The effects of microcrystalline cellulose as a dietary component for lactating dairy cows

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Microcrystalline cellulose (MCC) has several applications in food and pharmaceutical industries but the nutritional value for dairy cows and effects on *in vivo* digestion are not known. A feeding experiment was conducted using 24 dairy cows. The cows were offered MCC originating from unbleached softwood kraft pulp at 0, 10 or 100 g per kg diet dry matter (DM) to replace barley grain in the diet. The total daily DM intake was on average 25.6 kg and not significantly affected by the diet. Positive effects on rumen fermentation could not be demonstrated in a feeding situation where total mixed ration and a concentrate proportion of 0.50 on DM basis was used. Diet organic matter digestibility was not affected by MCC inclusion but fibre digestibility improved and the additional MCC fibre was virtually completely digested. The production potential of MCC was lower than that of barley grain as daily yields of energy-corrected milk, milk fat and protein, and milk protein concentration decreased when MCC replaced barley grain in the diet. Based on these results, MCC is not recommended as a dietary component for high-yielding dairy cows.

Key words: AaltoCell™, digestibility, MCC, rumen fermentation, milk production

Introduction

Cellulose consists of repeated β-D-glucose units and is the most abundant renewable source of carbohydrates worldwide (Habibi et al. 2010). Ruminants naturally utilize cellulose and hemicellulose as major components of their diets, but they typically originate from grasses (Van Soest 1994). Parent cellulose has crystalline and amorphous regions. Isolation of the crystalline regions has led to production of different shaped and sized variable functional ingredients including microcrystalline cellulose (MCC) (Habibi et al. 2010). MCC is defined as partially depolymerized cellulose prepared by treating α-cellulose (FAO 2018). The degradation of cellulose fibres derived from high-quality wood pulp with hydrochloric acid was the starting point of commercialization of MCC (Battista 1950). The degree of polymerization of native wood is approximately 10000 while it is typically less than 400 in MCC (Sjöström 1993). The typical particle size of commercial MCC products is in the range of 20–250 µm (Vanhatalo 2017). No more than 10 % of the material has a particle size of less than 5 µm (FAO 2018). In addition to carbohydrates, MCC includes variable proportions (10–40%) of lignin (Vanhatalo et al. 2014).

In wood material, cellulose chains are aggregated in microfibrils during biosynthesis. In ordered regions, cellulose chains are tightly packed together in crystallites. These elements are stabilized by a complex and strong intra- and intermolecular hydrogen-bond network which can vary widely (Habibi et al. 2010). In woody raw materials, cellulose strains are also bonded together by lignin, which makes it a cross-linking polymer (Nsor-Atindana et al. 2017). The bonds between lignin and cellulose and hemicellulose in plant cell walls impair the accessibility of carbohydrates to the rumen microorganisms (Susmel and Stefanon 1993, Ding et al. 2012). Thus, removing forage cell wall lignin, hemicellulose and other substances may improve the rate of digestion of cellulose matter (Van Soest 1994). Indeed, pulp from papermaking containing purified cellulose has high digestibility comparable to regular ruminant feeds (Saarinen et al. 1959).

MCC has many qualities which give it significant opportunities for multiple use (Habibi et al. 2010) and MCC is the only commercially produced crystalline cellulose (Nsor-Atindana et al. 2017). MCC is chemically inactive, stable and physiologically inert and it has notable binding properties. MCC has many promising applications in functional and nutraceutical food industry (Nsor-Atindana et al. 2017) and also paper and composite applications (Habibi et al. 2010). Interest towards MCC has risen as a result of its positive effects on gastrointestinal physiology of humans and due to its hypolipidemic effects observed in rats (Nsor-Atindana et al. 2017).

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There is only a limited number of experiments on the effects of MCC on the health and performance of monogastric animals (Wu et al. 2016) and even less related to digestion in ruminants. In an *in vitro* experiment by Stefański et al. (2018), MCC was readily digested by rumen microbes, which indicates that it is a suitable feed component for ruminants. There is no information of MCC concerning possible health effects and impacts on digestion in ruminants. To elucidate the effects of MCC on feed intake, rumen fermentation, diet digestion and production of dairy cows, *in vivo* experimentation is needed.

The objective of the present experiment was to evaluate the effects of MCC fed to dairy cows with inclusion rates of 0, 10 and 100 g kg^{-1} (on dry matter [DM] basis) on feed intake, rumen fermentation, diet digestion and milk production. The 10 g kg^{-1} DM inclusion rate represented the use of MCC as a feed additive and that of 100 g kg^{-1} as a feed material (i.e. replacing conventional feeds). We hypothesized that MCC inclusion could stabilize rumen fermentation by replacing barley starch with digestible fibre in the diet. According to our hypothesis there is no reduction in milk production in a diet containing MCC compared to the control diet due to increased intake and fibre digestion, which would compensate for the increased fibre content of the diet.

Materials and methods

Production of MCC

The MCC was produced at the XAMK Fiber Laboratory (Savonlinna, Finland) using methods patented by Aalto University (Dahl et al. 2011a, 2011b). In short, the raw material was unbleached softwood kraft pulp, which was taken from the chemical pulping line after the digester stage. Pulp was acidified at about 80 g DM kg^{-1} to a pH level of 1.8 and fed to a continuous digester where the hydrolysis occurred at 165 °C temperature. The reaction time was 30 minutes. The resulting MCC was washed so that the pH of the filtrate was 3.5 and thickened with a belt filter thickener into a DM concentration of 270 g kg^{-1}. The product name is AaltoCell™. The humid form of MCC was chosen for the feeding trial to fasten the production process, to reduce the cost of production and to prevent possible modifications of MCC functional properties due to drying. Moist MCC was stable and showed no signs of spoilage during the feeding trial.

Experimental animal and dietary treatments

The effects of MCC were studied in the research barn of Natural Resources Institute Finland (Luke) at Jokioinen using 24 multiparous (average parity 3.3 ± 1.13) Nordic Red dairy cows. At the beginning of the experiment the cows averaged 176 ± 59.3 days in milk and their average milk yield was 38.9 ± 5.59 kg d^{-1}. The average live weight (LW) and body condition score (scale 1 to 5) (Edmonson et al. 1989) at the beginning of the experiment were 682 ± 63.9 kg and 3.2 ± 0.34, respectively. Cows were housed in a free-stall barn and fitted with transponder collars that allowed identification in the milking parlour, scale and feeding area. Cows were milked in a 2×6 auto tandem milking parlour at 0700 and 1700 h. Experimental cows were divided into 4 blocks of 6 cows each according to parity and calving date. In the block, the cows were directed to the dietary treatment randomly. One block of six cows previously fitted with permanent rumen cannulae (Bar Diamond Inc., Parma, ID, USA) were used for rumen fluid collection. The use of animals in scientific experimentation was in line with Directive 2010/63/EU. The cows were fed according to an imbalanced change over design with three diets and two 21-day experimental periods so that each cow received two out of the three dietary treatments during the experiment and 16 observations per each dietary treatment were obtained. Both experimental periods included 14 days of diet adjustment before collection of data and samples during the last seven days.

The cows were fed total mixed rations (TMR) containing grass silage and concentrate in addition to MCC (Table 1). The experimental silage was made from primary growth of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) swards, grown at Jokioinen, Finland (60°49'N, 23°28'E) in 2017. The silage was slightly prewilted in the field, precision chopped and stored in a clamp. A formic acid based silage additive (760 g kg^{-1} formic acid and 55 g kg^{-1} ammonium formate, AIV 2 Plus; Eastman, Oulu, Finland) was applied at a rate of 5 l ton^{-1} grass. The concentrate components other than MCC were pelleted at the feed mill of Luke, Jokioinen to ensure exact amounts of each component into the TMRs.

Table 1. Recipes (g kg^{-1} DM) of the total mixed rations and the concentrate given at the milking parlour (MPC)

	Total mixed rations			MPC
	CON	MCCL	MCCH	
Microcrystalline cellulose (MCC)	0	10	100	0
Barley	110	109	0	310
Oats	90	89	68	0
Wheat	50	50	30	190
Sugar beet pulp	60	59	75	120
Rapeseed meal	177	175	214	355
Minerals[1]	13	13	13	25
Grass silage	500	495	500	

CON = Control without MCC inclusion; MCCL = diet containing 10 g MCC kg DM^{-1}; MCCH = diet containing 100 g MCC kg DM^{-1}. [1]Mineral premix (Lypsykivennäinen Tiineys+, Hankkija Ltd., Hyvinkää, Finland) declared as containing Ca (210 g kg^{-1}), P (15 g kg^{-1}), Mg (90 g kg^{-1}), Na (95 g kg^{-1}), Selenium (3bE8, 20 mg kg^{-1}; 3b8.11, 10 mg kg^{-1}), Vitamin E (3a700; 2000 mg kg^{-1}) and biotin (3a880; 30 mg kg^{-1})

Experimental treatments comprised three TMRs containing no MCC (control, CON) or MCC inclusion in the diet DM of either 10 (MCCL) or 100 (MCCH) g per kg diet DM. The aim in diet MCCH formulation was to replace rolled barley with MCC and the recipes of the TMRs are presented in Table 1. Total diet crude protein (CP) concentration was maintained the same by increasing rapeseed meal proportion and with minor adjustments in amounts of the cereals. The CON and MCCL cows received the same TMR, and MCCL cows received MCC top-dressed onto their TMR portions twice daily, at 0630 and 1830 h, so that the estimated DM intake from MCC was 10 g kg^{-1} diet DM. For MCCH, a separate TMR was prepared and fresh MCC was mixed into it in a TMR wagon and delivered manually at the same time when the automatic feeding wagon delivered TMR for CON and MCCL, at 0700, 1300, 1600 and 1800 h. To ensure *ad libitum* TMR intake, at least 5% daily refusals were allowed. In addition to the TMR, the cows received 0.6 kg of a concentrate in the milking parlour.

Measurements, sampling and analytical methods

In both experimental periods, measurements and samples were collected during days 15–21. The TMR refusals were recorded manually every day. Representative samples of grass silage were collected daily during the last week of each period, composited at the end of each experimental period and stored frozen (–20 °C) for chemical analyses. Thawed samples were dried at 60 °C, milled through a 1 mm sieve and analysed for DM, ash, CP, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), *in vitro* organic matter (OM) digestibility, acid insoluble ash (AIA) and iNDF. Concentrate samples were collected daily during the last week of each period and analysed for DM, ash, CP, ether extract and NDF, AIA and iNDF. Representative samples of MCC were collected and analysed for DM, ash, CP, NDF, ADF, ADL, *in vitro* OM digestibility, AIA and iNDF. For the measuring of OM digestibility the OM solubility was analysed with pepsin-cellulase enzyme.

The DM was determined by drying at 105 °C for 20 h and silage DM was corrected for the loss of volatiles according to Huida et al. (1986). The ash concentration of the feeds was determined by ashing at 600 °C for 2 h while CP content was analysed using a Dumas-type N analyser (Leco FP-428 N analyser, Leco Corporation, St Joseph, MI, USA). The CP concentration was obtained as N × 6.25. Ether extract in concentrates was determined after acid hydrolysis (HCl) according to Anon (1971). The NDF concentration was analysed according to Van Soest et al. (1991) and ADF according to Robertson and Van Soest (1981) using amylase for feeds containing starch and presented ash free. The ADL was analysed using AOAC Method 973.18 (AOAC 1990). The indigestible NDF (iNDF) concentration of feeds was measured by a 12-day ruminal *in sacco* incubation in nylon bags (Huhtanen et al. 1994). The analysis was not conducted for MCC because based on preliminary *in sacco* measurements there is no iNDF in it and a value of zero was used for it in further calculations.

The *in vitro* organic matter digestibility of the silage and MCC were analysed by the pepsin-cellulase solubility method (Nousiainen et al. 2003). The solubility values were converted to *in vivo* organic matter digestibility by using the equations presented by Huhtanen et al. (2006). For the silage, the equation for primary growth silage was used while the general equation was used for MCC. Fresh silage samples were also analysed for volatile fatty acids (VFA) (Huhtanen et al. 1998), lactic acid (Haacker et al. 1983), water soluble carbohydrates (Somogyi 1945) and ammonium-N (McCullough 1967).

Milk yield was recorded automatically during every milking. In statistical analyses, the milk yield records of the last week in each period were used. The milk samples of each cow for milk fat, protein, lactose, urea and total solids were collected during last two days of each period from four consecutive milkings. Constituent concentrations were calculated as a weighted mean according to milk yield. The analyses of milk protein, fat, lactose, urea and total solids were conducted at the laboratory of Valio Ltd. (Seinäjoki, Finland) using a MilkoScan FT6000 analyser (Foss Electric, Hillerød, Denmark).

Rumen fluid was sampled during the third day in the last week of both periods from the six rumen fistulated cows. Samples were taken at 1.5 hour intervals between 06.00 and 16.30 and analysed for pH, NH_3-N and VFA. Faeces of cows on diets CON and MCCH were spot sampled twice a day for 4 days.

The cows were weighed automatically by walk through static scale (Pellon Group Ltd, Ylihärmä, Finland) every day after morning and afternoon milking. The average body weight from the morning and afternoon weights were used in the calculations. The body condition scores of the cows were assessed using a scale of 1 to 5 (1=skinny to 5=very fat) with intervals of 0.25 (Edmonson et al. 1989) in the beginning of the study and in the end of both periods.

Calculations

The metabolizable energy (ME) content for grass silage was calculated as 0.016 × D-value (MAFF 1975, MAFF 1984). The ME concentration of the concentrates was calculated from digestible nutrients (MAFF 1975, MAFF 1984). The digestibility coefficients for the concentrate components were derived from the Finnish Feed Tables (Luke 2020). The amino acids absorbed from the small intestine (metabolizable protein; MP) and protein balance in the rumen (PBV) were calculated according to the Finnish protein evaluation system (Luke 2020). The energy corrected milk (ECM) yield was calculated according to Sjaunja et al. (1990).

The *in vivo* digestibility of the dietary nutrients (DN) were calculated using AIA (Van Keulen and Jung 1977) and iNDF (Huhtanen et al. 1994) as internal markers (M) according to the following equation:

Digestibility of DN (g g^{-1}) = 1 – (M, g kg DM^{-1} in diet / M, g kg DM^{-1} in faeces) × (DN, g kg DM^{-1} in faeces / DN, g kg DM^{-1} in diet)

The ME intake for feed efficiency calculations was estimated in three different ways. In the basal method, the ME values (MJ kg^{-1} DM) of the feeds, which were calculated on the basis of digestible nutrients, were multiplied by their intake (ME_{TAB}). The ME_{COR} refers to using the equation presented in Luke (2020) in order to correct the basal ME intake. The equation takes into account the negative associative effects of diet digestion and the feeding level of dairy cows and results in lower total ME intake than ME_{TAB}. According to ME_{COR} equation, DMI and diet ME concentration have negative effects on ME intake while diet CP concentration slightly improves it. The third method to estimate ME intake used was based on measured OM digestibility determined by the internal markers AIA (ME_{AIA}) and iNDF (ME_{iNDF}). One kg of digestible OM was assumed to provide 16 MJ ME for the cow (MAFF 1984). Since we did not measure digestibility of treatment MCC10, the mean value of CON was used for it. The N use efficiency in milk production (NUE) was calculated as kg N excreted in milk kg^{-1} N intake. Energy balance was calculated for each cow by subtracting the energy required for milk production and maintenance from the total energy intake. The ME (MJ) used for ECM production (5.15 × ECM, kg) and for maintenance (0.515 × kg $BW^{0.75}$) were based on Finnish requirements (Luke 2020).

Statistical analyses

The data was analysed using a MIXED procedure (SAS Inc. 2002–2012, Release 9.4; SAS Inst. Inc., Cary, NC, USA) of SAS with dietary treatment as fixed effect and cow as random effect. The model used was:

$$Y_{ijkl} = \mu + B_i + C(B)_j + P_k + T_l + \varepsilon_{ijkl},$$

where μ is the overall mean, B_i represents the block, $C(B)_j$ the cow within block, P_k the period, T_l is the treatment effect and ε_{ijkl} is the residual effect. The effects of experimental diets on variation in rumen fluid samples were assessed using the MIXED procedure with a model for repeated measurements and cow as a random effect.

Sums of squares for treatment effects were further divided into single degree-of-freedom comparisons to test for the significance of linear and quadratic effects of MCC inclusion. The contrast coefficients were adjusted to match the uneven inclusion levels on MCC. The tables include the least square means with treatment effects that are significant at $p<0.05$.

Results

The chemical composition and feed values of the experimental feeds are presented in Table 2. Grass silage was of good quality based on energy value (D-value 682 g kg^{-1} DM) and fermentation quality (pH < 4 and ammonia N 54 g of kg total N). The cellulose content, and as a consequence, the NDF content of MCC was very high but the ash and CP concentrations as well as *in vitro* digestibility were low. Consequently the feed values of MCC were low.

Table 2. Chemical composition of the experimental feeds

	Grass silage[1]	CON, MCCL	MCCH	MPC	MCC
Dry matter (DM), g kg^{-1}	217	879	878	876	286
In DM, g kg^{-1}					
Ash	92	84	83	74	1.2
Crude protein	133	207	243	213	12.5
Crude fat	40	34	34	30	nd[5]
Neutral detergent fibre (NDF)	513	263	244	216	937
Acid detergent fibre (ADF)	301	nd	nd	nd	892
Acid detergent lignin (ADL)	27	nd	nd	nd	23
Hemicellulose[2]	212	nd	nd	nd	45
Cellulose[3]	274	nd	nd	nd	869
Indigestible NDF	76	79	97	61	nd
Feed values					
In vitro organic matter digestibility, g g^{-1}	0.751	nd	nd	nd	0.404
D-value[4], g kg^{-1} DM	682	nd	nd	nd	404
Metabolizable energy, MJ kg^{-1} DM	10.9	11.8	11.8	12.2	6.5
Metabolizable protein, g kg^{-1} DM	81	127	126	123	41
Protein balance in the rumen, g kg^{-1} DM	12	50	47	40	-50

[1]Silage fermentation quality: pH 3.99, ammonia N (g kg^{-1} total N) 54, lactic, acetic, propionic and butyric acid, ethanol and water soluble carbohydrates 84, 18, 1.0, 0.05, 8.8 and 58 g kg^{-1} DM, respectively; CON = control without microcrystalline cellulose (MCC) inclusion; MCCL = diet containing 10 g MCC kg DM^{-1}; MCCH = diet containing 100 g MCC kg DM^{-1}; MPC = milking parlour concentrate; MCC = microcrystalline cellulose; nd = not determined; [2]Hemicellulose = NDF – ADF; [3]Cellulose = ADF – ADL; [4]D-value = digestible organic matter

The concentrations of nutrients in the experimental diets are presented in Table 3. There was a clear quadratic increase in diet NDF concentration with diet MCCH ($p<0.05$). Concentrations of ME_{TAB} ($p<0.001$) decreased quadratically ($p<0.001$) and ME_{COR} decreased linearly ($p<0.001$). ME_{iNDF} increased linearly ($p<0.05$) but there was no effect on ME_{AIA} of the increased MCC inclusion in the diet. Diet MP concentration decreased quadratically ($p<0.001$) while PBV increased quadratically slightly although significantly ($p<0.001$) when the proportion of MCC increased in the diet.

The feed intake of the cows on different treatments is presented in Table 4. The palatability of the experimental diets was good as the total DM intake was high on all treatments being on average 25.6 kg day^{-1}. Numerically the feed intake was highest at the highest MCC inclusion but the difference was not statistically significant. The NDF intake increased linearly ($p<0.001$) with increasing MCC inclusion, which was expected as MCC replaced barley in the diets. There were no differences in CP intake between the dietary treatments. Corrected ME intake decreased and ME_{iNDF} increased linearly ($p<0.05$) with increasing MCC inclusion in diet. There were now differences in MP intake between diets as they were balanced with increasing rapeseed meal inclusion on MCCH diet, but protein balance in the rumen was somewhat higher on MCCH compared to CON and MCCL.

Table 3. Diet composition of dairy cows fed microcrystalline cellulose (MCC)

	Diet			SEM	Statistical significance[1]	
	CON	MCCL	MCCH		Linear	Quad
Organic matter	918	916	917	0.1	<0.001	<0.001
Crude protein	171	169	166	0.1	<0.001	<0.001
Crude fat	37.8	37.4	32.7	0.01	<0.001	<0.001
Crude fibre	50.2	49.7	39.7	0.02	<0.001	<0.001
Neutral detergent fibre (NDF)	375	381	451	0.5	<0.001	0.017
ME_{TAB}[2]	11.5	11.4	10.8	0.001	<0.001	<0.001
ME_{COR}[3]	10.6	10.6	10.1	0.02	<0.001	0.325
ME_{AIA}[4]	10.6	10.6	10.5	0.04	0.244	0.426
ME_{iNDF}[5]	9.4	9.5	9.7	0.07	0.003	0.319
Metabolizable protein	101	100	97.6	0.04	<0.001	<0.001
Protein balance in the rumen	25.1	24.3	25.7	0.08	<0.001	<0.001

CON = control without microcrystalline cellulose (MCC) inclusion; MCCL= 10 g MCC kg DM^{-1}; MCCH = 100 g MCC kg DM^{-1}; [1]Significance of linear and quadratic (Quad) effects of MCC inclusion in the diet; [2]ME_{TAB} = metabolizable energy based on tabulated feed values according to Luke (2020); [3]ME_{COR} as ME_{TAB} but with applying the correction equation presented in Luke (2020); [4]ME_{AIA} and [5]ME_{iNDF} refer to ME intake calculated from digestibility measured individually for each cow using acid insoluble ash (AIA) and indigestible NDF (iNDF) as an internal marker (mean of CON used for cows fed MCCL).

Table 4. Feed and nutrient intake of dairy cows fed microcrystalline cellulose (MCC)

	Diet			SEM	Statistical significance[1]	
	CON	MCCL	MCCH		Linear	Quad
Intake, kg DM day^{-1}						
Total	25.4	25.2	26.0	0.43	0.197	0.650
Silage	12.4	12.2	12.8	0.22	0.139	0.391
Concentrate	12.9	12.7	10.7	0.19	<0.001	0.975
MCC	0	0.25	2.56	0.040	<0.001	0.919
Concentrate+MCC	12.9	13.0	13.2	0.22	0.273	0.963
Organic matter	23.3	23.1	23.9	0.40	0.214	0.680
Crude protein	4.33	4.25	4.32	0.073	0.863	0.469
Crude fat	0.960	0.943	0.852	0.0150	<0.001	0.753
Neutral detergent fibre	9.52	9.59	11.7	0.187	<0.001	0.574
ME^2 intake, MJ day^{-1}						
ME_{TAB}[3]	291	287	280	4.8	0.146	0.698
ME_{COR}[4]	270	267	263	4.1	0.018	0.434
ME_{AIA}[5]	268	267	274	4.4	0.292	0.768
ME_{iNDF}[6]	238	239	253	4.6	0.022	0.958
Protein intake, g day^{-1}						
Metabolizable protein	2563	2526	2541	42.8	0.897	0.570
Protein balance in the rumen	636	611	670	11.3	0.006	0.092

CON = control without microcrystalline cellulose (MCC) inclusion; MCCL = 10 g MCC kg, DM^{-1}; MCCH = 100 g kg DM^{-1}; [1] Significance of linear and quadratic (Quad) effects of MCC inclusion in the diet; [2]Metabolizable energy (ME); [3] ME_{TAB} based on tabulated feed values according to Luke (2020); [4]ME_{COR} as ME_{TAB} but with applying the correction equation presented in Luke (2020); [5]ME_{AIA} and [6]ME_{iNDF} refer to ME intake calculated from digestibility measured individually for each cow using AIA and iNDF as an internal marker (mean of MCC0 used for cows fed MCC10)

No statistically significant effects on rumen pH, ammonia concentration or rumen fermentation pattern could be detected, although ammonia concentration tended to increase with increasing MCC inclusion (Table 5). The sampling time significantly affected rumen fermentation. However, no interactions were detected between the dietary treatments and sampling time.

Table 5. Rumen fermentation of dairy cows fed microcrystalline cellulose (MCC)

	Diet			SEM	Statistical significance[1]		
	CON	MCCL	MCCH		Linear	Time	Diet×time
pH	6.22	6.17	6.28	0.089	0.669	0.043	0.267
Ammonia N, mmol l^{-1}	3.84	3.91	5.11	0.751	0.085	<0.001	0.612
Total acids, mmol l^{-1}	126.9	127.9	127.7	2.55	0.914	0.154	0.327
Proportions of volatile fatty acids							
Acetic acid (A)	0.633	0.628	0.630	0.0048	0.832	<0.001	0.303
Propionic acid(P)	0.191	0.191	0.189	0.0052	0.657	<0.001	0.284
Butyric acid (B)	0.136	0.132	0.135	0.0037	0.947	0.016	0.139
A+B P^{-1}	4.10	4.01	4.08	0.146	0.841	<0.001	0.285
A P^{-1}	4.67	4.77	4.69	0.142	0.872	<0.001	0.094

CON = control without microcrystalline cellulose (MCC) inclusion; MCCL = 10 g MCC kgDM^{-1}; MCCH = 100 g MCC kg DM^{-1}; [1]Significance of linear effects of MCC inclusion in the diet

There were no significant differences of MCC inclusion on digestibility of diet DM or OM determined with either AIA or iNDF method (Table 6). However, digestibility of CP showed a decrease with AIA method in response to MCC inclusion while no effect was detected when using iNDF as a marker. The digestibility of NDF increased clearly according to both markers (p<0.001). There was a clear difference in the absolute values between AIA and iNDF as AIA values e.g. for organic matter digestibility were on average 0.068 units higher than those derived from iNDF.

Table 6. Apparent diet digestibility of dairy cows fed microcrystalline cellulose (MCC) using acid insoluble ash (AIA) and indigestible NDF (iNDF) as internal markers

	Diet		SEM	Statistical significance
	CON	MCCH		
Dry matter				
AIA	0.701	0.697	0.0029	0.439
iNDF	0.617	0.635	0.0078	0.216
Organic matter				
AIA	0.721	0.717	0.0028	0.383
iNDF	0.643	0.659	0.0072	0.211
Crude protein				
AIA	0.678	0.662	0.0035	0.037
iNDF	0.587	0.593	0.0093	0.690
Neutral detergent fibre				
AIA	0.603	0.669	0.0056	<0.001
iNDF	0.496	0.601	0.0087	<0.001

CON = control without microcrystalline cellulose (MCC) inclusion; MCCH = 100 g MCC kg DM^{-1}

The effects of experimental treatments on milk yield and composition are presented in Table 7. Dietary MCC supplementation decreased linearly (p<0.05) the daily yields of ECM, fat and protein. These production variables did not, however, follow the results of apparent digestibility of DM or OM, which were not affected by the MCC inclusion. The concentrations of protein and urea decreased linearly (p<0.01and p<0.05, respectively) when the proportion of MCC increased in diet. These results were in line with the decreased apparent digestibility of CP when the amount of MCC increased in the diet. However, numerically the differences in milk production were small. Dietary inclusion of MCC decreased linearly (p=0.001) the feed efficiency of cows (Table 7). The cows produced on average 1.46 kg ECM per kg DMI and 0.14 kg ECM per kg metabolizable energy intake ($MEI_{COR,}$ $ME_{AIA,}$ ME_{iNDF}). Inclusion of MCC decreased linearly energy $balance_{AIA}$ and energy $balance_{iNDF}$ (p<0.05 and p=0.001, respectively), which was calculated by subtracting the energy required for milk production and maintenance from the total energy intake.

The inclusion of MCC did not affect body live weight of the cows. However, the average body condition score increased quadratically (p<0.01) and the change in it turned out positive and increased linearly (p=0.05) when the amount of MCC increased in the diet.

Table 7. Milk production, efficiency of milk production, body live weight and body condition score of dairy cows fed microcrystalline cellulose (MCC)

	Diet			SEM	Statistical significance[1]	
	CON	MCCL	MCCH		Linear	Quad
Production, kg day^{-1}						
Milk	34.7	35.6	34.7	0.40	0.074	0.885
Energy corrected milk	36.8	36.6	35.3	0.45	0.024	0.977
Yield, g day^{-1}						
Milk fat	1499	1500	1442	20.2	0.039	0.818
Milk protein	1290	1290	1231	15.5	0.008	0.783
Milk lactose	1575	1548	1518	20.2	0.094	0.477
Milk composition, g kg^{-1}						
Fat	42.0	42.3	41.8	0.45	0.615	0.703
Protein	36.2	36.5	35.7	0.13	0.003	0.129
Lactose	44.0	43.5	43.7	0.18	0.554	0.072
Total solids	13.3	13.3	13.2	0.50	0.117	0.654
Urea, mg 100 ml^{-1}	25.3	25.3	23.6	0.02	0.015	0.888
Efficiency of milk production						
NUE	0.297	0.304	0.286	0.0054	0.062	0.329
kg ECM/DMI	1.45	1.46	1.36	0.025	0.010	0.604
kg ECM/MEI$_{COR}$	0.136	0.137	0.135	0.0022	0.517	0.650
kg ECM/MEI$_{AIA}$	0.137	0.137	0.129	0.0022	0.011	0.744
kg ECM/MEI$_{iNDF}$	0.154	0.153	0.141	0.0027	0.001	0.957
Energy balance[2]$_{COR}$	12.9	10.3	13.0	3.66	0.795	0.615
Energy balance$_{AIA}$	11.4	10.6	24.4	3.88	0.014	0.708
Energy balance$_{iNDF}$	-18.7	-17.0	3.8	4.12	0.001	0.921
Body live weight						
Mean, kg	671	673	674	2.1798	0.386	0.539
Change, kg day^{-1}	-0.34	-0.26	-0.102	0.1400	0.266	0.763
Body condition score[3]						
Mean	3.16	3.30	3.28	0.030	0.149	0.008
Change	-0.04	0.104	0.16	0.050	0.045	0.088

CON = control without microcrystalline cellulose (MCC) inclusion, MCCL = 10 g MCC per kg dry matter, MCCH = 100 g MCC kg^{-1} dry matter; [1]Significance of linear and quadratic (Quad) effects of MCC inclusion in the diet; NUE = N use efficiency (kg N in milk / kg N intake); ECM = Energy corrected milk; DMI = Dry matter intake; MEI = Metabolizable energy intake; MEI$_{COR}$ = MEI calculated based on tabulated feed values according to Luke (2020) with applying the correction equation presented in Luke (2020).MEI $_{AIA}$and MEI$_{iNDF}$ refer to MEI calculated from digestibility measured individually for each cow using acid insoluble ash (AIA) or indigestible neutral detergent fibre (iNDF) as an internal marker (mean of CON used for cows fed MCCL). [2]Energy balance was calculated for each cow by subtracting the energy required for milk production and maintenance from the total energy intake. The ME (MJ) used for ECM production (5.15 × ECM, kg) and for maintenance (0.515 × kg BW$^{0.75}$) were based on Finnish requirements (Luke 2020). [3]Scale to 1 from 5 with 1 being the lowest and 5 the highest body fat content.

Discussion

To the best knowledge of the authors, MCC has not been used as a feed component to lactating dairy cows earlier so we used relatively low level of MCC in the diets to minimize the risk of digestive disorders. The MCCL diet represents a situation where MCC would be used as a feed additive while that of MCCH already has an impact on the nutrient intake from the basal diet. The MCC tended to form larger lumps, which did not totally mix with the other feeds during total mixed ration (TMR) preparation, but the final TMR was homogenous enough to prevent separation of MCC by cows during eating.

The general observations of the experiment revealed that MCC was readily consumed by the cows at both dosage levels with no indications of reduced voluntary feed intake or abnormalities in digestion, production or general behaviour of the cows.

Effects of MCC inclusion on feed intake and diet digestibility

Feed intake is a key factor in determining the amount of nutrient supply and subsequently milk production of dairy cows (Huhtanen and Nousiainen 2012). Dietary factors affecting feed intake on grass silage based diets are well known (Huhtanen et al. 2011) and include e.g. the proportion of silage in the diet and silage quality as well as concentrate composition. In our case, most factors were constant except the increased fibre concentration of the non-silage proportion of the diet, which based on the meta-analysis of Huhtanen et al. (2011) should increase total diet DM intake. We found no change in feed intake but numerically it was highest on MCCH in line with Huhtanen et al. (2011).

The high intake of MCCH is probably linked to the high fibre digestibility of MCC. Digestibility of conventional cellulose pulp (Saarinen et al. 1959) and dissolving pulp (Näsi 1984) have been found to be high, but information of MCC digestibility *in vivo* in ruminants was lacking. However, the *in vitro* gas production results of various MCC products revealed that rumen microbes readily digested MCC (Stefański et al. 2018).

We determined the *in vivo* digestibility of the diets using AIA and iNDF as internal markers. They are commonly used to determine diet total tract digestibility but there are some problems related to them. The concentration of AIA in the feeds is very low and it predisposes the method to analytical errors. Soil contamination of feeds may reduce the reliability of AIA (Huhtanen et al. 1994). The loss of feed particles from the nylon bags may cause incomplete recovery for iNDF (Huhtanen et al. 1994). This has probably been the reason for the lower digestibility values derived from iNDF compared to AIA marker.

The ability of a method to detect the differences between treatments may be more important than to produce the precise values. The R^2 values between ME intake based on digestibility with internal markers AIA and iNDF and milk ME output were 0.83 and 0.93, respectively. Based on these results iNDF may have been able to detect the differences between the dietary treatments in digestibility more accurately than AIA. There were only two observations for these calculations so more data is needed to confirm the conclusion. Although AIA and iNDF as internal markers differed from each other similarly as in our previous experiment (Savonen et al. 2020), they both showed that diet fibre digestion significantly increased when MCC was included in the diet. Calculated as the difference in fibre intake and digested fibre intake of diets CON and MCCH, the MCC digestibility was complete (0.96 and 1.06 when using AIA and iNDF, respectively).

MCC has not been used as a feed component to lactating dairy cows earlier so there are no digestibility results of MCC. However, different kinds of chemically treated wood materials have been used as a feed component for ruminants. Rations containing low acid (80 g kg^{-1} H_2SO_4) hydrolysed wood residues (with cellulose content of 480 g kg^{-1} DM) at rates of 0, 0.25, 0.50 and 0.75 had lower *in sacco* DM (0.56, 0.49, 0.45 and 0.37, respectively) and OM (0.55, 0.47, 0.44 and 0.37, respectively) digestibilities compared to the basal ration (Butterbaugh and Johnson 1974). The *in vivo* digestibility results for goats showed that the inclusion of the mixed hardwood kraft bleached pulp fines generated during sulphate process of commercial pulp and paper operations increased the DM digestibility (Millett et al. 1973). The effective breakdown of the lignin-cellulose (composition up to 280 and 980g kg^{-1} DM, respectively) matrix by chemical pulping and bleaching led to a significant enhancement of DM digestion (0.59 for basal ration to 0.78 with the peak proportion of 0.5 in ration) Millett et al. (1973).

Treatment of wood with high acid (230 g kg^{-1} H_2SO_4) concentration decreased the DM digestibility in lambs by forming large amount of artefact lignin reducing the palatability and digestibility leading to low digestibility (0.43 at the level of 200 g kg^{-1} DM) (Butterbaugh and Johnson 1974). These digestibility results of paper making residues are not directly comparable with the MCC examined in the current study. There is only a limited amount of amorphous cellulose in MCC compared to the paper making residues. The amorphous part has a more open structure and is more inclined to chemical, biological and mechanical treatments (Vanhatalo 2017).

One factor facilitating the efficient digestion of MCC may be the small particle size of it, which facilitates microbial access to the substrate. The degree of polymerization (DP) of MCC is 400 (Vanhatalo 2017) while that of native wood is approximately 10000 (Sjöström 1993). The typical particle size of commercial MCC products range between 20–250 µm (Vanhatalo 2017). For comparison, the particle size distribution of rations based on different silages ranged between 2440–2832 µm (Bayat et al. 2011).

We also determined the MCC digestibility using the *in vitro* pepsin cellulase method which has been developed and optimized for typical forages (mainly grass silages) used for ruminants (Nousiainen et al. 2003, Huhtanen et al. 2006). Based on the *in vitro* method, the OM digestibility of MCC was only 0.404 which is very similar to the values reported by Stefanski et al. (2018). According to Huhtanen et al. (2006), a typical OM digestibility value for grass silages would be around 0.75, but these values are not directly comparable. The fibre concentration of typical grass based forages is approximately half of the DM so the other half (i.e. cell solubles) is totally solubilized in the process. For typical forages, this means that the fibre digestion *in vitro* is clearly lower than *in vivo*. Using the numbers from Huhtanen et al. (2006) and assuming complete digestion of cell solubles, the *in vitro* and *in vivo* NDF digestibilities would be 0.5 vs 0.75, respectively. It is also important to notice that the *in vitro* analysis represents true digestibility as there is no endogenous nor metabolic excretion *in vitro*, which makes it essential to convert the *in vitro* values to *in vivo* values using corrections equations based on sufficient amount of reference data. Obviously, this was not possible for MCC and demonstrates the challenges of using traditional feed analysis methods for non-typical samples such as MCC.

Effects of MCC inclusion on milk yield and rumen functions

Feed energy value can be calculated based on digestibility (Luke 2020) and the energy intake is closely linked with milk energy output (Huhtanen and Nousiainen 2012). However, in this experiment, energy intake calculated based on all methods increased with MCC inclusion while that of milk output decreased. However, numerical differences as well as the range in dietary treatments were small. Part of the discrepancy may be explained by greater partitioning of feed energy into body tissues as indicated by the increased body condition score of the cows fed MCC.

After energy intake, protein supply is the second most important factor affecting milk production of dairy cows (Huhtanen and Nousiainen 2012). MCC contained virtually no protein, but in our design, the diets were balanced to be isonitrogenous by rapeseed meal supplementation so the decreased milk production linked with MCC supplementation should not be attributed to protein supply.

There are reports from feeding MCC to monogastric animals that benefits related to gastrointestinal tract function have been found (Nsor–Atindana et al. 2017). Subacute rumen acidosis (SARA) is a common and serious problem in intensive dairy production when high concentrate diets are used to increase milk production. The risk of ruminal pH to drop drastically can be reduced by diets which contain adequate amount of dietary fibre with sufficiently large particle size (Krause and Oetzel 2006). According to the hypothesis of our study, MCC inclusion could stabilize rumen fermentation by replacing barley starch in the diet with digestible fibre. However, we found no differences in rumen fermentation between the dietary treatments, which probably reflects the high digestibility and the small particle size of MCC. Further, the fibre concentration in the control diet was high with a reasonable proportion of concentrate at 0.5 of total diet DM which was delivered as TMR so the potential to show improvements in rumen pH was not very high.

The economics of MCC use are dominated by the cost of MCC relative to the conventional feeds it would replace in the diet, but other factors may modify this simple relationship. Although we could not demonstrate benefits of including MCC into a dairy cow diet, it might be justified to evaluate the potential of MCC to stabilize rumen fermentation in a more challenging dietary situation. Other potential benefits of using MCC include possibility to use forest based feed materials which are not directly competing with human edible food resources. From nutrient management point of view, a feed material with virtually no N or P in it could help increase the nutrient use efficiency in milk production as e.g. N use efficiency in milk production is mainly governed by the amount of N intake (Huhtanen et al. 2008). Wood based feed materials could be used as an emergency feed (Rinne and Kuoppala 2019) if the prizes of the traditional feeds would increase due to unexpected weather conditions or other disturbances in feed supply.

Conclusions

This study demonstrated that humid MCC can be included into dairy cow diets and it is palatable to dairy cows. However, it decreased milk production slightly when replacing barley grain in the diet. Beneficial effects on ruminal digestion could not be demonstrated under the conditions of the current experiment. Further studies would be needed to evaluate the effects of long-term usage of MCC. The influence of MCC under more challenging dietary conditions should also be examined for example without TMR feeding and with higher concentrate to forage ratio to assess if it could stabilize rumen fermentation.

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