

Effect of ruminal mechanical stimulating brushes on the performance of lactating Holstein dairy cows

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Radko Loucka¹, Filip Jancik^{1}, Junichi Takahashi², Vaclav Jambor³, Ludek Barton¹, Petr Homolka^{1,4}, Ivana Knizkova¹, Veronika Koukolova¹, Petra Kubelkova¹, Petr Kunc¹, Yvona Tyrolova¹, Alena Vyborna¹*

¹Institute of Animal Science, 815, 104 00 Prague - Uhřetín, Czech Republic

²Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan

³NutriVet s.r.o., Videnska 1023, 691 23 Pohorelice, Czech Republic

⁴Czech University of Life Sciences Prague, Faculty of Agrobiolgy, Food and Natural Resources, Dpt. of Microbiology, Nutrition and Dietetics, Kamycka 129, 165 00 Prague 6 - Suchbát, Czech Republic

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*Corresponding author: jancik.filip@vuzv.cz

Abstract

The aim of this study was to confirm the hypothesis that artificial brushes administered to the rumen can partially replace the function of structural fibre, and increase milk production or quality. To mitigate the risks of feeding low levels of physically effective neutral detergent fibre (peNDF) to cattle, the administration of ruminal mechanical stimulating (RMS) brushes was examined in 22 high-yielding lactating Holstein dairy cows. The cows were divided into an experimental group equipped with RMS brushes and a control group without RMS. Cows were fed four experimental total mixed rations (TMR) consisting of fixed amounts of alfalfa silage, maize silage, rush corn cob mix silage and different proportions of brewer's grains, concentrate and wheat straw. The TMRs had the following 4 peNDF contents: 10.9 %, 13.0 %, 12.6 % and 14.0 %. The duration of the experiment was 18 weeks. All cows were fed TMRs with a low structural fibre content near levels associated with a risk of subacute ruminal acidosis (SARA). For the RMS brush group, 3 RMS brushes were inserted orally into the rumen using a special applicator. The effects of RMS brushes on feed intake, rumen fermentation and milk production were evaluated. Ruminant fluid (250 mL) was taken using a stomach tube for pH, volatile fatty acids and ammonia nitrogen analysis. A significant increase was found for the feed intake and milk yield of the RMS group fed the TMRs with 13.0 % peNDF although no relationship between peNDF content and RMS TMRs was found. No significant increase in milk quality, rumen pH or rumen fermentation metabolites was declared for the group with RMS brushes. Although RMS brush technology only partially reduced the requirements for peNDF, it may decrease the risk of SARA.

Key words: rumen function; ruminant nutrition; ruminal mechanical stimulating brushes; milk quality; milk yield

Introduction

The effect of ruminal mechanical stimulation (RMS) brushes on rumen fermentation was studied in dairy cows (Golder et al., 2017), and in Holstein steers (Matsuyama et al., 2000; Horiguchi and Takahashi, 2000, 2002, 2004). Studies on the effect of ruminal mechanical stimulation (RMS) brushes on rumen fermentation in dairy cattle have been reported by Golder et al. (2017), Matsuyama (2002) and Horiguchi and Takahashi (2000). According to the patent (EP0609045A2), the administration of RMS brushes into the rumen of dairy cows will be expected to increase milk production by 0.9 to 3.5 litres of FCM (fat corrected milk) milk per dairy cow per day.

The neutral detergent fibre (NDF) content in diets is related to rumination and cud-chewing stimuli and thus rumen fermentation and digestion (Jancik et al., 2010; Koukolova et al., 2010). Structural fibre has a significant effect on the motion activity of the rumen. This activity depends on physically effective neutral detergent fibre (peNDF) in TMR. The peNDF is determined by multiplying the content of the NDF in DM and the % residue on the 8- and 19-mm sieves of the Penn State Particle Separator (PSPS). Beauchemin and Yang (2005) confirmed that particle length is a reliable indicator of the rumination period, but it is not necessarily an indicator of rumen acidosis. Acidosis is defined as a state of high pathological acidity in the blood. This state includes situations of ruminal or systemic acidity and represents the most important nutritional disorder in dairy cattle and beef cattle in feedlots. The duration of which the pH remained below the threshold value 5.8 in a 24-h period was used as an indicator to characterize subacute ruminal acidosis (SARA) by Valente et al. (2017). Plaizier (2004) determined that a peNDF of 12.5 % DM or lower resulted in a rumen pH indicative of SARA.

Rumen acidosis could be reduced by using ruminal mechanical stimulating (RMS) brushes, European patent EP0609045A2, commercially known as Rumenfibe (RF; Meiwa-Sangyo Co., Ltd, Kyoto, Japan). This device administered as three individual brushes per dairy cow could be used for stimulating the physical function of the rumen mucosa. According to this patent entitled "Method and device for improving milk secretion of cattle", the yield of dairy cows could be increased by 0.9 to 3.5 litres of FCM/cow/day. In addition, this device can replace high-fibre (hay, straw) feed by a minimum of 1.1 kg per day in the ration or allow for feeding of more feeds with relatively low peNDF values, such as grain, Brewers' grains, and distillery grains; specifically, these feeds can be increased by approximately 1.25 kg compared to the standard amount without metabolic disturbances.

Mechanical stimulation of rumination by the intra-ruminal administration of inert fibre particles was studied by Campion and Leek (1996). They used inert fibre particles in a series of experiments to quantify the effects of some of the physical characteristics of dietary long fibres on rumination. When different masses of loose, inert polyethylene particles that had been chopped into 10-mm lengths were

placed in the rumen, the time spent ruminating was found to be directly proportional to the mass of fibre placed in the rumen. The authors concluded that inert polyethylene fibres mimicked the peripheral excitatory effects of dietary fibre by stimulating mechanoreceptors (epithelial receptors) located in the cranial portions of the reticulorumen. Smith (1971) examined the RMS device (Rumen Reticulator) in cows. The device was a 4 and 1/2-inch rod with four tentacles on each end, made entirely of nontoxic plastic. Data from their trials indicated no effect of the Rumen Reticulator on feed intake, milk or milk fat production, nor the percentage of fat in the milk. Furthermore, Golder et al. (2017) examined the effects of RMS brushes on rumen fermentation and subsequent milk production in early lactation dairy cows from a commercial pasture-based herd in the Australian spring and summer. Consequently, RMS brushes did not affect rumen fermentation properties or milk production. However, plasma biological antioxidant potential as an indicator of oxidative stress increased in cows with RMS brushes.

The present paper evaluated the beneficial effect of administration of RMS brushes on the performance of high-yielding dairy cows in early lactation fed on TMR varying in low structural fibre. The hypothesis was that artificial brushes applied to the rumen could partially replace the function of structural fibre and increase milk production or quality.

Materials and methods

The experiment was carried out on the Experimental Farm of the Institute of Animal Science in Prague (50°05'N, 14°27'E; altitude of 287 metres above sea level; 8.4 °C daily mean temperature; 526 mm average precipitation). The temperature and relative humidity inside the barn were continuously measured during the experiment. The average daily values are shown in Table 1.

Table 1. Air temperature and relative humidity in the experimental barn (mean; \pm SD)

Period	T (°C)	RH (%)	THI
P1	17.0 \pm 4.9	66.0 \pm 17.5	61.7 \pm 6.58
P2	19.0 \pm 6.1	67.4 \pm 18.4	64.7 \pm 7.83
P3	21.4 \pm 5.1	58.2 \pm 17.9	67.6 \pm 6.02
P4	23.3 \pm 6.3	51.9 \pm 18.8	69.7 \pm 6.61
P5	17.8 \pm 5.5	65.4 \pm 18.3	62.9 \pm 7.38

T, Temperature; *RH*, Relative Humidity; *THI*, Temperature Humidity Index ($THI = 0.8 * T + RH / 100 * (T - 14.4) + 46.4$)

The feeding experiment was carried out in accordance with the practices outlined in Act No. 183/2017 Sb. Protection of animals against cruelty. The experimental meth-

odology (project MZe NAZV QJ1510391) was approved by the Czech Ministry of Agriculture (13320/2015-MZE-17214). A total of 22 Holstein dairy cows were included in the experiment. The cows were divided into experimental (RF) and control (C) groups by the pairing method according to parity, days after calving, milk yield and liveweight, so that each cow pair was comparable at the beginning of the first experimental period (Table 2).

Table 2. Parameters for the inclusion of dairy cows in the experimental (RF) and the control (C) groups (mean; \pm SD)

Group	Lactation	Days after calving	Milk yield (l/day)	LW of cows (kg)
RF	2.5 \pm 0.9	81.5 \pm 41.2	45.1 \pm 7.9	613 \pm 49.1
C	2.9 \pm 0.9	79.7 \pm 31.1	44.3 \pm 5.7	614 \pm 57.0

Values were not significantly ($P > 0.05$) different between groups, \pm , standard deviation, LW - liveweight

The experiment started on May 4, 2015. In the first preparatory period, lasting 3 weeks, dairy cows were acclimated to the barn and feeding technology. Five experimental periods (P1 - P5) were implemented, each lasting 3 weeks. At the beginning of the second (P2) period, 3 individual artificial RMS brushes were administered *per os* using a custom designed applicator by Meiwa-Sangyo to the rumen of cows in the RF group. Each RMS brush consisted of synthetic polymer bristles held in place with a metal component and was enclosed in a paper capsule that dissolved after insertion (Golder et al., 2017).

The cows were stabled in an experimental barn equipped with tensometric feeding troughs (Insentec, Marknesse, NL) connected to a computer system. The intake of feed was continuously monitored individually for each cow during the experiment. Sawdust bedding was used to avoid the eating of straw by animals. The yield and quality of milk and the live weight (LW) of cows were monitored daily. The composition of the experimental TMRs is shown in Table 3.

Cows in both experimental groups were fed *ad libitum* with unrestricted access to feed and water. The TMR1 was fed during the adaptation period, P1 and P2, TMR2 was fed in P3, TMR3 was fed in P4, and TMR4 was fed in P5. The concept of feeding rations was as follows: TMR1 did not contain straw, TMR2 contained 1.9 % of straw, TMR3 had an increased malt content (from 11.5 % to 18.8 %) and reduced concentrate content (from 17.3 % to 13.2 %), and TMR4 contained no straw (from 19 % to 0 %). For the estimation of the chemical composition of the diet, we used optimized nutritional software, a product of the feed consulting company AgroKonzulta Zamberk based on NRC (2001). Samples were taken every week at the same time. The chemical composition of each TMR was repeatedly analysed 3 times in each experimental period. The fresh samples were dried for 24 hours at 50 \pm 2 °C and subsequently milled to pass through a 1-mm sieve. The DM

(# 934.01), ash (# 942.05), crude protein (# 976.05), starch (# 920.40), neutral detergent fibre (# 2002.04) and acid detergent fibre (# 973.18) contents were determined according to the methods of the Association of Official Analytical Chemists (AOAC, 2005). The aNDF in maize silage was determined according to the procedure of Van Soest et al. (1991). The nonfibre carbohydrates (NFCs) were calculated as described by Van Soest et al. (1991). NEL was calculated according to Van Es (1978). Table 3 shows the composition of the concentrate feed mixtures (DO1 and DO2) and the average nutrient content in the TMRs and the particle length in the TMRs measured using a Penn State Particle Separator (PSPS) with 8-mm and 19-mm sieves (S. Kunc, Strojirna Postupice, IČO 16579038). The peNDF was determined by multiplying the content of the NDF in the DM and the % residue on the 8-mm and 19-mm sieves of the PSPS.

The intake of TMRs and milk yield were measured daily. The chemical composition of feed, ruminal fluid samples,

Table 3. Characteristics of total mixed rations used in experiment

Index	TMR1	TMR2	TMR3	TMR4
Alfalfa silage (%)	23.3	21.1	24.4	27.7
Maize silage (%)	35.9	35.6	32.0	29.6
Maize silage CCM (%)	8.7	10.6	8.5	8.9
Brewers' grains (%)	11.7	11.5	18.8	18.8
DO1 concentrate (%)	18.4	17.3	0.0	0.0
DO2 concentrate (%)	0.0	0.0	13.2	13.8
Energy concentrate (%)	1.2	1.2	0.8	0.8
Promel mineral (%)	0.8	0.8	0.4	0.4
Wheat straw (%)	0.0	1.9	1.9	0.0
Sum	100	100	100	100
Dry matter (DM) (g/kg)	460	481	449	450
Protein (g/kg DM)	189	169	159	162
NDF (g/kg DM)	317	362	356	353
ADF (g/kg DM)	174	206	205	202
Starch (g/kg DM)	297	286	262	277
NFC (g/kg DM)	343	323	344	353
NEL (MJ/kg DM)	7.55	7.24	6.47	6.56
Ca/P ratio	2.36	2.24	2.67	2.66
Na/K ratio	3.51	3.44	3.30	3.56
% in the upper 19 mm sieve of PSPS	7.7	8.9	7.6	9.1
% between the 8 and 19 mm sieve of PSPS	26.7	27.1	27.9	30.5
% in the bottom of PSPS	65.6	64.0	64.5	60.4
peNDF	10.9	13.0	12.6	14.0

TMR - total mixed ration; CCM - Corn Cob Mix; DO1 - mixed grain concentrate for the first part of lactation (Composition: Wheat 30 %, Triticale 16 %, Soya 17 %, Rapeseed meal 16 %, AminoPlus protein 6 %, Fat protected against rumen degradation 6 %, Premin DO1 mineral 8 %, Sodium bicarbonate 1 %); DO2 - mixed grain concentrate for the second part of lactation (Composition: Wheat 55.8 %, Soya 10 %, Rapeseed meal 27 %, Premin DO2 mineral 6 %, Sodium bicarbonate 1.2 %); aNDF, ADF - acid detergent fibre, NDF - neutral detergent fibre, NFC - nonfibre carbohydrates, NEL - net energy of lactation, PSPS - Penn State Particle Separator, peNDF - physically effective NDF

quality of milk and behaviour were measured for all cows at the end of each period. Milk quality was analysed in the accredited (ČSN EN ISO/IEC 17025:2005) laboratory (MILCOM a.s., Prague, CR) using the following methods: fat in % by the butyrometric method (ČSN ISO 2446), protein in % by the Kjeldahl method (ČSN 57 0530) and urea in mg/L milk by an infrared analyser. Rumen fluid was sampled using a stomach tube (length: 240 cm; diameter: 2.5 cm; depth of insertion: 180 cm) four hours after morning feeding. A total of 250 mL of ruminal fluid was taken, and 1 mL of toluene was added for preservation. Then, the samples were transported to the laboratory, the pH was measured and the ruminal fluid was centrifuged at 1200 rpm for 5 minutes (rotor diameter: 170 mm). The resulting supernatant was saved in a PE bottle and frozen until analysis. Rumen fermentation properties were analysed as follows: pH potentiometrically using inoLab level 1 (INOLAB), volatile fatty acids (mmol L ruminal fluid) by a capillary electrophoresis method (Kvasnicka, 2000) using an ITP/CZE analyser ION-OSEP 2003 (RECMAN) and ammonia nitrogen (mg N/100 g ruminal fluid) spectrophotometrically using Biochrom Libra s22 (BIOCHROM).

All cows in the experiment were monitored daily for clinical signs of acidosis, i.e., bubbly scours, dull demeanour, lameness, not getting pregnant, treatment for mastitis, and treatment of limbs, or average conditions.

Feeding behaviour was measured every 3 days in each period from 7 am to 7 pm. The numbers of cows that were drinking, eating, chewing while standing, chewing while lying, standing and lying were recorded every 10 minutes. The behaviour data were evaluated by a simple T-test.

Other statistical data were analysed using the general linear model (GLM) procedure of SAS (SAS Institute, Cary, NC, USA, 1999). Model 1 included the fixed effect of the experimental group was used for the data in Table 2. Model 2 included the fixed effect of the experimental group, but alternatively the linear regression of the overall mean at P1 for each of the parameters examined was used for the data in Tables 4-7.

Model 1

$$Y_{ij} = \mu + A_i + e_{ij}$$

where:

Y_{ij} = observed variable;

μ = overall mean;

A_i = fixed effect of experimental group; and

e_{ij} = random error.

Model 2

$$Y_{ijk} = \mu + A_i + Bx_j + e_{ijk}$$

where:

Y_{ijk} = observed variable;

μ = overall mean;

A_i = fixed effect of experimental group;

Bx_j = linear regression of the observed variable at the experimental period P1; and

e_{ijk} = random error.

Results and discussion

For the evaluation of SARA risk, Plaizier (2004) identified that the critical content of peNDF in TMR for the development of SARA is less than 12.5 % on a dry matter basis. We compiled feed rations so that TMR1 was below 12.5 % peNDF, and the other TMRs were at or just above this level. In the present study, the peNDF in TMR1 was lower than the value considered to induce a risk of SARA. The other TMRs had peNDF values ranging from 12.6 to 14.0%. These were slightly higher than that in TMR1 and at the border of the critical values for SARA (Table 3). Despite of different chemical composition of TMRs, there was no significant influence of the separation between the 19 mm and 8 mm sieves in PSPS analysis. These results cannot be directly compared with those reported by Golder et al. (2017), as their RMS brush experiment took place with dairy cows on pasture, and the peNDF was 27.6 %. In the present study, we attempted to prepare peNDF contents by changing the feed composition in the rations. Yang and Beauchemin (2005, 2006) prepared peNDF contents in experimental diets by cutting the length of maize silage. In a feeding trial using 6 lactating dairy cows equipped with rumen fistulas, they reported that the total chewing time and ruminating time linearly increased with an increase in dietary peNDF, whereas the influence of dietary peNDF on ruminal pH and fermentation was minimal. Moreover, Yang and Beauchemin (2009) examined the effect of peNDF content in rations prepared based on the ratio of forage to concentrate. Consequently, they demonstrated that the risk of acidosis in dairy cows was mitigated by the increase in peNDF content in the diet. By contrast, in low forage diets (35:65 forage:concentrate on DM basis) the increased length of forage particles could not alleviate the risk of SARA because digestibility of the diet was relatively high and the changes in chewing activity were assumed to be marginal.

In our study, samples were taken to determine the chemical composition of the TMRs in each period. The particle size of TMRs in each portion was measured by using the PSPS separator with 8- and 19-mm sieves. The particles smaller than 8 mm at the bottom of the PSPS layer in the PSPS were assumed to be non-structural carbohydrates and cause acidosis due to the reduction in rumen mobility (Table 3). The peNDF value in every TMR examined fell within the range from 10.9 (TMR1) to 14.0 (TMR 4) despite the different compositions as shown in Table 4. Table 4 shows that feeding TMR2 resulted in relatively higher feed intake (6.2%) and milk production (6.4 %) for the RF group than for the control group ($p < 0.05$). The differences in live weight between the experimental and control groups of cows were not statistically significant. The results show the influence of feed energy intake on milk yield but not body weight.

As declared by Forbes (2007), despite many decades of research, there is still no unified theory on how animals control their feed intake with 3 RMS brushes and select feeds with different nutritional characteristics in relation to their physiological states. Forbes (2007) provided evi-

dence that the digestion of ruminants was not as simple as often presented.

The milk yield in the present study (Table 4) was higher than that reported by Golder et al. (2017) for the RMS brush experiment in pasture-based dairy cows. However, the expected increase in annual milk yield of dairy cows (patent EP0609045A2) by 0.9 to 3.5 litres of FCM milk/cow/day could not be confirmed for the RMS brush groups in either of the experiments.

There was no significant ($P > 0.05$) effect of RMS brushes on milk quality (Table 5) in relation to fat, protein and urea contents. This was in agreement with the results reported by Golder et al. (2017). The limit of urea in milk (300 mg/L) was exceeded in the periods P1 and P4. Since the protein content in the milk was lower than 3.2 %, it can be suggested that the TMR in P1 and P4 contained less energy and excess protein. TMR of P2 and P3 had, deficient energy but sufficient protein. Only TMR4 of P5 fulfilled both energy and protein requirements. As we expected, all TMRs (except TMR4) were confirmed to be borderline in terms of inducing the risk of acidosis. However, the differences

between the groups were not significant. There were no beneficial or detrimental effects of RMS brushes on rumen fermentation parameters, which was also declared by Golder et al. (2017).

No differences ($P > 0.05$) between groups were observed in any of the rumen fermentation parameters (Table 6 and Table 7). However, Horiguchi and Takahashi (2000) reported a postprandial (after feeding) increase in total VFA concentration from 6 hours to 12 hours in Holstein steers administered RMS brushes and fed a high-concentrate diet, compared with control animals without administration of RMS brushes. The major products of fermentation in the rumen are volatile fatty acids (VFAs), predominantly acetate, propionate, and butyrate, which are absorbed directly from the rumen mucosa. The VFAs could also negatively affect the ruminal environment. Some acetate absorbed in the rumen is used as a source of energy for activity of the rumen wall, and the rest is available for fat synthesis in adipose tissue and the mammary gland. Propionate is taken up almost completely by the liver, where it is used for gluconeogenesis (the ruminant would otherwise be very

Table 4. Feed intake and milk production

Period	Liveweight (kg/cow)				Feed intake (kg/cow.day)				Milk production (kg/cow.day)			
	RF	C	s.e.	P-value	RF	C	s.e.	P-value	RF	C	s.e.	P-value
P1	621	621	0		55.9	55.9	0		43.9	43.9	0	
P2	625	629	2.7	0.338	52.5	52.2	0.93	0.797	42.2	41.8	0.68	0.643
P3	629	623	4.3	0.367	44.7	42.1	0.65	0.010	36.8	34.6	0.58	0.015
P4	631	624	4.7	0.300	43.3	41.2	0.91	0.124	34.0	32.6	0.86	0.281
P5	629	626	12.9	0.872	43.4	42.1	1.34	0.516	30.8	30.2	1.55	0.777

RF - experimental group, C - control group

Table 5. Description of milk quality during experiment for tested groups

Period	Fat (%)				Protein (%)				Urea (mg/L)			
	RF	C	s.e.	P-value	RF	C	s.e.	P-value	RF	C	s.e.	P-value
P1	4.20	4.20	0		3.06	3.06	0		347	347	0	
P2	4.05	3.93	0.17	0.663	3.09	3.14	0.04	0.434	258	235	12.4	0.199
P3	4.47	4.28	0.15	0.402	3.10	3.17	0.04	0.236	277	265	10.0	0.417
P4	4.91	4.40	0.20	0.122	2.92	2.94	0.03	0.612	335	324	18.7	0.718
P5	4.03	4.15	0.20	0.677	3.26	3.39	0.05	0.108	276	261	15.8	0.524

RF - experimental group, C - control group

Table 6. Values of rumen pH and fermentation metabolites during experimental periods for tested groups

Period	pH				NH ₃ -N				VFA			
	RF	C	s.e.	P-value	RF	C	s.e.	P-value	RF	C	s.e.	P-value
P1	6.44	6.44	0		14.6	14.6	0		116	116	0	
P2	6.31	6.33	0.11	0.909	14.6	13.3	0.42	0.569	126	120	3.0	0.126
P3	6.37	6.42	0.08	0.669	14.4	13.7	0.36	0.693	124	124	3.0	0.993
P4	6.43	6.43	0.07	0.960	13.4	13.3	0.27	0.347	121	117	2.6	0.295
P5	6.76	6.75	0.10	0.938	13.5	13.9	0.61	0.561	104	107	5.2	0.697

RF - experimental group, C - control group, N-NH₃ - ammonia nitrogen (mg N/100 g rumen fluid); VFA - volatile fatty acids (mmol/L of rumen fluid)

Table 7. Rumen fermentation metabolites according to VFA (mmol/L of rumen fluid)

Period	Acetic acid				Propionic acid				Butyric acid			
	RF	C	s.e.	P-value	RF	C	s.e.	P-value	RF	C	s.e.	P-value
P1	76.4	76.4	0		22.5	22.5	0		16.1	16.1	0	
P2	82.8	72.8	1.87	0.100	24.5	23.0	0.96	0.262	18.1	17.9	0.64	0.768
P3	78.6	76.6	1.83	0.449	23.4	21.9	0.72	0.181	18.2	17.6	0.51	0.462
P4	80.6	80.2	1.77	0.884	24.1	23.7	0.73	0.689	18.7	19.3	0.74	0.541
P5	68.7	71.7	3.29	0.532	19.2	20.2	1.19	0.597	15.3	14.6	0.98	0.593

RF - experimental group, C - control group

Table 8. The basic ethological activities of the cows (in %) throughout the trial

Period	Lying		Standing		Chewing/lying		Chewing/standing		Eating		Drinking	
	RF	C	RF	C	RF	C	RF	C	RF	C	RF	C
P1	28	28	27	27	23	23	5	5	15	15	2	2
P2	20	23	22	22	23	27	9	8	15	13	11	7
P3	23	25	22	22	21	21	17	10	15	16	6	6
P4	25	31	24	24	19	19	13	8	17	16	2	2
P5	25	26	24	24	19	20	12	10	16	16	4	4

RF, experimental group, C, control group

deficient in glucose, which is required for lactose synthesis and for use in the brain). Butyrate is mainly used by the rumen wall (Forbes, 2007).

The RMS brushes administered to Holstein steers fed a low fibre diet did not affect the rumination time but increased the rumen digesta passage rate and ruminal propionic acid production in a study by Horiguchi and Takahashi (2002). This effect was not detected in our study. The results of Horiguchi and Takahashi (2004) suggested that digestibility and rumen fermentation status were not affected by RMS brush administration when the organic cell wall content of feeding diets was approximately 17 % in dry matter with rice straw over 2 cm in length. This result was also observed in our experiment because diets fed in our experiment had sufficient structure.

As shown in Table 7, the major VFA was acetic acid, therefore, sufficient digestible NDF was present in the TMR. Moreover, Matsuyama et al. (2000) observed that the postprandial changes in pH, an VFA and ammonia nitrogen contents in the ruminal fluid were smaller in Holstein steers with RMS brushes than in control animals without RMS brushes when fed a diet with a roughage-to-concentrate ratio of 7:93 on a dry matter basis. In their experiment, it seemed that the ruminating motion stimulated by RMS brushes accelerated saliva flow into the rumen, which buffered the pH in the ruminal fluid after intake of feed. Thus, in the present paper, the significantly ($P < 0.05$) higher feed intake and milk yield in dairy cows with RMS brushes compared to control animals without RMS brushes presumptively supported that RMS brushes functioned as dietary peNDF. These observations agreed with the results of the feeding trials using lactating dairy cows fed

different peNDF contents as determined by the PSPS system reported by Yang and Beauchemin (2006).

For the mitigating effect of RMS brushes on the risk of SARA, the most important key factor is a pH decline associated with an increase in organic acid production, particularly lactic acid, in the rumen. According to Valente *et al.* (2017) the threshold value to characterize SARA is a pH below 5.8 for a duration of 24 hours. As shown in Table 6, the average pH of ruminal fluid was not below 6.31 in any observed period. Although the composition and dilution of the TMR tested was at the limit of the peNDF critical value (12.5), SARA was not detected. Therefore, a mitigating effect of RMS brushes on the risk of SARA was not demonstrated. To date, the influence of administration of RMS brushes on the behaviour of dairy cows has not been reported. Campion and Leek (1996) examined rumen mechanical stimulation by the intra-ruminal administration of inert fibre particles in sheep and their effect on rumination. They used an inert fibre particle in a series of experiments to quantify the effects of some of the physical characteristics of long dietary fibre on rumination. When different masses of loose, inert polyethylene particles chopped into 10-mm lengths were placed in the rumen, the time spent ruminating was found to be directly proportional to the mass of fibre placed in the rumen. By inserting a fixed mass of particles with different lengths, the optimum length of inert fibre for rumination to occur was confirmed to lie between 7 and 30 mm. They concluded that the inert polyethylene fibre mimicked the peripheral excitatory effects of dietary fibre by stimulating mechanoreceptors (epithelial receptors) located in the cranial portions of the reticulo-rumen. No dairy cows were prematurely weaned or died.

One cow from the RF group and 4 cows from the C group were not easily impregnated and furthermore 3 cows from RF group and 5 cows from the C group were unable to get pregnant.

In the present study, a few differences were observed in ethological behaviour between the RF and C groups in each experimental period (Table 8). The chewing time during standing (at the expense of lying) in the group with RMS brushes was longer than that in the C group. According to Cook et al. (2007), the duration of lying behaviour decreased during the daytime with increasing temperature-humidity index (THI), but the lying duration at night was unaffected by THI. Although it is generally accepted that cows stand more in alleys and stalls during periods of heat stress, no previous study has followed changes in the daily time budgets for the lying, standing, drinking, milking, and feeding activities of cows under different climate conditions. The weather in the spring and summer when the present study was carried out was warmer and drier than in previous years. However, the average outdoor air temperature and relative humidity (and RHI, respectively) at that time were not as high as when the cows were under heat stress (Table 1). There are different formulas to calculate the THI, but we used the simplest and the most common one as follows: $THI = 0.8 \cdot T + RH \cdot (T - 14.4) + 46.4$, where T = ambient or dry-bulb temperature in °C and RH = relative humidity expressed as a proportion i.e., 75 % humidity is expressed as 0.75. Heat stress conditions indicated with mean daily values of $THI > 72$ were determined during the spring and summer seasons in all analysed regions (Gantner et al., 2011). No period had a THI higher than 72, so the cows were not under heat stress.

Golder et al. (2017) hypothesized that pasture-based dairy cattle with RMS brushes would have improved plasma oxidative stress parameters in spring and summer, mitigating the temperature stress from weather with high daily temperatures. However, the mitigating effect of RMS brushes on heat stress in dairy cows was not clearly demonstrated, except when BAP (biological antioxidant potential) was used as a plasma oxidative stress index due to an unusually cool summer climate at that time. Thus, it might be necessary to design an experiment with a more suitable THI condition, as well as a more sensitive detector for the heat stress index, to demonstrate the mitigating effect of administration of RMS brushes on heat stress under natural conditions.

Feeding dairy cows approaching the risk of SARA may increase milk production, but the risk of SARA and its negative effects on animals should be considered. Although RMS brushes might be a technology to reduce the risk of SARA, thus far, the positive effects of RMS brush administration on the mitigation of SARA were not confirmed in the present study. The most important role of rumination in a ruminant is regurgitation of fibrous components and the buffering action of saliva flow into the ruminal fluid, neutralizing the acidic state and activating fibrolytic microbes. It seems that the role of RMS brushes in rumination is dependent on the peNDF content in the feed. In the present study, minimal peNDF in all experimental TMRs did

not increase the risk of SARA, contributing to the unclear efficacy of RMF brushes. Consequently, administration of RMS brushes may fulfil its potential as a prophylactic device for peNDF in high-grain feeding environments such as feedlots or in high-concentrate feeding environments for high-performance lactation cows.

Conclusions

The hypothesis that artificial brushes applied to the rumen can partially replace the function of structural fibre and increase milk production or quality was not confirmed. Feed intake and milk production were not affected by the group or the interaction between groups, with the exception of period P3 with TMR2, when peNDF was 13 %. There appeared to be no effect of RMS brushes on milk composition or rumen fermentation properties. Thus, in the present study, negative impacts of RMS brushes in the rumen of high-yielding dairy cows were not observed, although a positive effect was also not demonstrated. This issue requires more research.

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Utjecaj četki za stimulaciju buraga na proizvodna svojstva mliječnih holstein krava u laktaciji

Sažetak

Cilj istraživanja bio je potvrditi hipotezu da umjetne četke za stimulaciju buraga (RMS četke) mogu djelomično zamijeniti funkciju strukturnih vlakana te povećati proizvodnju ili kvalitetu mlijeka. Kako bi se umanjili rizici hranidbe niskom razinom fizikalno učinkovitih neutralnih deterdžent vlakana (peNDF) na goveda, ispitivana je primjena četki za stimulaciju buraga kod 22 visoko proizvodne holstein mliječne krave. Krave su podijeljene u eksperimentalnu skupinu u kojoj su primijenjene RMS četke i kontrolnu skupinu bez primjene. Krave su hranjene sa četiri eksperimentalna potpuno izmiješana obroka s nepromjenjivim količinama silaže lucerne, kukuruzne silaže, silaže cijelog klipa kukuruza te različitih količina pivskog tropa i pšenične slame. Sadržaj peNDF u obrocima je bio redom 10,9, 13,0, 12,6 i 14,0 %. Istraživanje je trajalo 18 tjedana, a sve su krave hranjene obrokom s niskim sadržajem strukturnih vlakana povezanim s rizikom pojave subakutne ruminalne acidoze (SARA). U eksperimentalnoj skupini, tri RMS četke oralno su umetnute u burag posebnim aplikatorom. Tijekom istraživanja, promatran je utjecaj primjene RMS četki na konzumaciju, buražnu fermentaciju i proizvodnju mlijeka. Uzorak buražnog soka uzorkovan je želučanom sondom te je u njemu određen pH i sadržaj hlapljivih masnih kiselina te amonijskog dušika. Primjena RMS četki značajno je povećala konzumaciju i proizvodnju mlijeka samo kada je eksperimentalna skupina hranjena obrokom s 13,0 % peNDF, te nije utvrđena jasna povezanost između sadržaja peNDF obroka i primjene RMS četki. Kvaliteta mlijeka, pH buraga i sadržaj produkata fermentacije nisu značajno povećani primjenom RMS četki. Iste tehnologija RMS četki samo djelomično smanjila potrebe za peNDF, ona može smanjiti rizik pojave SARA.

Ključne riječi: funkcija buraga; hranidba preživača; četke za mehaničku stimulaciju buraga; kvaliteta mlijeka; proizvodnja mlijeka

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