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Original article

The effectiveness of fibrolytic enzymes and active yeast on improving reticulorumen pH in dairy cows

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Abstract

Exogenous fibrolytic enzymes (EFE) and yeast are feed supplements that improve forage digestion in rumen, but their influences on physical reticulorumen parameters are not well studied. This study was designed to evaluate the effect of the EFE:endo-β-xylanase ($37x10^4$ U/cow/day), endocellulase ($45x10^4$ U/cow/day), endo-β-glucanase ($12x10^4$U/cow/day), and active yeast – *Saccharomyces cerevisiae CNCM-1077* ($10x10^9$CFU/cow/day) supplements on reticulorumen pH (RpH) and temperature (RT) in dairy cows. Nine Lithuanian Red cows were allocated into three groups (3 cows/group): control group (C) – farm diet without supplementation, enzyme group (E) – farm diet supplemented with EFE, enzyme and active yeast group (EY) – farm diet supplemented with EFE and active yeast. The feeding trial lasted for 60 d. All cows were equipped with reticuloruminal telemetric pH and temperature sensor device. Data provided by the device were used to calculate the mean RpH (RpH/24h), the mean minimal RpH ($_{min}$RpH/24h) and mean of the time that RpH was below the threshold value of 6.0 (RpH<6.0/24h, min.). The highest RpH/24h (6.37±0.22) was observed in group EY and it was by 1.62% ($p<0.05$) and 1.27% ($p<0.001$) higher as compared with groups E and C, respectively. Also $_{min}$RpH/24h (6.24±0.24) was highest in group EY and values were by 0.63% ($p<0.001$) and 0.65% ($p<0.001$) higher as compared with groups C and E, respectively. The shortest duration of RpH<6.0/24h, was recorded in group EY, and it was by 57.76% ($p<0.05$) and 47.87% shorter as compared with groups C and E, respectively. In conclusion, feed supplementation with EFE and *Saccharomyces cerevisiae CNCM-1077* had beneficial effect on RpH.

Key words: exogenous fibrolytic enzymes, active yeast, reticulorumen pH, dairy cow

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Introduction

In commercial dairy farms roughage-based diets become more attractive as compared to high concentrate-based feeds, which may cause animal health, welfare and performance problems (Zebeli et al. 2007). Highly specialized digestive system of ruminants allows them to convert hardly digestive non-starch polysaccharides into energy, necessary for the animal, but this process also has some limitations (Varga and Kolver 1997). Efficient forage degradation in rumen is dependent on intra-ruminal conditions and animal health status, which could be described by rumen temperature and rumen fluid pH.

Rerticulorumen pH (RpH) measurement is a relatively fast indicator for describing the performance of rumen ecosystem and its ability to utilize fiber, also to prevent animal diseases (Antanaitis et al. 2018, Denwood et al. 2018). The availability of continuously monitored data from intraruminal boluses gives new opportunities for characterizing RpH and temperature all over the day (Jonsson et al. 2019), seasons (Antanaitis et al. 2016) or predicting patterns related to animal health (Denwood et al. 2018). Continuous measurement of RpH has become a helpful tool for defining acid-base status and its interactions with factors, such as diet composition (Denwood et al. 2018). Rumen temperature could be used for detecting excessive fermentation products (volatile fatty acids (VFA) and lactic acid) (Antanaitis et al. 2018).

Rumen microorganisms responsible for fiber degradation become less abundant when rumen pH drops below 6.2 (Varga and Kolver 1997) and their function is more limited below pH 6.0 (Hoover 1986). The diets with highly fermentable carbohydrates can challenge optimal ruminal conditions by decreasing ruminal pH even below 5.5 (Kolver and Veth 2002, Krause and Oetzel 2006, Bramley et al. 2008).

To achieve high energy supply from fibrous feeds, a few biological technologies have been developed, such as the application of exogenous fibrolytic enzymes (EFE) and active yeasts (Adesogan et al. 2019). The most promising effect of EFE is related to enzymes not normally found in the rumen. The supplementation of diet with endo-β-1,4-glucanase increases digestion of barley straw and alfalfa hay *in vitro* (Badhan et al. 2014). By mode of action, EFE shifts carbohydrate utilization in rumen by releasing more soluble sugars from fiber, thus leading into an increased VFA production and altered acetate: propionate ratio (Tricarico and Dawson 2005).

The other way to improve fiber utilization in rumen and animal performance is to use a probiotic yeast. *Saccharomyces cerevisiae* is currently widely used in animal feeds with the main purpose to prevent rumen flora disorders and disturbances, accelerate fiber degradation and improve ruminal condition for the fermentation (Fonty and Chaucheyras-Durand 2006).

We hypothesized that the use of EFE, as well as EFE and yeast supplements, would affect reticulorumen pH (RpH) and temperature (RT) of dairy cows in a commercial organic farm. It was expected that EFE would increase the risk of subacute ruminal acidosis (SARA), but EFE and active yeast supplementation would keep the RpH and RT in the optimum range. The aim of this study was to determine the effect of EFE and EFE and active yeast on RpH and RT. Therefore, this study aimed to evaluate the supplementation effect on RpH and RT at different keeping conditions (outdoor and indoor) and estimate their dynamics over the trial.

Materials and Methods

This study was performed in a commercial organic dairy farm (700 cows) in Lithuania. Animal welfare and experimental procedures were carried out in accordance with the Lithuanian State Food and Veterinary Service Permission No. G2-60, 2017.02.15. Fifteen clinically healthy Lithuanian Red cows (average 21 (±7) days in milk and producing 31.3 (±2.1) kg/d of milk) were selected for the experiment. Seven days before the trial, all cows were equipped with reticuloruminal telemetric sensor device "SmaXtec Premium Bolus" (SmaXtec animal care GmbH, Graz, Austria) according to the guidelines for bolus start-up preparation and oral inclusion techniques provided by the manufacturer for RpH and RT measurements. Detailed system information was described by Gasteiner et al. (2012). The measurements were taken throughout the experiment.

The cows were kept indoors in the free stall type barn for 18 h/d and outdoors on pasture for 6 h/d. Milking occurred twice daily at 6 a.m. and 6 p.m. The farm diet included total mixed ration (TMR) and pasture grass. The TMR of 14.4 kg dry matter (DM) cow/day was offered twice a day (at 7 a.m. and 5 p.m.) indoor. The outdoor grazing on pasture was *ad libitum*/6h. The diet of 20 kg/cow/day was balanced to meet maintenance and production needs (Table 1). The accessibility to water was *ad libitum*/24h.

The feeding trial lasted for 60 days (August-September). After seven pre-trial days of RpH measurements, the most biased cows were excluded and the remaining cows were enrolled in the trial. The cows (n=9) were allocated into three homologous groups (3 cows/group).

All animals received the same farm diet during the trial. The cows of the control group (C) were fed diet without supplements. The diet of group E was sup-

Table 1. The farm diet ingredients and diet chemical composition for dairy cows.

Item	Value
Ingredient	% of diet DM
Grass silage[1*]	26
Corn silage[1*]	9
Straw[1*]	3
High-moisture corn[1*]	5
Ground concentrate mixture[1,2]	29
Pasture grass[3*]	28
Farm diet chemical composition	
DM, %	40.5
OM, % of DM	71.1
CP, % of DM	15.08
NDF, % of DM	43.11
ADF, % of DM	27.46

DM – dry matter, OM – organic matter, CP – crude protein, NDF – neutral detergent fiber, ADF – acid detergent fiber.
[1] Ingredients of TMR.
[2] Composition of the concentrate mixture. Dry grains: wheat*, triticale*, oats*; dry ground peas* and ground beans*, dry rapeseed*, soybean meal*, sodium bicarbonate†, feed chalk†, salt†, vitamins† and trace mineral† premix.
[3] Pasture grass: ryegrass, red clover, white clover, alfalfa grass.
*Plants cultivated and harvested in the same organic farm
†Feed components suitable for organic farming

plemented with EFE mixture of endo-β-xylanase 37x10^4 U/cow/day, endocellulase 45x10^4 U/cow/day, endo-β-glucanase 12x10^{4}U/cow/day (Vilzim®NSP, UAB "Biorro", Lithuania). License for use of EFE in organic farming No. PAG-K-17-012 was issued by Public enterprise "Ekoagros" (Lithuania). Group EY received the same diet as group E and additionally was supplemented with active dry yeast *Saccharomyces cerevisiae CNCM-1077*, 10x10^{9}CFU/cow/day (Levucell SC, Lallemand Animal Nutrition, France). The corresponding treatment was administered by top-dressing forages and mixed by using TMR mixer – feeder "Kuhn Euromix I" (Bucher Industries AG, Switzerland), then delivered to the cows.

To confirm the hypothesis, the data provided by the devices were used to calculate the mean RpH of day (RpH/24h), mean minimal RpH of day ($_{min}$RpH/24h), the mean time of day in minutes that RpH was below threshold value of 6.0 (RpH<6.0/24h) and the mean RT of day (°C/24h).

The data of six hours' period (6 hours/day) were analysed, to evaluate the effect of EFE and EFE and yeast supplementation at outdoor and indoor keeping conditions The data of the outdoor (from 11 a.m. to 5 p.m.) and indoor (from 9 p.m. to 3 a.m.) periods was selected for the analysis (RpH/6h, $_{min}$RpH/6h, RpH<6.0/6h and RT,°C/6h).

Recorded data were grouped into six periods (I –VI) of 10 days each, to assess the dynamic of RpH and RT response to the supplementation.

Data were analyzed using IBM Statistic SPSS version 15 (license No. 9900457; IBM. the USA). The results are presented as mean and standard deviation of the mean (mean±SD), 95% confidence interval (CI) and the difference between treatment groups means (Δ). Normal data distribution was tested with the Kolmogorov – Smirnov test. For comparison between trial groups an independent sample Kruskal – Wallis test was used. For inside – group treatment effect estimation a related samples Friedman's two-way ANOVA was applied. To describe trends of RpH and RT throughout the trial a linear regression and Pearson's correlation coefficient were used. In tests α was set to 0.05 and β – 0.95. The results were considered statistically significant when p≤0.05.

Results

The supplementation with EFE and EFE and active yeast mixture affected RpH (Table 2 – total trial). Group EY had RpH/24h higher by 1.27% (p<0.001) compared to C and higher by 1.62% (p<0.05) compared to group E. Also $_{min}$RpH/24h in group EY was higher by 0.63%

Table 2. Statistical characteristics of indicators (mean±SD) in investigated dairy cows.

Items		Supplementation			P-value	Difference		
		C	E	EY		$\Delta_{(E-C)}$	$\Delta_{(EY-C)}$	$\Delta_{(EY-E)}$
Total trial								
RpH/24h		6.29±0.22	6.33±0.21	6.37±0.22	0.001	0.04**	0.08**	0.04*
	95% CI	6.28; 6.30	6.32; 6.34	6.36; 6.38				
$_{min}$RpH/24h		6.14±0.25	6.20±0.23	6.24±0.24	0.001	0.06**	0.10**	0.04**
	95% CI	6.13; 6.15	6.19; 6.21	6.23; 6.25				
RpH<6.0/24h, min.		165.5±223.6	134.3±191.8	69.9±111.3	0.046	-31.2	-95.6*	-64.3
	95% CI	132.6;198.4	106.1;162.5	53.58;86.3				
RT,°C/24h		38.9±0.7	38.9±0.7	38.9±0.9	0.221	0	0	0
	95% CI	38.9; 38.9	38.8; 38.9	38.9; 39.0				
Outdoor period								
RpH/6h		6.36±0.22	6.41±0.21	6.46±0.21	0.001	0.05	0.10	0.05
	95% CI	6.35; 6.38	6.39; 6.43	6.44; 6.47				
$_{min}$RpH/6h		6.22±0.25	6.27±0.25	6.33±0.22	0.001	0.05	0.11	0.06
	95% CI	6.19; 6.24	6.25; 6.29	6.31; 6.35				
RpH<6.0/6h, min.		27.17±50.88	27.39±49.60	5.50±23.41	0.001	0.22	-21.67	-21.89
	95% CI	19.68; 34.65	20.09; 34.68	2.06; 8.94				
RT, °C/6h		39.12±0.61	38.99±0.59	39.04±0.75	0.071	-0.13	-0.08	-0.05
	95% CI	39.06; 39.17	38.94; 39.04	38.98; 39.11				
Indoor period								
RpH/6h		6.19±0.21	6.23±0.19	6.28±0.20	0.001	0.04*	0.09**	0.05*
	95% CI	6.17; 6.21	6.21; 6.24	6.26; 6.29				
$_{min}$RpH/6h		6.03±0.24	6.10±0.21	6.14±0.23	0.001	0.07	0.11**	0.04
	95% CI	6.01; 6.05	6.08; 6.12	6.12; 6.16				
RpH<6.0/6h, min.		70.00±91.46	52.50±77.62	35.28±57.70	0.022	-17.50	-34.72*	-17.22
	95% CI	56.55; 83.45	41.08; 63.92	26.79; 43.76				
RT, °C/6h		39.13±0.57	39.09±0.58	39.07±0.87	0.058	-0.04	-0.06	-0.02
	95% CI	39.08; 39.18	39.04; 39.14	39.00; 39.15				

C – control group, E – group supplemented with EFE; EY – group supplemented with EFE and active yeast
** $p<0.001$, * $p<0.05$

($p<0.001$) and 0.65% ($p<0.001$) as compared with groups C and E, respectively. The RpH<6.0/24h was shorter by 57.76% ($p<0.05$) and 47.87% in group EY as compared with groups C and E, respectively.

A similar tendency was observed during the indoor period (Table 2 - indoor). The RpH/6h was by 1.45% ($p<0.001$) higher in group EY compared to group C and by 0.8% ($p<0.05$) higher than in group E. The $_{min}$RpH/6h was higher by 1.82% ($p<0.001$), but RpH<6.0/6h was shorter by 49.6% ($p<0.05$) in group EY as compared with group C. No statistically significant differences of $_{min}$RpH/6h and RpH<6.0/6h were observed between groups C, E, and EY. No statistically significant supplementation effect on RpH was observed during the outdoor period (Table 2 – outdoor).

Comparing the outdoor period to the indoor period, the RpH/6h was higher by 2.75%, 2.89% and 2.71% ($p<0.001$) and the $_{min}$RpH/6h was higher by 3.06%, 2.78% and 3.09% ($p<0.001$) in groups C, E, and EY, respectively. But the RpH<6.0/6h was shorter by 61.19%, 47.83% and 84.41% ($p<0.001$) in groups C, E, and EY, respectively (Table 2).

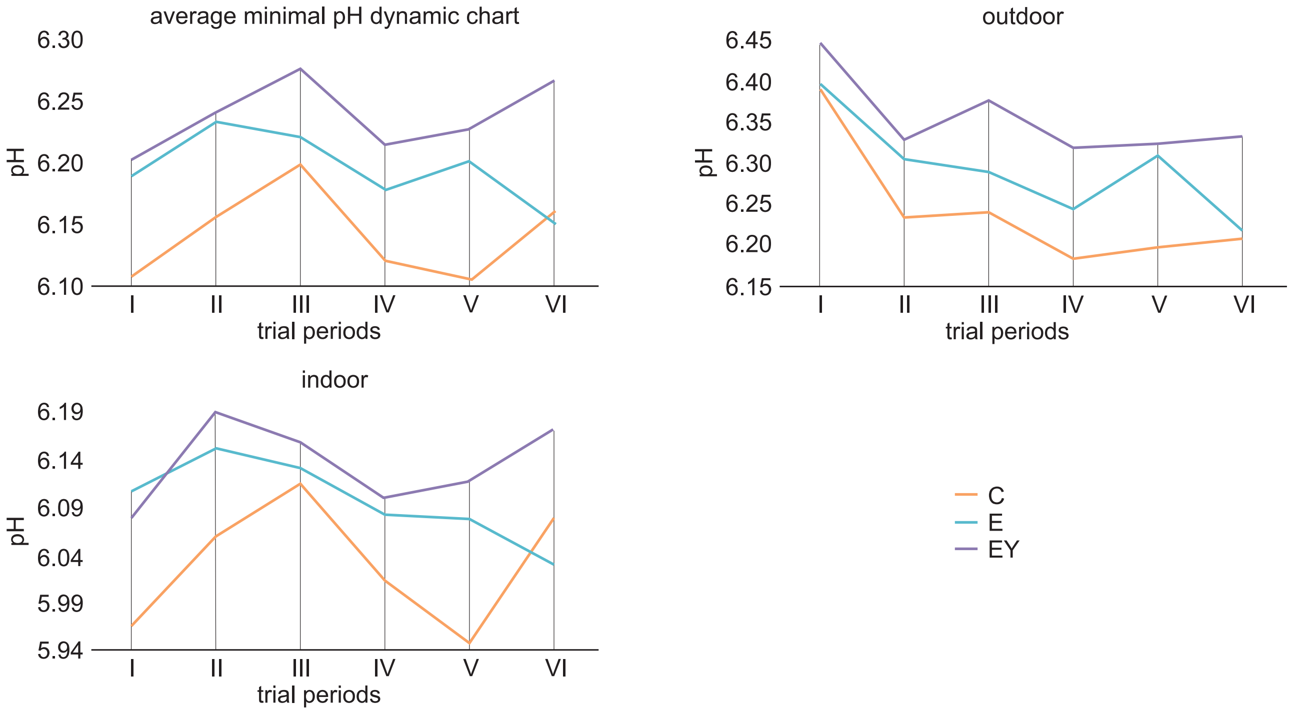

Fig. 1. The dynamics of dairy cows mean $_{min}$RpH in different periods (10d. each). Total trial – RpH/24h; outdoor period – RpH/6h; indoor period – RpH/6h. C – control group, E – group supplemented with EFE; EY – group supplemented with EFE and active yeast

The results of linear regression analysis were identical for both RpH and minRpH in all groups. According to our opinion $_{min}$RpH value is more specific to reflect lower RpH which is associated with cow health condition (e.g. SARA appearance), therefore for reticulorumen pH adaptation and feedback to feed supplementation assessment, the results of linear regression and comparison between periods will be based only on $_{min}$RpH. During the trial small but steady decrease of $_{min}$RpH/24h was observed in group E (y=-0.0094x+6.2293, R^2=0.3545) (Fig. 1. Total). During the indoor period the decrease of $_{min}$RpH/6h in group E was moderate (y=-0.0186x+6.1634, R^2=0.6615) (Fig. 1. Indoor). During the outdoor period $_{min}$RpH/6h slightly decreased (y=-0.0079x+6.2431, R^2=0.4143) in group C (Fig. 1. Outdoor).

Strong positive trend of RpH<6.0/24h over trial was estimated in group E (y=11.152x+95.244, R^2=0.8984), moderate negative trend in group EY (y=9.9905x+104.91, R^2=0.4312) and no trend in group C (Fig. 2. Total). During the outdoor period, strong positive trend (y=3.8190x+13.8, R^2=0.8331) of RpH<6.0/6h was observed in group C and slight positive trend (y=1.7429x+21.289, R^2=0.35) in group E, while group EY had moderate negative trend (y=-1.0571x+9.2, R^2=0.5154) (Fig. 2. Outdoor). During the indoor period, strong positive trend (y=6.8095x+28.667, R^2=0.96) of RpH<6.0/6h was observed in group E (Fig. 2. Indoor).

Mean RT during the trial was equal between groups. The RT in group E was higher by 0.28% ($p<0.05$) during the indoor period as compared to the outdoor period (Table 2). The RT did not show significant differences over the whole trial.

Discussion

Our trial results suggest that supplementing diet with EFE and active yeast can increase RpH and significantly reduce time when rumen pH is below the threshold of 6.0. Supplementing diet only with EFE can significantly reduce RpH. Different outcome from supplemented groups suggest that mostly active yeast is responsible for a positive effect on RpH. By mode of action, yeast can influence RpH by stimulating lactic acid-degrading bacteria and rumen protozoa (Fonty and Chaucheyras-Durand 2006). Published results on yeast impact on rumen pH are contradictory. Chung et al. (2011), AlZahal et al. (2014), Malekkhahi et al. (2016), Ambriz-Vilchis (2017) did not find active yeast influence on rumen pH, but Guedes et al. (2007) and Thrune et al. (2009) found a positive effect of yeast supplementation. These differences could be related to different *Saccharomyces cerevisiae* strains used in trials.

The published data on cow diet supplementation with EFE are controversial. A few studies with EFE carried out by Beauchemin et al. (1999) and Chung et al. (2012) demonstrated no significant effect on ruminal fermentation or mean ruminal pH. However, in studies

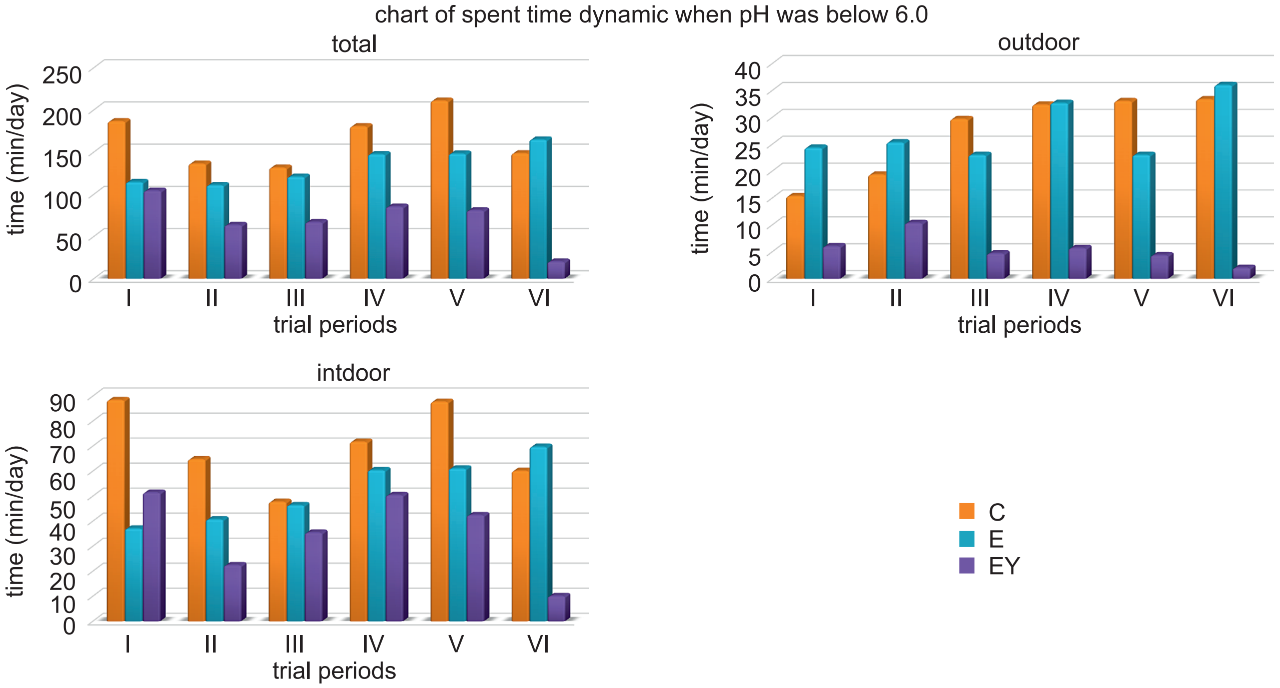

Fig. 2 The dynamics dairy cows of RpH <6.0 in different periods (10 days each). C – control group, E – group supplemented with EFE; EY – group supplemented with EFE and active yeast

carried out by Krueger et al. (2008) and Yang et al. (1999) EFE application increased neutral detergent fiber (NDF) digestibility. In our trial RpH had tendency to decrease in animals fed diet only with EFE supplementation. This observation could be related to enzyme-mediated digestibility via increased microbial colonization of feed particles or due to direct cell wall hydrolysis that have been reported to increase VFA production and concentration (Yang et al. 1999, Arriola et al. 2011). To confirm the theory that EFE promoted decrease of RpH by increased VFA production additional VFA measurements are necessary.

The higher RpH values were recorded during the outdoor period as compared to indoor, but no supplementation effect during the outdoor period was registered. Only in group EY the increased RpH/6h and reduced RpH<6.0/6h was detected during the indoor time (Table 2 – indoor). Controversial results were published by Gasteiner et al. (2015) when during barn feeding mean RpH was higher (6.44±0.14) compared to grazing (6.25±0.22). In our trial, the average outdoor RpH/6h and $_{min}$RpH/6h of all three treatment groups was 6.41 and 6.27, respectively, while Gasteiner et al. (2012) in grazing cows found a mean RpH was 6.36 and mean $_{min}$RpH was 5.95. Kolver and Veth (2002) concluded that cows' low ruminal pH is associated with higher VFA concentrations, positively related to forage NDF and negatively related to nonstructural carbohydrate. Higher RpH rates in the outdoor period could be related to grass composition keeping in mind that this trial was performed in late summer and beginning of autumn when grazing grass naturally has higher lignin concentration. The lignin could increase rumination and salivation, which acts as a buffer and equalize RpH across the experimental groups, but additional measurements of lignin and rumination are necessary. The grass composition effect on RpH is supported by results of RpH<6.0/6h. In outdoor period it varied from 5.50 min./6h in group EY to 27.39 min./6h in group E while Gasteiner et al. (2012) recorded average time below RpH threshold of 6.0 for 333 min in cows grazing 7h/d. Rumen pH threshold between 5.2 and 5.6 for >176 min./day is defined as harmful for animals and associated with SARA (Gozho et al. 2005, Alzahal et al. 2008). In this trial, no evidence of SARA was observed.

Both supplements (EFE, EFE and active yeast combination) affected the rumen pH. Highest RpH value in group E was recorded during the period III and then gradually decreased. (Fig. 1. Total). The highest RpH value in group EY started to increase from 7.0 (period III) up to 7.38 (period VI) (Fig. 2. Total). In agreement with the results of AlZahal et al. (2014) the minimum time required for feed supplementation to show beneficial effect is approximately 3 weeks of continuous use.

Indoor period RT was slightly higher than the outdoor period. Similar RT results were observed by Antanaitis et al. (2016), when night RT was higher by 0.31°C than during daytime. RT could be influenced by animal water intake (Bewley et al. 2008), heat stress (Lees et al. 2018), fast carbohydrates fermentation in rumen (Alzahal et al. 2008), circadian rhythm and season (Antanaitis et al. 2016). No significant effect of EFE or EFE and yeast on RT was recorded during this study.

Conclusion

The trial results revealed that combination of EFE and *Saccharomyces cerevisiae CNCM-1077* as feed supplements to a high roughage diet has beneficial effect on RpH. RpH values were within an optimal range for fiber digestion in the rumen and prevented from the SARA. Supplementing diets exclusively with exogenous fibrolytic enzymes for a prolonged period could reduce RpH and increase the risk of health problems associated with lower RpH. None of the supplements affected the RT.

References

Adesogan AT, Arriola KG, Jiang Y, Oyebade A, Paula EM, Pech-Cervantes A, Romero JJ, Ferraretto LF, Vyas D (**2019**) Symposium review: Technologies for improving fiber utilization. J Dairy Sci 102: 5726-5755.

AlZahal O, Kebraeb E, France J, Frotscher M, McBride BW (**2008**) Ruminal temperature may aid in the detection of subacute ruminal acidosis. J Dairy Sci 91: 202-207.

AlZahal O, Dionissopoulos L, Laarman AH, Walker N, McBride BW (**2014**) Active dry Sac-charomyces cerevisiae can alleviate the effect of subacute ruminal acidosis in lactating dairy cows. J Dairy Sci 97: 7751-7763

Ambriz-Vilchis V, Jessop NS, Fawcett RH, Webster M, Shaw DJ, Walker N, Macrae AI (**2017**) Effect of yeast supplementation on performance, rumination time, and rumen pH of dairy cows in commercial farm environments. J Dairy Sci 100: 5449-5461.

Antanaitis R, Juozaitienė V, Rutkauskas A, Televičius M, Stasiulevičiūtė I (**2018**) Reticulorumen temperature and pH as indicators of the likelihood of reproductive success. J Dairy Res 85: 23-26.

Antanaitis R, Žilaitis V, Juozaitienė V, Stoškus R, Televičius M (**2016**) Changes in reticulorumen content temperature and pH according to time of day and yearly seasons. Pol J Vet Sci 19: 771-776.

Arriola KG, Kim SC, Staples CR, Adesogan AT (**2011**) Effect of fibrolytic enzyme application to low- and high-concentrate diets on the performance of lactating dairy cattle. J Dairy Sci 94: 832-841

Badhan A, Wang Y, Gruninger R, Patton D, Powlowski J, Tsang A, McAllister T (**2014**) Formulation of enzyme blends to maximize the hydrolysis of alkaline peroxide pretreated alfalfa hay and barley straw by rumen enzymes and commercial cellulases. BMC Biotechnol 14: 31.

Beauchemin KA, Yang WZ, Rode LM (**1999**) Effects of grain source and enzyme additive on site and extent of nutrient digestion in dairy cows. J Dairy Sci 82: 378-390.

Bewley JM, Einstein ME, Grott MW, Schutz MM (**2008**) Comparison of reticular and rectal core body temperatures in lactating dairy cows. J. Dairy Sci 91: 4661-4672.

Bramley E, Lean IJ, Fulkerson WJ, Stevenson MA, Rabiee AR, Costa ND (**2008**) The definition of acidosis in dairy herds predominantly fed on pasture and concentrates. J Dairy Sci 91: 308-321.

Chung YH, Walker ND, McGinn SM, Beauchemin KA (**2011**) Differing effects of 2 active dried yeast (Saccharomyces cerevisiae) strains on ruminal acidosis and methane production in nonlactating dairy cows. J Dairy Sci 94: 2431-2439

Chung Y, Zhou M, Holtshausen L, Alexander TW, Mcallister TA, Guan LL, Oba M, Beauchemin KA (**2012**) A fibrolytic enzyme additive for lactating Holstein cow diets: ruminal fermentation, rumen microbial populations, and enteric methane emissions. J Dairy Sci 95: 1419-1427.

Denwood MJ, Kleen JL, Jensen DB, Jonsson NN (**2018**) Describing temporal variation in reticuloruminal pH using continuous monitoring data. J Dairy Sci 101: 233-245.

Fonty G, Chaucheyras-Durand F (**2006**) Effects and modes of action of live yeasts in the rumen. Biologia 61: 741-750.

Gasteiner J, Guggenberger T, Häusler J, Steinwidder A (**2012**) Continuous and long-term measurement of reticuloruminal pH in grazing dairy cows by an indwelling and wireless data transmitting unit. Vet Med Int 2012: 236956.

Gasteiner J, Horn M, Steinwidder A (**2015**) Continuous measurement of reticuloruminal pH values in dairy cows during the transition period from barn to pasture feeding using an indwelling wireless data transmitting unit. J Anim Physiol Anim Nutr 99: 273-280.

Gozho GN, Plaizier JC, Krause DO, Kennedy AD, Wittenberg KM (**2005**) Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. J Dairy Sci 88: 1399-1403.

Guedes CM, Gonçalves D, Rodrigues MAM, Dias-Da-Silva A (**2008**) Effects of a Saccharomyces cerevisiae yeast on ruminal fermentation and fibre degradation of maize silages in cows. Anim Feed Sci Technol 145: 27-40.

Hoover WH (**1986**) Chemical Factors Involved in Ruminal Fiber Digestion. J Dairy Sci 69: 2755-2766.

Jonsson NN, Kleen JL, Wallace R, Andonovic I, Michie C, Farish M, Mitchell M, Duthie CA, Jensen DB, Denwood MJ (**2019**) Evaluation of reticuloruminal pH measurements from individual cattle: Sampling strategies for the assessment of herd status. Vet J 243: 26-32.

Kolver ES, de Veth MJ (**2002**) Prediction of Ruminal pH from Pasture-Based Diets. J Dairy Sci 85: 1255-1266.

Krause KM, Oetzel GR (**2006**) Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim Feed Sci Technol 126: 215-236.

Krueger NA, Adesogan AT, Staples CR, Krueger WK, Kim SC, Littell RC, Sollenberger LE (**2008**) Effect of method of applying fibrolytic enzymes or ammonia to bermudagrass hay on feed intake, digestion, and growth of beef steers. J Anim Sci 86: 882-889.

Lees AJ, Lees JM, Lisle A, Sullivan M, Gaughan JB (**2018**) Effect of heat stress on rumen temperature of three breeds of cattle. Int J Biometeorol 62: 207-215.

Malekkhahi M, Tahmasbi AM, Naserian AA, Danesh-Mesgaran M, Kleen JL, AlZahal O, Ghaffari MH (**2016**) Effects of supplementation of active dried yeast and ma-late during sub-acute ruminal acidosis on rumen fermentation, microbial population, selected blood metabolites, and milk production in dairy cows. Anim Feed Sci Technol 213: 29-43.

Thrune M, Bach A, Ruiz-Moreno M, Stern MD, Linn JG (**2009**) Effects of Saccharomyces cerevisiae on ruminal pH and microbial fermentation in dairy cows: Yeast supplementation on rumen fermentation. Livest Sci 124: 261-265.

Tricarico JM, Dawson KA (**2005**) Influence of supplemental endoglucanase or xylanase on volatile fatty acid production from ruminant feed by ruminal in vitro cultures. Arch Anim Nutr 59: 325-334.

Varga GA, Kolver ES (**1997**) Microbial and animal limitations to fiber digestion and utilization. J Nutr 127: 819S-823S.

Yang WZ, Beauchemin KA, Rode LM (**1999**) Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. J Dairy Sci 82: 391-403.

Zebeli Q, Tafaj M, Weber I, Dijkstra J, Steingass H, Drochner W (**2007**) Effects of varying dietary forage particle size in two concentrate levels on chewing activity, ruminal mat characteristics, and passage in dairy cow. J Dairy Sci 90: 1929-1942.