal pH was discussed by Beauchemin & Yang (2005), who suggested that the varying results could be due to other factors influencing more, such as diet fermentability and DMI.

2.5.4 Digestibility

Reduction of FPS in a feed ration can both enhance and reduce the digestibility of fiber, or the two factors can outplay each other, leading to lack of effect. When decreasing the FPS of a forage feedstuff, the surface area available for microbes increases and therefore microbial degradation can be reinforced (McDonald, 2011). On the other hand, a smaller FPS can lead to a higher ruminal passage rate, which leaves the cellulolytic microbes with less time for degradation (McDonald, 2011). The previous research on FPS's interaction with diet digestibility has resulted in varying conclusions and several explanations have been presented as reasons. For instance, the work of Nasrollahi *et al*. (2015) suggested that there can be an interaction between forage source and FPS. They found that when a TMR was forage based (>50 %, DM basis) without inclusion of corn silage, the DM digestibility of the feed ration increased when FPS was decreased. However, they could not confirm an overall effect for FPS in forage based diets on DM digestibility when corn silage was included.

Depressed fiber digestibility has been associated with decreasing FPS in earlier literature (Shaver *et al.*, 1986). In this study, long alfalfa hay was replaced by grounded alfalfa hay in pelleted form and an organic matter (OM) digestibility decrease by 3.2 percentage units was noted. In consistency with Nasrollahi *et al.* (2015), more recent research showed that a reduction of FPS in a ration with 50 % DM from forage resulted in increased DM digestibility of the feed ration (Kononoff & Heinrichs, 2003). In this study, the authors made the suggestion that this could be because of an increased available surface area for microbial degradation since the rumen passage rate was unchanged. Zebeli *et al.* (2012) also proposed that a moderate decrease in FPS could contribute to the improvement of fiber degradation.

However, there have been multiple studies that has concluded that FPS does not affect DM digestibility of the feed ration (Yang *et al.*, 2001a; Krause *et al.*, 2002a; Krause & Combs, 2003; Alamouti *et al.*, 2009). Other factors has been suggested to influence more, *e.g.* forage to concentrate ratio or grain processing (Yang *et al.*, 2001a). Alamouti *et al*. (2010) analysed digestibility using acid insoluble ashes (AIA) content in feed and in *faeces*. They could not find an effect of FPS on DM digestibility when an alfalfa hay based TMR was fed.

2.6 Dry matter content of the feed ration

The typical DM content of a TMR offered to a high producing dairy cow ranges from 40 – 60 % of the feed ration (Eastridge, 2006). The DM in a TMR feed ration is a result of the DM content of the feedstuffs used and their proportion in the mix, if not manipulated by the addition of water. Altering the DM content of a TMR by the addition of water into the mix has been used as a method for prevention of sorting of the feed ration (Shaver, 2002). Adding water to TMR has also been suggested as a method for limiting the nutrient intake of dairy cows in late lactation because of its negative influence on DMI (Felton & DeVries, 2010).

Table 2. Dry matter (DM) content of TMR and effects on dry matter intake (DMI) expressed as kg day^{-1}, milk yield expressed as kg day^{-1}, pH expressed as average daily ruminal pH and digestibility expressed as DM digestibility. *Feed ration not offered as TMR, DM content and effects expressed for silage consumption. "NS" = non-significant. "-" = not measured in the study

Reference			Effects			
		DM (%)	DMI	Milk yield	pH	Digestibility
Storm & Kristensen (2010)	Diet 1	53.8	NS	NS	NS	NS
	Diet 2	44.3				
Felton & DeVries (2010)	Diet 1	56.3	Decreased when DM decreased	NS	-	-
	Diet 2	50.8				
	Diet 3	44.1				
Miller-Cushon & DeVries (2009)	Diet 1	57.6	Decreased when DM decreased	NS	-	-
	Diet 2	47.9				
Fish & DeVries (2012)	Diet 1	61.7	NS	NS	-	-
	Diet 2	51.9				
Leonardi *et al*. (2005a)	Diet 1	80.0	NS	NS	NS	-
	Diet 2	64.4				
Gordon *et al.* (1965)*	Diet 1	38.6	NS	NS	-	Increased on Diet 2
	Diet 2	51.1				
	Diet 3	57.5				

2.6.1 Feed intake

Previous literature is indicative towards that lowering DM content from over 50 % DM to less than 50 % DM in a TMR can be limiting to dairy cows' DMI (Table 2; Miller-Cushon & DeVries, 2009; Felton & DeVries, 2010. Further, Eastridge (2006) stated that a DM content below 40 % in a TMR reduces DMI. When the DM content of a silage based TMR was altered by the addition of water (Table 2) the daily DMI was reduced by with 3.2 kg DM, even though the wetter ration increased feed intake in fresh weight (Miller-Cushon & DeVries, 2009). This was supported by a later study that compared TMR's with different DM content (Table 2) where a positive linear relationship between DM and DMI could be determined as well as that fresh feed intake was higher for the wetter ration (Felton & DeVries, 2010).

Therefore, it would seem that cows which receive a TMR with a lower DM content can have a higher fresh feed consumption than if offered a TMR with a higher DM content (Miller-Cushon & DeVries, 2009; Felton & DeVries, 2010). Both studies observed that even though the cows consumed more in kg fresh feed when offered the wetter ration, it was not enough to reach the same DMI as the dryer ration. One theory that has been made about increased fresh feed intake in cows when wet feed was compared to drier feed, is that cows try to compensate for the reduction in feed DM by consuming more fresh feed to cover their energy demands (Mertens, 1994). A reduction in DMI when a ration with added water is fed, has also been considered to be a result from a more extensive ruminal filling effect (Miller-Cushon & DeVries, 2009; Robinson *et al*., 1990). It was further suggested that the reasons for the increased ruminal filling effect were limitations in ruminal capacity to transport the water from the feed ration (Robinson *et al*., 1990; Miller-Cushon & DeVries, 2009). Another theory that regards ruminal fill, suggested by Robinson *et al*. (1990), was the “bulk effect” of the feedstuff due to intracellular water. It was proposed that this effect caused a higher ruminal fill until the feed was digested and water held inside the feed could be transported from the rumen. This can be a larger concern when the ration includes a high proportion of forage, because of its relatively high content of intracellular water and structural cell walls.

Lowering the DM content in TMR’s to over 50 % of DM content does not seem to lower dairy cows’ DMI according to the reviewed literature (Table 2).When the DM content of an alfalfa haylage and corn silage based TMR was lowered from 61.7 % to 51.9 %, a difference in DMI could not be detected (Fish & DeVries, 2012). In earlier experiments, when comparing TMR’s with 35 % and 45 % DM content and then 45 % with 60 % DM content no differences effect of DM content on DMI could be detected (Robinson *et al*., 1990). This was also the case when TMR’s with DM content 52 % was compared with 40 % DM (Lahr *et al*., 1983).

2.6.2 Milk production and composition

Dry matter content of TMR rations does not seem to affect milk yield or milk composition (Lahr *et al*., 1983; Robinson *et al*., 1990; Kellems *et al*., 1991; Leonardi *et al*., 2005a; Storm & Kristensen, 2010; Felton & DeVries, 2010; Fish & DeVries, 2012). Fish & DeVries (2012) discussed that they were not surprised by the lack of effect on milk yield and milk composition in their experiment, as there was no effect of DM content in the feed ration on DMI. Some authors have seen tendencies towards that adding water to the diet can give a higher milk fat percentage (Leonardi *et al.*, 2005a) or a lower protein percentage (Felton & DeVries, 2010). However, no further evidence has been found in the present literature review.

Felton & DeVries (2012) saw that even though DMI was reduced when DM content of the TMR was reduced, milk yield and milk composition were maintained. This resulted in a linear increase in milk production efficiency of both milk yield and 4 % fat corrected milk when DM content in the ration was reduced. The feed ration was however formulated for a higher milk production than the actual yield in the experiment and even the lowest DMI observed could cover the cows’ energy demands. The same phenomenon was seen by Miller-Cushon & DeVries (2009), where lower DM content in the TMR (Table 2) decreased DMI, although both milk yield and milk constituent proportions were maintained. Each treatment period in their change over experiment lasted 21 days and the authors hypothesised that if the experimental periods would have been longer, the decreased DMI would probably eventually have an effect on the milk production.

2.6.3 Rumen environment and digestibility

The DM content of the feed seem to have limited or no effect on rumen environment. No difference in ruminal fluid pH was found when TMR’s with a DM content of 52 % and 40 % DM (Lahr *et al*., 1983) were compared. No difference in ruminal pH could either be found when DM content of a TMR was lowered by 20 percentage units and offered to dairy cows (Table 2; Leonardi *et al*., 2005a). Further, there was no influence of diet on rumen pH values when Storm

& Kristensen (2010) compared diets with different DM contents (Table 2).

When it comes to DM content's influence on digestibility in feed rations, the research seems to be scarce. A study performed in the 60's concluded a small, yet significant, increase in DM digestibility on the intermediate DM concentration when silage was ensiled at different DM levels (Table 2; Gordon *et al.*, 1965). However, when the authors performed a consequent similar experiment, the results could not be further endorsed. The limited influence of DM content was further confirmed when more recently, a drier TMR ration was compared to a wetter ration (Table 2) and DM content had no influence on total tract digestibility (Storm & Kristensen, 2010).

2.7 Hygienic quality of the feed ration

2.7.1 Microbial activity in silage

When exposing silage to air, there is an increase in microbial activity (Woolford, 1990). When the activity by microorganisms is increased, it causes the feed temperature to rise (Kung *et al.*, 1998). One definition used for aerobic stability of a feedstuff is the amount of time before the temperature in the feedstuff elevates over 2 °C above ambient temperature (Kung *et al.*, 1998). However, temperature in itself is not certain proof of that feed spoilage has occurred. Elevated pH and signs of deterioration (*e.g.* visible mould) contributes to assurance of that spoilage is present (Kung, 2010). Another way to determine that feed spoilage is onset in the feed bunk is by identifying characteristic changes in the feed, for example yeast counts (Kung *et al.*, 1998). Yeast has been seen to be the first microorganism that proliferates during aerobic exposure of silage (Lindgren *et al.*, 1985).

Microorganisms in the silage degrade DM content and therefore decrease both the nutritive (Woolford, 1990) and the hygienic quality, referred to as feed spoilage. It has been stated that TMR's with a lower DM content are more unlikely to remain stable during higher ambient temperatures (Eastridge, 2006). Unwanted microorganisms that proliferates in silages during aerobic conditions are *e.g.* moulds and yeasts (Driehuis & Oude-Elferink, 2000; Kung, 2010). Many moulds are known to produce mycotoxins, of which some has the capability to end up in the meat or milk products as well as be hazardous to animal health (Driehuis & Oude-Elferink, 2000) in the form of respiratory or metabolic disorders (Wilkinson, 1999). Impaired hygienic quality of silage has also been shown to decrease the voluntary DMI of silage in dairy cows (Wichert *et al.*, 1998).

2.7.2 Microbial activity in TMR

When mixing TMR, several conditions that are known to be favourable for microorganisms occur (Seppälä *et al.*, 2012). Firstly, silage is mixed with starch rich concentrates, which are easily utilised substrates for microbes. Moreover, there is a risk that residues from earlier mixes are still present in the mixer, as well as mixing silage with concentrates increases the time before the TMR reaches the feed bunk which both increases the risk of microbial growth. Seppälä *et* *al.* (2012) concluded that when seven days old TMR or grass silage showing signs of deterioration was included in a freshly mixed TMR the aerobic stability was reduced compared with when TMR with only good quality silage was used. It was concluded that the diets with the shortest aerobic stability also had the highest microbial counts. The TMR's consisted of grass silage of different hygienic quality together with rapeseed expeller, concentrates and brewers' grains. The lowest microbial concentrations were found when good quality grass silage was used and brewer's grains were excluded from the diet.

An increase in feed temperature has previously been linked to increased microbial activity and spoilage of a TMR (Kung *et al.*, 1998). In the study, the TMR rations could increase between 10-18 °C in temperature during a 24 hour period (Kung *et al.*, 1998). The addition of water to a TMR can actually result in initially lower temperatures of the feed at feed out (Felton & DeVries, 2010). The reason for this was thought to be that temperature of the water added was lower than temperature of the feedstuff.

It has been concluded that TMR was less aerobically stable and contained higher yeast counts when compared with pure silage samples (Kung, 2010). This was thought to be a result from that the silage in a TMR has already been exposed to oxygen for some time before being mixed (Kung, 2010). In the same study, where 30 freshly mixed TMR's were sampled within 1 hour of mixing and then kept in 22 °C, it was shown that TMR could spoil in less than 12 hours. This was supported by the work of Cogan *et al*. (2017) where microbiological analyses were performed on silages and readily mixed TMR's from 39 farms. Samples were taken on average 4.5 hours after mixing and there was a tendency for TMR to contain higher concentrations of both yeasts and moulds compared with pure grass silage (Cogan *et al.*, 2017). A large variation was found in the study, but grass silages averaged 2.04 and 2.32 $\log_{10}$ colony forming units (cfu) g^{-1} for yeasts and moulds respectively, while the TMR's averaged 4.27 and 3.50 $\log_{10}$ cfu g^{-1} respectively. One suggestion proposed about the higher yeast counts in TMR was that the inclusion of highly fermentable substrates, in combination with extra air going through the feed ration during mixing, might have accelerated the growth.

3. Material and methods

3.1 Animals and housing

The study was performed at Lövsta research facilities outside Uppsala, Sweden. The animals used in the experiment were 40 cows of the breeds Swedish Holstein and Swedish Red in early to mid-lactation with an average of 48 ± 19 (mean ± SD; range: 17-86) days in milk (DIM) at the start of the experiment. Both multi- and primiparous cows were used. Four of the cows were fitted with a ruminal cannula. The cows were kept in a loose housing system and batch milked twice daily at 05.00 and 16.00 in an automatic milking rotary (AMR). Feed was offered *ad libitum* in 19 separate feeding mangers on weighing scales (Biocontrol A/S, Rakkestad, Norway) to be able to measure feed intake for each individual cow.

The cows were blocked into seven blocks according to parity, where the primi- and multiparous cows were separated, and calving date. Cannulated cows were all multiparous and in a separate block. Each cow within the blocks was randomly allocated to one of two groups before the study started. The experiment was a changeover design were the groups were assigned to one of two treatments at the beginning of the experiment and switched treatment half way through. The two periods were three weeks each, with two weeks of adaption to the diet and one week for measurements and recordings. In total, the experiment lasted for six weeks.

3.2 Treatments

The treatments consisted of two different diets, TMR and CTMR. Both diets were based on the same forage and concentrates (Table 4) and similar in chemical composition, The CTMR diet was altered in DM content and FPS. Forage to concentrate ratio was 60:40 on a DM basis. The forage was a second harvest grass silage chopped to 2 cm theoretical length of cut and preserved in a bunker silo. The concentrate was in the form of crushed pellets and composed from ingredients that were available as organically certified by KRAV (Table 3).

Table 3. Ingredients and proportions of concentrate SLU Sund

Ingredients	**%**
Wheat	37.3
Oats	28.0
Soya expeller KRAV	14.1
Wheat bran	10.5
Soya bean KRAV	7.0
Limestone	1.3
Sugar beet molasses	1.0
Sodium chloride (NaCl)	0.5
PRX KO 0,2%	0.2
Magnesium oxide (MgO)	0.05
PRX E-VIT 20	0.05

CTMR diet forage was mixed in a vertical augers fitted with knives (SiloKing, Tittmoning, Germany) for 60 min to reduce FPS. Forage used in the TMR diet was obtained straight from the bunker silo. The forage was then placed in a vertical auger mixer without knives (DeLaval, Tumba, Sweden) before the addition of concentrate for both diets. Then water was added to adjust DM for the CTMR diet. The dry matter content of the TMR was in average 58.1 % and the CTMR diet DM was altered by the addition of water to 37.0 %. The mixer was placed on a weighing scale and all ingredients were added automatically according to a preprogramed recipe. The recipe had the ingredients specified in kg fresh weight. The principles of CTMR described in the introduction and described in this method were not identical. In the present study’s CTMR, concentrates were not soaked beforehand but added after the forage at the same time as water to the mixer, since the mixer could not manage to store water without leaking. Also, no corn silage was used.

The feed was distributed automatically in the feeding mangers three times a day for TMR diet and two times a day for CTMR diet with the goal to provide *ad libitum* access to feed for the animals. To distribute *ad libitum* access of the feed, the amount of feed required to achieve this was altered during the first adaption weeks until orts of a manageable amount was provided. DM in roughage were measured weekly for adjustment of diet recipes.

Table 4. Nutritional composition of feedstuffs concentrate SLU Sund and grass silage forage

Concentrate			**Forage**		
DM	%	88.12	DM	%	41
Energy	MJ ME kg DM^{-1}	13.4	Energy	MJ ME kg DM^{-1}	10.7
Crude protein	g kg DM^{-1}	170	Crude protein	g kg DM^{-1}	165
Crude fat	g kg DM^{-1}	55	NDF	g kg DM^{-1}	452
Crude fiber	g kg DM^{-1}	66	Ca	g kg DM^{-1}	8.2
Ash	g kg DM^{-1}	62	P	g kg DM^{-1}	2.2
Ca	g kg DM^{-1}	8	Mg	g kg DM^{-1}	2.3
P	g kg DM^{-1}	6	K	g kg DM^{-1}	27.3
Mg	g kg DM^{-1}	3			
K	g kg DM^{-1}	9			
Na	g kg DM^{-1}	3.2			

3.3 Measurements and recordings

All cows were assessed for body condition score (BCS) three times during the experiment. The assessments were made during first week, week four and after the end of the experiment (during week 7). A BCS protocol from Geno Avl og Semin was used for the assessment. All cows were graded on a scale from 1 to 5 on four measuring points and the values were then calculated to an average score per cow.

A Penn State Particle Separator was used to calculate the particle size distribution of the rations. The PSPS was used in its original appearance with two sieves and a pan. The upper sieve's holes were 19 mm and the holes of the lower sieve 8 mm in diameter. The samples were taken from the feed table directly after feedout at four occasions during both sampling weeks. The PSPS was shaken horizontally five times, then rotated one quarter of a turn and this procedure was repeated eight times. Then the feed particles in the different fractions of the PSPS were weighed and dried and then weighed again. The proportions of the different fractions in fresh and dried weight was calculated.

DMI was measured by retrieving data from the feed mangers that registered in kg feed per cow and day and then recalculated to DMI by using the calculated DM content of the rations. The data were collected for each day of the last week of each treatment period. Intake of drinking water was measured by the usage of water cups fitted with water meters and transponder sensors (Biocontrol A/S, Rakkestad, Norway). Total water intake was calculated by using registered water intake from water cups, feed intake and DM content of diets.

Digestibility was measured by analysis of acid insoluble ash (AIA) in feed and in *faeces*. Feed samples were collected in duplicates from both treatments diets in both experimental periods. Spot samples of *faeces* was collected three times for each cow during both measurement periods on three different days and stored in a freezer room. The three samples from the same dietary treatment were then thawed and mixed, and a single 180 g sample was frozen again for storage until further analysis. The samples were then freeze dried, ground to pass a 1 mm sieve, combusted in 550 °C, boiled in hydrochloric acid to remove acid soluble components and then filtrated.

pH was measured by sampling of ruminal liquid from the cannulated cows during both measuring weeks. One sample was taken for every clockwise hour of the day, resulting in 24 registrations per cow. Sampling for all hour specific registrations was not necessarily performed subsequently for 24 hours but could be obtained on any day during the measuring week. Ruminal fluid was extracted from rumen digesta through the cannula and collected in a tube. Measuring of pH was performed using a pH meter (pHenomenal, VWR, United Kingdom). pH value of the ruminal liquid was noted with two decimals.

Milk yield was recorded at every milking by the AMR. Milk components were determined by analysis of milk samples from both morning and evening milking on two consecutive days during the sampling weeks. The milk was collected by the use of milk samplers that collect the milk during the whole milking. Analysis was performed at the laboratory at Swedish University of Agricultural Sciences (SLU) with a Delta Combiscope (Combiscope FTIR 300, Delta instruments, the Netherlands) and MIR-analysis with Fourier transformation. Samples were analysed for fat, protein and lactose proportions. Yields of fat, protein and lactose content were calculated using each component's proportion and the corresponding milk yield to the sample.

Hygienic quality of the feed was assessed by the sampling of both feeds at 0, 12 and 24 hours after feed out. Samples will be referred to as CTMR0, CTMR12, CTMR24 and TMR0, TMR12, TMR24 in the results. Samples were collected from four feeding mangers within each treatment diet and placed in a plastic box which was shaken to mix the samples. Between each sample, the plastic box was sterilised with ethanol. Approximately 300 g sample were taken from different areas of the plastic box and put in a sealed plastic bag. The samples were then stored in a fridge until taken to the laboratory. At the laboratory 30 g were collected from the 300 g samples for further analysis.

The 30 g feed samples were diluted with 270 ml distilled water to receive a dilution with concentration 10^{-1} and then homogenised in a stomacher blender for 480 seconds. 1 ml of the 10^{-1} dilution were then diluted with 9 ml of distilled water, and then again, to retrieve dilutions with concentrations 10^{-2} and 10^{-3} respectively. 0.1 ml of the liquids was put on agar plates in three replicates, which led to final dilutions of 10^{-2}, 10^{-3}, and 10^{-4} on the plates. The malt extract agar supplemented with 0.12 M lactic acid (50ml L^{-1}) plates were aerobically incubated in 25 °C for 72 hours and then counted for yeast and mould colonies. The average yeast and mould counts from the three replicates were then recalculated to an average log cfu^{-1}. This sampling and analysis was repeated six times during the study.

3.4 Statistical procedures

The data observations from milk yield, feed intake and water intake were summarised as total sums per cow per day. Average diurnal pH and digestibility used in the data set were summarised as one average observation per sampling week. All eight observations from particle size distribution samplings were used in the statistical data. Time below pH 5.80 was summarised as hours per day and cow. The experimental data for all observation parameters except pH and particle size distribution was run in the statistical programme SAS 9.4.

Average diurnal pH was analysed using a paired two-tailed t-test in Excel, comparing diurnal averages of all cows for sampling period one and two. Particle size distribution was also analysed with a paired two-tailed t-test in Excel, where four particle assessed feed samples from each dietary treatments and each period were compared. The experimental data for feed intake, milk yield, ECM yield and water intake were analysed with the procedure Mixed and class variables animal, block, breed, period and treatment. Breed, block, treatment group and treatment were fixed factors. Animal was considered a random variable. The covariance between samples within cow was modelled with a spatial power covariance structure.

Yeast and mould concentrations were analysed separately from all six sampling occasions for each time unit and treatment (CTMR0, CTMR12, CTMR24, TMR0, TMR12 and TMR24). The data were analysed using the procedure GLM with treatment and time unit as class variables and fixed factors.

4. Results

One animal was absent due to illness during the first measuring week. Two animals were taken out of the experiment due to mastitis during the third day of the second measuring week and excluded from the data for the last three days. These incidents accounts for registrations missing in the statistical material.

4.1 Particle size distribution of the diets

The particle size distribution of both diets was assessed in both periods (Table 5). The results for both periods within a treatment followed a similar pattern. In the control diet TMR, proportions of particles was close equal on all three levels. The increased mixing of forage and water addition to the CTMR diet resulted in a reduced fraction retained on the upper sieve ($p=<0.001$) and increased fraction in the lower sieve ($p=<0.001$), compared with the TMR diet.

There was a tendency towards more particles retained in the pan for the TMR diet (p=0.076).

Table 5. Particle size distribution of treatment diets CTMR and TMR assessed with a Penn state particle separator (PSPS) with two sieves. CTMR = re-chopped forage and water added to achieve dry matter content 37 %, TMR = forage from the bunker silo and average dry matter content 58.1 %

PSPS sieves	**CTMR**	**SEM**	**TMR**	**SEM**	**P-value**
Upper, >19mm, %	5.1	0.6	31.6	2.2	<0.001
Lower, 19-8 mm, %	64.9	1.3	34.0	1.3	<0.001
Pan, <8mm, %	30.1	1.4	34.3	1.0	0.076

Table 6. Effects of treatment diets CTMR and TMR on dairy cows in early to mid-lactation. CTMR = re-chopped forage and water added to achieve dry matter content 37 %, TMR = forage from the bunker silo and average dry matter content 58.1 %

		CTMR	**SEM**	**TMR**	**SEM**	**P-value**
Feed intake	kg DM day^{-1}	26.8	0.6	28.6	0.6	<0.001
Water intake	kg day^{-1}	98.9	2.6	109.6	2.6	<0.001
Total water intake	kg day^{-1}	144.3	3.0	136.3	3.0	<0.001
pH, diurnal average	n	5.74	0.14	5.76	0.07	0.802
Time below pH 5.80	h	14.94	1.60	14.63	1.60	0.902
Body condition score	n	3.39	0.08	3.37	0.08	0.513
Digestibility	% of DM	62.1	0.01	61.35	0.01	0.187
Milk yield	kg day^{-1}	35.1	0.4	35.2	0.4	0.495
Milk yield	kg ECM day^{-1}	33.8	0.8	34.7	0.8	0.151
Milk constituents						
Fat	%	3.71	0.01	3.83	0.01	0.239
	kg day^{-1}	1.28	0.04	1.36	0.04	0.062
Protein	%	3.25	0.01	3.24	0.01	0.621
	kg day^{-1}	1.13	0.03	1.15	0.03	0.189
Lactose	%	4.78	0.01	4.77	0.01	0.748
	kg day^{-1}	1.68	0.04	1.71	0.04	0.237

4.2 Feed and water intake

The dietary treatments affected feed intake (p=<0.001). Cows on the control diet TMR, consumed more kg DM per day than cows on experimental treatment CTMR (Table 6). Water consumption from water cups was affected by dietary treatments (p=<0.001). The registered water intake was lower when CTMR was fed compared to when TMR was fed (Table 6). There was also a difference in total water intake between treatments (p=<0.001). When cows were fed CTMR, total amount of water intake increased (Table 6). In total, water intake increased with

8 kg per day and cow when fed CTMR compared to when fed the TMR diet.

4.3 Body score condition

The body condition score (BCS) was similar across treatments (p=0.513). The BCS's were almost identical for CTMR and TMR diets respectively (Table 6). In general, the herd gained slightly in body condition score during the first period and lost slightly during the second period. Overall, there was no difference in BCS from the beginning until the end of the experiment.

4.4 Milk production and milk constituents

The average milk yield for CTMR and TMR diets was around 35 kg for both diets (Table 6) and was not affected by diet (p=0.495). Production of milk components fat, protein and lactose (Table 6) was not affected by dietary treatments in either yield as kg per day or proportion as percentage of kg milk. There was a tendency (p=0.062) towards a higher milk fat yield when TMR was fed compared to when CTMR was fed.

4.5 Digestibility

The AIA-content of diet forage showed large differences between periods and the reason for that is unknown. This led to a large effect of period in the statistical results. All samples were run as duplicates and therefore it was a low risk of an analysis error. The average digestibility of the diets CTMR and TMR was similar (Table 6) and no effect of diet was found. However, a relationship between period and dietary treatment (p=0.05) was detected, where digestibility of CTMR diet was increased by two percentage units compared to TMR diet in the second experimental period.

4.6 Rumen environment

The average daily ruminal pH was very similar for cannulated cows fed either CTMR or TMR diets (Table 6) and there was no difference between diets (p=0.802). The highest pH values was found in early morning for both diets (Figure 1). The lowest pH was found midmorning and during the afternoon when fed CTMR and TMR respectively (Figure 1). The red line marking pH 5.80, indicated in Figure 1, is at which point rumen degradation of fiber is considered to be depressed, according to Shriver *et al.* (1986). The time ruminal pH was below pH 5.80 was not affected by dietary treatments (p=0.901) and was close to approximately 15 hours for both CTMR and TMR (Table 6).

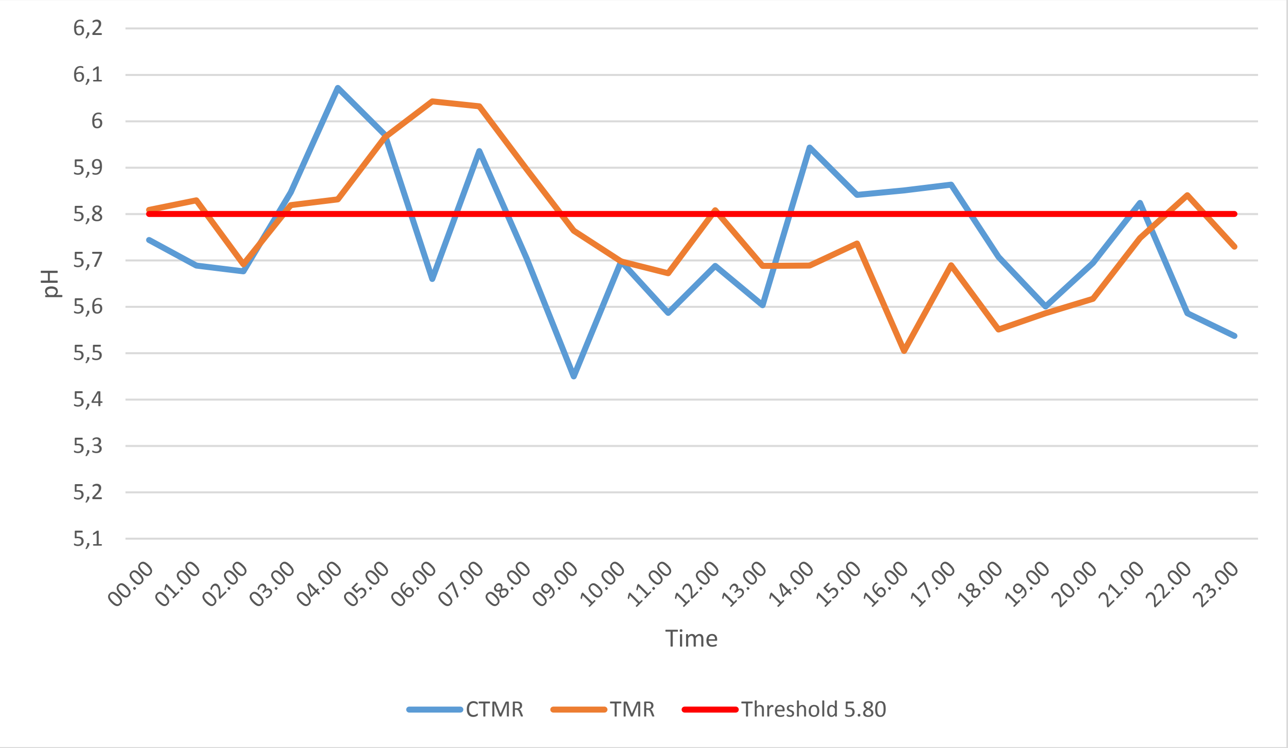

Figure 1. Fluctuations of the diurnal ruminal pH for cannulated cows on diets CTMR or TMR over 24 hours. Red line indicates pH at which fiber degradation is depressed (Shriver *et al*., 1986).

4.7 Hygienic quality

The outside ambient average diurnal temperatures were 4.3, 1.4, 5.4, -2.0, 0.2 and 0.3 °C respectively during the six sampling occasions (SMHI, 2018). The inside temperature in the barn was not measured.

4.7.1 Yeast and mould counts

The sample obtained from both CTMR and TMR diet 24 hours after feed out were significantly higher in yeast concentration than all other samples (Table 7). The yeast counts in the samples for both were affected by time (p=0.001), meaning that the concentrations of yeast in the feed samples increased as time after feed out increased. There was no differences between the dietary treatments (p=0.548) concluding that the reduced FPS and lower DM content of the CTMR feed ration did not affect yeast counts compared to TMR. However, there was a tendency for an interaction between diet treatment and time (p=0.076) on yeast concentrations in the feed. The yeast concentrations increased faster over time in the CTMR diet compared with in the TMR diet.

The concentration of mould in feed was also affected by time (p=0.004). Therefore, concentrations of mould increased equally in both feed rations when time after feedout passed. No effect of treatment could be detected (p=0.456). There was no interactions between time and treatment (p=0.467). However, CTMR24 had numerically higher mould concentrations

(3.41 log cfu g^{-1}) than the TMR24 sample that contained 2.78 log cfu g^{-1}, yet not significant (p=0.158).

Table 7. Yeasts and mould concentrations of dietary treatments CTMR and TMR sampled at 0, 12 and 24 hours after feed out. Different superscripts within a column differ (p=<0.05). CTMR = re-chopped forage and water added to achieve dry matter content 37 %, TMR = forage from the bunker silo and average dry matter content 58.1 %

	Yeasts, log cfu g^{-1}		**Moulds, log cfu g^{-1}**	
	Mean	**SD**	**Mean**	**SD**
CTMR0	4.43^{a}	0.292	1.94^{a}	0.708
CTMR12	4.48^{a}	0.049	2.39^{a}	0.156
CTMR24	5.95^{d}	0.885	3.41^{b}	0.800
TMR0	4.81^{abc}	0.788	2.01^{a}	0.627
TMR12	5.05^{abc}	0.702	2.37^{a}	0.922
TMR24	5.38^{cd}	0.660	2.78^{ab}	0.922
Standard Error	0.259		0.307	
$P_{treatment}$	0.548		0.456	
P_{time}	<0.001		0.004	
$P_{treatment*time}$	0.076		0.469	

5. Discussion

In the present study, the effect of DM content and FPS was investigated as one factor and therefore it was not possible to determine either if one of them affected the results or if the factors interacted. It would seem, according to literature used in this study that FPS previously has had a somewhat larger effect (Table 1) on parameters investigated in the current trial; feed intake, milk production, rumen environment and digestibility, than DM content of the feed ration (Table 2). However, not possible to prove in the current study.

5.1 The CTMR protocol

The CTMR diet in the present study was not manipulated exactly according to the guidelines reported in Kristensen (2015). According to the original protocol, all concentrates and dry feedstuffs should be added first into the mixer and soaked with water for a minimum of one hour, depending on type of feedstuffs. When concentrates and dry feedstuffs are sufficiently soaked, fibrous feed components are added and the feed is mixed for 15-20 minutes before corn silage is added and the feed is mixed for another 15-20 minutes. In this study, the protocol for CTMR was that forage was separately mixed in an auger with knives for 60 minutes and then added to a mixer without knives. When forage had been added, concentrates and water was included simultaneously into the mixer. Also, no corn silage was used in the current trial, as in the originally described CTMR (Kristensen, 2015).

Even though concentrates dissolved, adhered to the forage and FPS was decreased in the present study, which also were the intentions with the original CTMR practice, the practical performance in the present study differed from how it was suggested by Kristensen (2015). From the pictures in Kristensen (2015), the CTMR in this study seemed to have somewhat more structure left. Therefore, it is possible that the CTMR used in this study was not fully comparable to the one intended by Kristensen (2015) and the different results in this study compared to the results from the “real life-farms” could be explained by this. However, it is suggested even though means of procedure differed, it resulted in a similar end product and thereby can be compared to CTMR.

5.2 Particle size distribution of the diets

The particle size distribution in the TMR and the CTMR differed when assessed in a PSPS. The proportion on the lower (19-8 mm) sieve almost doubled at expense of the upper sieve (>19 mm) and the pan (<8 mm) for the CTMR diet compared to the TMR diet. The diets varied in appearance as well. The TMR was noticeably drier and coarser than the CTMR, which was moister, finer in structure and concentrates were more dissolved. If a handful of feed was picked up, concentrates would fall more easily out of the TMR than out of the CTMR.

Previous studies that estimated particle size distribution in TMR’s with different DM contents also noticed that the proportion of particles in the lower sieve increased and decreased in the pan when DM was reduced (Felton & DeVries, 2010; Miller-Cushon & DeVries, 2009). This was considered by the authors of the studies to be a result from smaller particles adhering to larger particles. This seem likely in the present study as well, as the CTMR was considered to a stickier appearance than the TMR. It is also possible that the mixing reduced the particle size from larger than 19 mm to in between 19-8 mm and therefore cause the increase in the lower sieve. It is probably a results of these two factors combined. The proportion of the upper sieve was reduced in the CTMR compared with the TMR. It would be probable that this is a consequence of the increased mixing of forage, since increased mixing of forage will shred the longer forage particles in the feed ration. That would be consistent with many previous studies, which showed that increased mixing of forage gives a lower proportion of particles in the PSPS’ most upper sieve (Table 1). Therefore, the result from the increased mixing of forage and addition of water was as could have been expected.

5.3 Feed intake

Nasrollahi *et al.* (2015) found through their meta-analysis a link between F:C of the feed ration, FPS and DMI. In the study it was concluded that when forage constitutes more than 50 % of DM in a feed ration, a decreased FPS can increase DMI. In consistency with this, all studies in the current literature review that had more than 50 % DM forage in TMR ration experienced an increase in DMI when particle size of the ration was reduced (Table 1). When forage make out the majority of feed ration DM, it seem reasonable that reduction of FPS would affect DMI positively since forage is a more structural feedstuff than concentrates and therefore should give a higher ruminal fill. On the contrary, in this study the dietary treatment with a larger FPS resulted in a higher DMI.

In the present study, DMI was decreased when cows were fed the CTMR diet compared to TMR (Table 6). The CTMR diet was the diet where the forage had been rechopped, and therefore had a smaller FPS. The TMR diet which had larger FPS increased the DMI with almost 2 kg DM per day. On the contrary to the suggestions made by Nasrollahi *et al.* (2015), these findings occurred even though forage proportion of the diet constituted 60 % of feed ration DM. The findings are also inconsistent with the previous studies on TMR's with more than 50 % DM forage (Kononoff & Heinrichs, 2003; Maulfair *et al.*, 2010; Leonardi *et al.*, 2005b) where DMI increased when FPS decreased. There have been multiple studies presented in the present literature review that has not been able to prove a link between particle size and DMI (Table 1). However, there have not been any previous studies that has shown a relationship between decreased FPS and decreased DMI. This led to the suspicion that the effect of reduced FPS might have been overridden by the other feed ration trait that was manipulated in the present study, the DM content.

According to Mertens (1994), dairy cows will try to compensate the energy dilution caused by lowered DM in the feed ration by the consumption of more fresh feed in order to cover their energy demands. This would explain the increased fresh matter intake from the CTMR ration, that the cows felt urged to increase their intake because of the lower energy content per kg fresh feed in the CTMR. It is although also possible that the CTMR diet was less palatable than the TMR diet since there was a tendency towards higher concentrations of yeasts and therefore caused the CTMR intake to not reach the same DMI. These two factors combined could also possibly explain why the fresh feed intake was higher but not enough to reach same DMI level for the CTMR diet as for the TMR diet.

None of the reviewed studies that explored TMR DM content's effect on DMI tested a diet with DM content as low as in the present study, which was altered by the addition of water to 37 % DM. However, all studies that lowered the DM content to below 50 % except one, reported that a wetter ration led to a decreased DMI (Miller-Cushon & DeVries, 2009; Felton & DeVries, 2010). This is in consistency with the results from the present study where the diet with DM content below 50 % decreased DMI. Feed intake in terms of kg fresh weight consumed by the animals, was higher for the CTMR diet in the present study even though DMI was lower. The intake of water from water cups was lower for the CTMR diet. Even if the water cup intake was lower, total water intake was higher when CTMR was fed. This meant that the increase in total water intake per day was caused by the added water to the CTMR feed ration. These findings are consistent with the work of Miller-Cushon & DeVries (2009) and Felton & DeVries (2010), who also saw an increase in fresh matter feed intake but a reduction in DMI when DM was lowered by water addition to diet. On this matter, it has been suggested that this might be due to the added water lingering in the rumen and limiting feed intake (Robinson *et al.*, 1990; Miller-Cushon & DeVries, 2009). It has also been suggested that the intracellular water content in feedstuffs can limit DMI (Robinson *et al.*, 1990). However, in the present study the same forage was used in the same proportions for both experimental diets and intracellular water content should therefore not affect the results. It is suggested that in the present study the difference in DMI caused by diet was due to the filling effect of the added water.

5.4 Milk production

Milk yield has not been affected when TMR FPS or DM content has been altered in most previous research used in the current literature review. It is not surprising that studies that showed no differences in DMI lacked differences in milk yield since there the energy intake would be the same, as suggested by Fish & DeVries (2012). It was hypothesised in the present study that milk yield would increase, as a consequence of increased feed intake when cows were fed the CTMR diet. As the results for feed intake were opposite to the hypothesis, that the TMR diet resulted in increased daily DMI instead of CTMR diet, it would have been expected to see a consequent increase in milk yield on the TMR diet.

However, milk yield was not affected by diet even if DMI was increased on the TMR diet. As an increase in DMI was found in the present study it is somewhat surprising that an increase in milk yield could not be detected. It was expected that increasing DMI would lead to an increased energy intake and thus show a response in milk yield or that the energy would have been utilized for fat tissue synthesis. Yet, a change in BCS could not be detected (Table 6) when cows received the different dietary treatments. The difference in DMI for the dietary treatments in combination with the maintained milk yield actually means that there was a higher milk production efficiency when cows were fed CTMR, which is consistent with the studies performed by Felton & DeVries (2012) and Miller-Cushon & DeVries (2009).

That an increase in DMI did not lead to an increase in kg milk per day, resembling the results of the present study, has been seen in several studies before. Both Maulfair *et al.* (2010) and Kononoff & Heinrichs (2003) measured an increase in DMI but could not find a correspondent increase in milk yield when FPS of the feed ration was reduced. The same effect was experienced by Felton & DeVries (2012) and Miller-Cushon & DeVries (2010) when altering the DM content, which reduced DMI although was not followed by a consequent drop in milk yield. In the study by Felton & DeVries (2012) the rations were generously formulated, even the lowest DMI covered the energy demands of the cows in the study. This was also the case in the present study, the diets' energy content more than covered the needs of the cows actual milk yield. Therefore it is suggested that with time, cows would gain in BCS. The increased energy intake should however present in some way eventually, and it seems more reasonable that the animals potentially would have gained in BCS if the experiment had lasted longer, as suggested by Miller-Cushon & DeVries (2009).

In the present study, proportions and yield of milk constituents were not affected by the dietary treatments which is consistent with the majority of previous studies considered in the literature review. However, reduced milk fat percentages has previously been linked to a smaller particle size of TMR (Shaver *et al.*, 1986; Krause & Combs, 2003; Nasrollahi *et al.*, 2015). That a reduction in milk fat percentage and yield could not be detected in the present experiment might be due to the high inclusion of forage in both diets. Feeding 40 % instead of 60 % DM forage has previously shown to reduce milk fat content in milk (Olsson *et al.*, 1998). However, there was a tendency towards a lower milk fat content when cows were fed CTMR. Therefore it is hypothesised that the high feeding of forage might have dampened the effect of a smaller FPS.

It would be interesting to see how a lower forage inclusion in a CTMR would affect milk fat content in a similar study. Also, it has been recommended that a mean particle size of 6.4 mm would be enough to avoid a lower milk fat content (Woodford *et al.*, 1986). A majority of feed particles for both diets were retained on the two sieves of the PSPS (>8 mm) in the present study. Thus, it might be able to assume that the mean geometrical particle size of both diets were larger the previously recommended value, also contributing to the maintenance of milk fat content.

5.5 Rumen environment

The previous results of FPS in TMR diets on rumen environment has differed, with both results that has suggested that a smaller FPS gives a lower ruminal pH and results that has not been able to prove a connection between the parameters (Table 1). In the studies where reduced particle size has resulted in a lower pH (Krause *et al.*, 2002b; Beauchemin *et al.*, 2003), forage proportion of the TMR has been lower than in the present study, leading to the assumption that FPS might be of larger importance when forage constitutes less than 60 % of ration DM. Those studies has also had lower fractions of particles on the >19 mm sieve of the PSPS (Table 1) than in the current study (Table 5), indicating a smaller FPS. The mean ruminal pH in the present study was slightly low for both diets though, under the threshold previous studies used for SARA (Duffield *et al.*, 2004) and under benchmarks used for detecting impaired rumen environment (Krause *et al.*, 2002b; Beauchemin *et al.*, 2003; Krause & Combs, 2003). These low values are somewhat surprising, considering the relatively high inclusion of forage in both diets. A suggestion is that the cows might have been able to sort both rations on an equally high level, even though the manipulation of the CTMR diet was carried out in belief that it would decrease the occurrence of sorting behaviour. When Figure 1 was observed, it seemed like the CTMR curve fluctuations were steeper than in the TMR curve. It is possible that the reduced FPS resulted in a faster eating time, as previously seen in Kononoff & Heinrichs (2003).

It has also been questioned whether the daily mean ruminal pH really gives a good indication of the effects of particle size on rumen environment, as multiple studies has shown that diurnal mean pH was similar between diets but differed in time pH spent below 5.80 (Yang *et al.*, 2001b; Krause *et al.*, 2002b; Beauchemin *et al.*, 2003). However, in the present study both mean diurnal ruminal pH and time below pH 5.80 of the cannulated cows was practically identical (Table 6), and not affected by treatments. These findings are consistent with Beauchemin & Yang (2005), who could not prove that either mean diurnal pH or time below pH 5.80 differed when comparing different particle size distributions. According to them, the reason for this might be other parameters influencing, like diet fermentability. The inclusion of readily degradable carbohydrates in the current study was lower than in most studies reviewed. It is possible that results from this study are not completely comparable to those experiments that used a larger proportion of concentrates in the feed ration or a different processing technique.

It is also worth noting that most of the studies that measured the time ruminal pH was below 5.80 used indwelling pH-meters and therefore received continuous data that were registered with short intervals, during one single day. This was the intention in this study as well, however the indwelling pH-meters were unavailable during the experimental period. In the present study pH was measured manually by extracting spot samples of ruminal fluid once an hour. All hour specific registrations were not made on the same day but could be collected on any day during the sampling week. There might have led to fluctuations of pH that was not registered, and thus not detected, and therefore could have affected the results.

5.6 Digestibility

In the present study, digestibility was measured using the AIA method. Forage and concentrates were analysed separately for AIA content. When samples were analysed, higher AIA values was found for the forage in one period. The reason for this was unknown, but this affected the results and gave a large effect of period. The same forage from the same bunker silo was used for both diets during both periods. A relationship between period and treatment was found in period two where the DM digestibility of CTMR was improved. As a difference between treatments only could be found in one period, these findings are not very assuring that there was an actual effect of diet on digestibility. However, since *faeces* was spot sampled during three days of each measuring week and then blended to one sample per dietary treatment, it is also a possibility that samples were not representative for the actual DM digestibility of the feed.

The average DM digestibility of the diets was not affected by the dietary treatments (Table 6), which is in consistency with Alamouti *et al.* (2010) who also used AIA content as a digestibility marker, as well as other studies that altered particle size of TMR (Yang *et al.*, 2001a; Krause *et al.*, 2002a; Krause & Combs, 2003). Since DMI was lowered and milk production maintained in the current experiment, it was suspected that the digestibility would have been improved when the CTMR diet was fed, especially since forage proportion exceeded 50 % of ration DM and FPS was reduced, as seen in Kononoff & Heinrichs (2003). Even though forage proportion was large in this current trial, it could not be proven that the digestibility was enhanced by the reduction of FPS. If the DM digestibility was not affected by the dietary treatments, it is possible that the FPS was not reduced enough for an improvement to occur. It is also possible that the FPS was so small that the passage rate increased to the extent that fiber degradation was depressed because of the higher outflow from the rumen. According to Nasrollahi *et al.* (2015) DM digestibility in diets where forage constitutes more than half of feed ration DM increase when forage particle size is reduced. Yang *et al.* (2001a) could not prove that reduced FPS improved diet DM digestibility. They could however prove that the F:C ratio had an effect on DM digestibility, where a TMR with more than 50 % DM from forage decreased the DM digestibility. It seem reasonable that the reduction in digestibility caused by a large forage proportion could be mitigated by reduction of forage particle size. This was further endorsed by the only study in the present review that had more than 50 % DM from forage and measured effect of FPS on digestibility in their experiment (Kononoff & Heinrichs, 2003). Therefore it could also be possible that CTMR did increase DM digestibility, even if this could not be endorsed by the results.

It is also possible that the absence of differences in digestibility for the diets might have been because of that the reduction of particle size led to a combination of increased passage rate and increased microbial availability, factors that can outplay each other, as suggested by McDonald (2011). It is possible that reduction of FPS in the present study increased passage rate in the rumen but the effect was dampened or blocked by the filling effect of water from the diet due to the lower DM content of CTMR, leading to the same ruminal retention time as the TMR diet. Another possible explanation could be the low mean ruminal values obtained in the present study. Cows on both diets had mean pH values below the limit that has been suggested to depress fiber digestibility (Shriver *et al.*, 1986). If fiber digestibility was depressed because of ruminal pH and pH was influenced by another factor than dietary treatment, such as that potentially suffering from SARA, it is still possible that one of the diets was more digestible than the other.

5.7 Hygienic quality

When mixing a TMR, favourable conditions for microbes occur (Seppälä *et al*., 2012; Cogan *et al*., 2017). As the CTMR was wetter than the TMR, it is possible that even more favourable conditions were presented to the microorganisms and allowed a higher deterioration rate. Therefore the hypothesis in the current study was that a TMR with added water, *i.e.* the CTMR, would increase concentrations of yeast and mould, indicating a higher deterioration rate.

Hygienic quality of the feed ration, as in terms of quantities of yeast and mould concentrations, was not affected by the addition of water and increased mixing of forage. The concentrations of yeast and moulds were affected by time (Table 7), meaning that as both feeds were subjected to aerobic exposure, the microbial concentrations increased. These results were not surprising, as both moulds and yeasts are aerobic microorganisms. The concentrations of yeast and mould during the first two samplings at 0 and 12 hours are similar to those obtained by Cogan *et al.* (2017) who sampled TMR in average 4.5 hours after feedout. There was a tendency towards an interaction between treatment and time for yeast content in the diets, suggesting that the proliferation rate of yeasts in CTMR diet over time was higher than in TMR (Table 7), but there was no corresponding relationship for mould concentrations. Lindgren *et al*. (1985) showed that yeast was commonly the first microorganism to increase its activity when silage was exposed to air. It is possible that the tendency for higher yeast counts in CTMR over time could be an indication towards that CTMR was more prone to spoil than TMR, however inhibited by the cold environmental temperatures during the experimental period.

During the sampling weeks, weather conditions at the location were cold. The facilities of the experiment was semi-insulated, meaning that temperatures inside were never at freezing point but yet colder than on a summer day. Also, silage was stored in bunker silos outside. As most microbial proliferation is favoured by higher temperatures, this might have been inhibiting to potential differences between the dietary treatments. When TMR's were sampled at one hour after feedout and then kept in 22 °C, it was shown that they could spoil in less than 12 hours (Kung, 2010). It would be interesting to see further research on the hygienic quality of CTMR compared to TMR during summer conditions, when deterioration rates would be higher. This is of importance, as impaired hygienic quality of silage is known to lower DMI of dairy cows

(Wichert *et al.*, 1998) as well as to increase the risk of animal health disorders (Wilkinson & Davies, 2013; Driehuis & Oude-Elferink, 2000).

The frequency of feedout occasions was different for the two treatment diets, the TMR diet was freshly mixed and fed three times a day whilst the CTMR diet was distributed two times a day. This was due to the fact that the DM consumption of TMR was higher as well as that more kg fresh feed could be transported by the feed wagon at each occasion for the CTMR. To be able to measure hygienic quality in a comparable way, the 12 hour TMR sample was taken out of the feeding mangers just before the second feedout and stored in a plastic box just next to the feed bunk until the 12 hours sampling. Even though those samples were kept in identical environment and temperature as the CTMR feed until sampled, the stirring, additional warmth and saliva caused by the cows when feeding was excluded by this measure. This could potentially have affected the results. Further, this also affected the 24 hour sampling since one more batch of fresh feed was incorporated into the feed residues that was sampled in the TMR compared to the CTMR. It might be possible that the extra feedout occasion interfered with the result and therefore lowered the true concentrations of yeast and mould in the residues of TMR. This is another possible reason for the tendency towards the increased yeast concentrations in relation to time in the CTMR.

6. Conclusion

Compact total mixed ration decreased dry matter intake in dairy cows and increased water intake compared to when a total mixed ration was fed. Milk yield, milk constituents, rumen environment and the hygienic quality was not affected by dietary treatments. The effect of dietary treatments on digestibility could not be fully established. To be able to differentiate the effects or detect an interaction between forage particle size and dry matter content in a compact total mixed ration it is suggested that in potential following studies, factors are studied separately. More research is needed to evaluate the long term effects, as well as the effect of different forage to concentrate ratios, when compact total mixed ration is fed. To establish the hygienic quality of CTMR during summer conditions, further research is required.

7. References

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