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Original article

Impact of feed supplement with alfa-amylase and beta-glucanase on ingestive-related biomarkers registered with real-time sensors

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Abstract

The aim of this study was to investigate the impact of feed supplements with alfa-amylase and beta-glucanase (Optipartum C+ 200) on ingestive-related behaviour biomarkers registered with real-time sensors: rumination behaviours and reticulorumen parameters (pH and temperature). Cows (n=20) in the treatment group (TG) were fed with Optipartum C+ 200 (Enzymes feed supplement: Alfa-Amylase 57 Units; Beta-Glucanase 107 Units) from 21 days before calving until 30 days after calving with a feeding rate of 200 g/cow/day. Cows (n=22) in the control group (CG) were fed a feed ration without feed supplement. Measurements started from 6 days before calving and continued until 21 days after calving. The following indicators were registered: with the RumiWatch System: Rumination time; Eating time; Drinking time; Rumination chews; Eating chews; Drinking gulps; Bolus; Chews per minute; Chews per bolus. With the SmaXtec system: the temperature, pH of the contents of the cows' reticulorumens, and cows' walking activity.

According to our results, feed supplementation with alfa-amylase and beta-glucanase (Optipartum C+ 200) in the TG group resulted in increases in the following parameters: 9% rumination time and eating time, 19% drinking time, 11% rumination chews, 16% eating chews, 13% number of boluses per rumination, 5% chews per minute and 16% chews per bolus. The rumination time showed a strong, positive relation with rumination chews and bolus indicators in both groups (TG and CG) ($p<0.001$); while the rumination time in both groups of cows showed an opposite direction and was negatively related to eating time and eating chews ($p<0.05$).

We found a 1.28 % lower reticulorumen pH and a 0.64 % lower reticulorumen temperature in cows fed with the supplement compared with cows in the control group. Cows in TG were 8.80% more active than those in the CG group. For improvement of ingestive-related behaviour we suggest adding a feed supplement with alfa-amylase and beta-glucanase (Optipartum C+ 200).

Key words: alfa-amylase, beta-glucanase, reticulorumen, rumination

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Introduction

There are several studies on the we of enzyme additives in the dairy nutrition industry. There is a need to search for new enzymes, as well as to utilize bioinformatic tools in order to design specific enzymes that work in certain environmental conditions and substrates (Shepley et al. 2020). The majority of the research conducted with ruminant nutrition has been based on the use of fibrolytic enzymes, amylases and proteases, mainly multienzyme complexes composed of xylanases, cellulases, amylases and pectinases (Grant et al. 1995). Positive effects have been recorded when using exogenous enzymes on agro-industrial and agroforestry wastes used as animal feed that increased the bioavailability of nutrients and digestibility as well as helping in eliminating some anti-nutritional factors. Although endogenous enzymes are involved in the digestion of animals, they do not have the ability to degrade, and to take advantage of, all their nutritional components; therefore, the use of exogenous enzymes is a trend with beneficial results on production and animal yield (Edwards et al. 2004, Šilinskas et al. 2020). However, the application of enzymes in the feeding of ruminants has been developing only slowly. One of the reasons is the complexity of the digestive system, mainly the four compartments, of these animals, and the existence of ruminal microorganisms that excrete enzymes and are fermenting make it difficult to analyze the data obtained. Fibrolytic enzymes are generally used for improving the digestibility of forage cell walls, increasing the availability of the starch present in cereals and improving the productivity of dairy cattle (Yang et al. 2006). Industries focusing on the production of food for animals should concentrate on investing in research and development and in the design of new enzymes, which can play a main role in the improvement and nutritional quality of feed. By developing thermostable enzymes, the industry will simplify the application of the pre-granulation of dry product and will promote the addition of the enzyme in granulated diets (Bowman et al. 2003). The diet, and the enzymatic action to improve its digestibility, contributes to intestinal development and it' s effect on the productive performance. Applying such a diet has the potential to reduce the use of antibiotics by decreasing the incidence of diseases and mortality. The addition of exogenous enzymes into ruminant diets has shown positive effects in lactating dairy cows and growing cattle. In one study, dairy cows received forage treated with a fibrolytic enzyme additive and had a higher dry matter intake and produced 5-25% more milk (Lewis et al. 1996). An improvement in the energy balance of transition dairy cows was seen and an increased milk production in small ruminants was recorded (Lewis et al. 1996). Positive effects were registered in feedlot cattle as fibrolytic enzymes improved live weight gain by as much as 35% and the feed conversion ratio by up to 10% (Šilinskas et al. 2020).

Multiple devices have been implemented in the dairy industry to automatically monitor the behaviour and physiological parameters of dairy cows (Grant et al. 1995). By simply monitoring the ingestive-related parameters such as rumination or feeding, much information can be acquired about the productivity, welfare and health of the animal (Vallejo et al. 2016). Direct visual observation is mostly used for the validation of animal behaviour technologies, but the drawback is that it is time consuming and subject to human error (Elghandour et al. 2016). Pereira et al. (2021) state that the RumiWatch system was able to evaluate rumination, standing, grazing and lying behaviours with high precision and accuracy, and that the RumiWatch system may be used as a benchmark instead of visual observation to validate animal behaviour technologies. In our previous studies we found that the increase of reticulorumen pH and temperature before calving can act as biomarkers of cow health status after calving. There is a positive correlation between reticulum pH and temperature before calving, which can serve as biomarkers of diseases after calving. A noticeable decrease in cow walking activity before calving can serve as an indicator of diseases after calving (Antanaitis et al. 2020).

It was hypothesized that the treatment with exogenous enzymes may have an impact on ingestive-related biomarkers such as reticulum contents pH and temperature, rumination and eating parameters and also cow walking activity registered with real-time sensors.

Accordingly, the aim of this study was to investigate the impact of feed supplements with alfa-amylase and beta-glucanase (Optipartum C+ 200) on ingestive-related behaviours registered with real-time sensors: rumination and eating, reticulorumen parameters (pH and temperature), and walking activity.

Materials and Methods

Location and animals

This study was conducted during 2021.05.01 – 2021.08.31 at a Lithuanian dairy farm with 500 dairy cows (55.911381565736, 21.881321760608195). Average calving was 50 cows per month. None of the cows in the study had trouble calving or had any health issues. From the whole herd 42 cows were selected. The following criteria for the selection of cows were applied: cows had to be within their 21 days before calving, werw of the Lithuanian black and white breed, having two or more lactations, with 570 ± 45 kg body

Table 1. Components of the total mix ration (TMR) feeding per a cow per day.

Feedstuff	Fresh dairy cows	Dry cows
Barley grain, 74% DM (kg)	3	0
Corn grain 56% DM (kg)	3	0
Rapeseed meal 36% protein (kg)	2	1.2
Soy meal is 46% protein (kg)	1	0
Beetroot molasses (kg)	0,5	0
Grass silage 27% DM (kg)	20	8
Maize silage 27% DM (kg)	24.5	1.2
Wheat straws (kg)	0.6	7.5
Nordic Fat (Bergafat) 300 (kg)	0.240	0
Water (kg)	0.1	4.3
Grain mixture (kg)	5	
Mineral-vitamin supplement for dairy cows (kg)	0.300	0
Mineral vitamin supplement for dry cows (kg)	0	0.150

Table 2. Chemical composition of feeding rations for dry and fresh dairy cows.

Parameters	Fresh dairy cows	Dry cows
Dry matter (%)	45.0	46.0
Dry matter intake (DM) (kg DM/d)	27.5	12.1
Net energy lactation (MJ/kg DM)	6.42	4.38
Crude protein (g/kg DM)	172	108
Crude Fat (g/kg DM)	47	25
Fatty acids (g/kg DM)	34	9
Protein balance in rumen (g/kg DM)	23	10
Neutral detergent fiber (g/kg DM)	291	629
Starch (g/kg DM)	205	25
Acid detergent fiber (ADF) (g/kg DM)	180	170
Acid detergent lignin (ADL) (g/kg DM)	20	18
Sugar (g/kg DM)	62	25

weight and average productivity of past lactation of 10500 ± 560 kg milk per year, and the average of feed intake of dry cows was 11 kg DM/day, and fresh cows was 27 kg DM/day. All these criteria were similar between groups. The study was performed with 15 days of adaptation period followed by 27 days (6 d before and 14 d after calving) of experimental period. Cows (n=20) in the treatment group (TG) were fed with Optipartum C+ 200 feed supplement. The supplement (200g/cow/day), in powder form, was mixed with the concentrate feed in a total mix ration (TMR) from 21 days before calving until 30 days after calving. Cows in the control group (n=22) (CG) were fed a feed ration without the feed supplement. TG and CG cows were kept separately in different pens. The cows were fed with TMR at 07:00 am and 07:00 pm (twice per day) and were kept in a loose housing system. The feed ration (Table 1) was balanced according to the nutrient requirements of dairy cattle (NRC 2001) with the NorFor® programme (Agro Food Park 15, 8200 Aarhus N, Denmark, to fit the energy and nutrient requirements of a 550-650 kg Holstein cow (Table 2). Water was available *ad libitum*.

Composition of Optipartum C+ 200 feed supplement: Trace elements: Zinc sulphate monohydrate (3b605) 996 mg; Manganous sulphate monohydrate

Table 3. Parameters registered with a real-time sensors measured using a RumiWatch noseband sensor (ITIN + HOCH GmbH, Fütterungstechnik, Liestal, Switzerland) and their description.

Parameter	Equipment	Description
Rumination time (RT)	RumiWatch System (RWS)	Time spent for rumination chews including chewing interruptions up to 5 s
Eating time (ET)	RumiWatch System (RWS)	Time spent for eating chews, including interruptions between eating chews up to 5 s
Drinking time (DT)	RumiWatch System (RWS)	Time spent for drinking, including interruptions between drinking gulps up to 5 s
Rumination chews (RC)	RumiWatch System (RWS)	Chews during rumination for mechanical breakdown of the regurgitated materials into finer particles using the molars
Eating chews (EC)	RumiWatch System (RWS)	Total number of prehension bites and mastication chews while eating
Drinking gulps (DG)	RumiWatch System (RWS)	Total number of drinking gulps while drinking
Bolus (B)	RumiWatch System (RWS)	Number of boluses per rumination
Chews per minute (CM)	RumiWatch System (RWS)	Chews for one minute
Chews per bolus (CB)	RumiWatch System (RWS)	Chews performed during rumination between the regurgitation and swallowing of one bolus
Temperature	SmaXtec system	Temperature of reticulorumen content
pH	SmaXtec system	pH of reticulorumen content
Activity	SmaXtec system	Walking activity of cows
Temperature without drinkcycles	SmaXtec system	Temperature of reticulorumen content without drinking cycles

Fig. 1. Cow with RumiWatch System (A), Software (B)

(3b503) 955 mg. Enzymes: Alfa-Amylase 57 Units; Beta-Glucanase 107 Units. Technological: Sepiolite (E562) 3750 mg Sensory additives; Aromatic substances: (CAS No. 89-83-8 / Thymol / Flavis No. 04.006) 62.5 mg. Calcium carbonate-Sodium sulfatup to 1 kg. Analytical constituents: Protein 7.1%, Oils and Fats 1.4%, Fibre 9.5%, Ash 4.8%, Sodium 0.1%, Calcium 0.5%, Phosphorus 0.22%, Starch and sugars 39.42%.

Measurements

Sensors were placed on the 21st day before calving. Recording of measurements was started on day 6 before calving to allow for adaptation and was continued until the 21st day after calving. The indicators were reordered and are presented in Table 3. Variables were developed and validated by Zehner et al (2017) and Alsaaod et al (2015). The RumiWatch sensor (RWS) consists of a noseband halter and a built-in pressure detector together with a liquid-filled pressure tube (Fig. 1A). The pressure sensor transmits the signal to the data logger, which is safely stored in a hard plastic box on the halter. A wireless data transmitter then connects to the RumiWatch Manager software and acquires live data (Fig. 1B). The classifications performed by the algorithms are based on the identification of specific pressure peak clusters, produced by the movements of the jaw, which are differentiated between the behavioural characteristics.

SmaXtec system

The data for cow reticulum contents temperature and pH and cow walking activity were recorded using specific SmaXtec boluses (Fig. 2A). This technology allows for continuous real-time monitoring of various

Fig. 2. SmaXtec system: bolus (sensor) (A) and SmaXtec messenger® computer software (B).

Table 4. Results of the RumiWatch noseband sensor readings of dairy cows; means (M) and standard errors (SE) by groups of cows.

Indicators	CG			TG			P
	M	SE	95% CI	M	SE	95% CI	
RT (min/h)	24.04	3.42	22.81-25.27	26.41	3.31	25.25-27.58	>0.05
ET (min/h)	5.53	0.94	5.22-5.83	6.10	0.98	5.78-6.42	>0.05
DT (min/h)	0.79	0.11	0.74-0.85	0.97	0.12	0.91-1.02	>0.05
RC (n/h)	1521.89	232.13	1438.27-1605.52	1718.71	226.94	1638.92-1798.50	>0.05
EC (n/h)	317.13	62.21	296.91-337.35	376.86	64.71	355.65-398.07	>0.05
DG (n/h)	157.89	33.3	145.89-169.90	142.67	29.42	132.47-152.87	>0.05
B (n/rumination)	25.51	3.66	24.19-26.83	29.26	3.75	27.94-30.58	>0.05
CM (n/min)	74.54	0.96	74.19-74.88	78.58	0.76	78.31-78.85	<0.01
CB (n/rumination)	13.67	1.46	12.90-14.45	16.25	1.74	15.45-17.05	>0.05

CG - control group; TG - treatment group; RT - Rumination time (time in minutes spent for rumination chews); RC - Rumination chews (chews during rumination for mechanical breakdown of the regurgitated materials); ET - Eating time (time in minutes spent for eating chews); DT - Drinking time (time in minutes spent for drinking); EC - Eating chews (number of prehension bites); DG - Drinking gulp (total number of drinking gulps while drinking); B - number of boluses per rumination); CM - Chews per minute (chews for one minute); CB - Chews per bolus (chews per formed during rumination).

data such as ruminal pH and temperature. According to the manufacturer's instructions, the boluses were inserted into the reticulorumen using a specific tool. Specially designed antennas (SmaXtec animal care technology®) were used to receive the data from the indwelling boluses (SmaXtec animal care GmbH, Graz, Austria) (Fig. 2). At the beginning of the experiment, the pH probes were calibrated with the provided pH 4 and pH 7 buffer solutions. The data were registered every 10 min daily. All data were collected and displayed using SmaXtec messenger® computer software (Fig. 2 B).

Data analysis and statistics

Statistical data analysis was performed using the SPSS 20.0 (SPSS, Inc., Chicago, IL, USA) program package. The data was presented using methods of normal distribution analysis, the Kolmogorov-Smirnov test, and descriptive statistics. A linear regression equation was calculated to determine the statistical relationship between RumiWatch noseband sensor readings (dependent variables) between a day before and after calving (independent indicator) and also repeated measures ANOVA followed by linear trend analysis. The Pearson correlation coefficient was calculated to determine the linear relationship between rumination time and other RumiWatch variables.

One-way analysis of variance was used for analysis of data. Multiple comparisons of group means were calculated using Tukey's test. The differences were considered as significant at $p<0.05$.

Results

Impact of feed supplement Optipartum C+ 200 on ingestive-related behaviours biomarker registered with real-time sensors (rumination behaviour)

Cows in TG showed higher average values, but not statistically significant values, for the following indicators: rumination time (9% higher), eating time (9% higher), drinking time (19% higher,), rumination chews (11% higher), eating chews (16% higher), num-

Table 5. Evaluation of changes in RumiWatch noseband sensor readings between a day before and after calving (independent indicator) using linear regression and for a relation estimation using repeated measures ANOVA followed by linear trend analysis

Dependent indicator	CG			TG		
	Y	R^2	p	Y	R^2	p
RT	-23.869x + 30.679	0.8367	0.001	2.4845x + 17.982	0.9198	0.001
ET	-0.389x + 6.6149	0.5697	0.215	0.3082x + 5.0492	0.5658	0.007
DT	-0.0029x + 0.8038	0.8589	0.918	0.0054x + 0.9196	0.0088	0.825
RC	-184.83x + 2027.9	0.9355	0.001	206.64x + 1012.9	0.934	0.001
EC	-32.956x + 516.56	0.3777	0.012	31.933x + 290.88	0.4063	0.002
DG	-4.956x + 170.54	0.4306	0.292	16.996x + 85.2	0.6087	0.001
B	-2.355x + 32.185	0.8086	0.001	2.062x + 22.41	0.721	0.001
CM	-0.9973x + 77.278	0.921	0.001	0.2459x + 77.633	0.2739	0.176
CB	0.472x + 11.94	0.2743	0.437	0.755x + 13.555	0,5966	0.031

CG - control group; TG - treatment group; RT - Rumination time (time in minutes spent for rumination chews); RCR - umination chews (chews during rumination for mechanical breakdown of the regurgitated materials); ET - Eating time (time in minutes spent for eating chews); DT - Drinking time (time in minutes spent for drinking); EC - Eating chews (number of prehension bites); DG - Drinking gulp (total number of drinking gulps while drinking); B - number of boluses per rumination) CM - Chews per minute (chews for one minute); CB - Chews per bolus (chews performed during rumination).

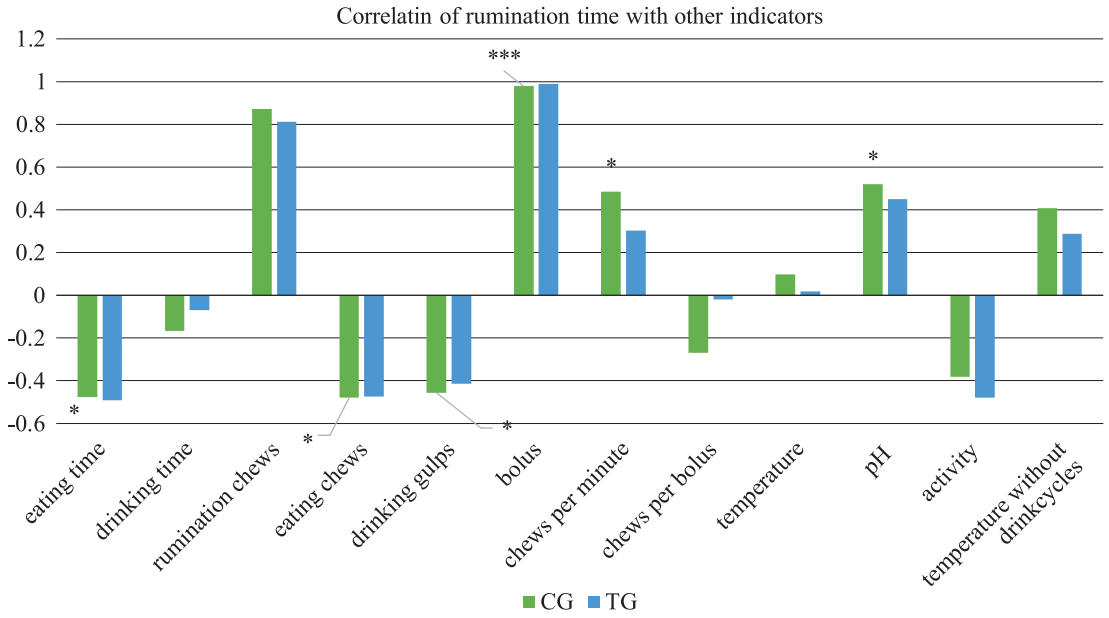

Fig. 3. Correlation.
CG- control group; TG - treatment group. Correlation coefficients are statistically reliable:
* p<0.05, ** p<0.01, *** p<0.001.

ber of boluses per rumination (13% higher), chews per bolus (16.00%) and chews per minute (5% higher, p<0.01) (Table 4).

A description of the change of the observed parameters during the days of the experiment according to the linear regression equation is given in Table 5. The analysis indicated that the change of parameters (rumination time, drinking time, eating time, rumination chew, eating chew, drinking gulp, bolus, chews per minute, chews per bolus) during the experiment for cows of the CG, can be characterized by a linear regression equation with a statistically significant regression coefficient and R^2 from 0.2743 (chews per bolus) to 0.9355 (rumination chew); there were similar trends in the TG group, where a statistically significant R^2 ranged from 0.4063 (eating chews) to 0.934 (rumination chew).

The rumination time showed a correlation with rumination chews and bolus indicators in the two groups of cows, where a statistically significantly positive cor-

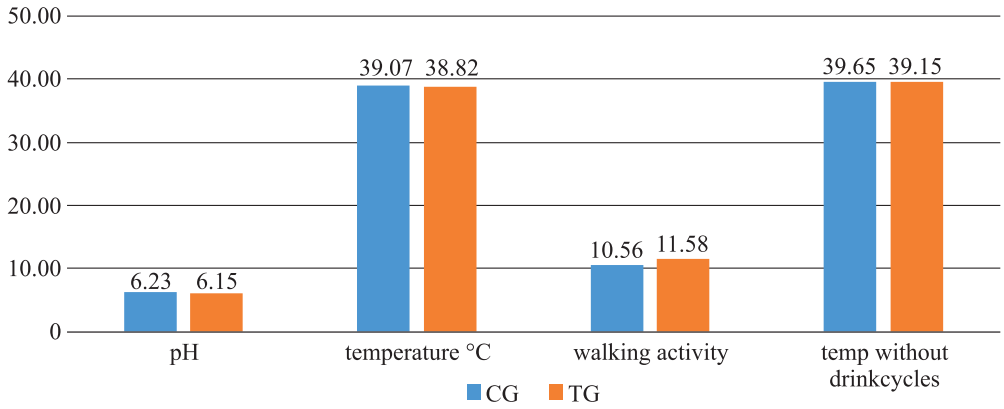

Fig. 4. Analysis of reticulorumen pH, temperature, walking activity of cows and temperature without drinking cycles according to testing groups; CG - control group; TG - treatment group

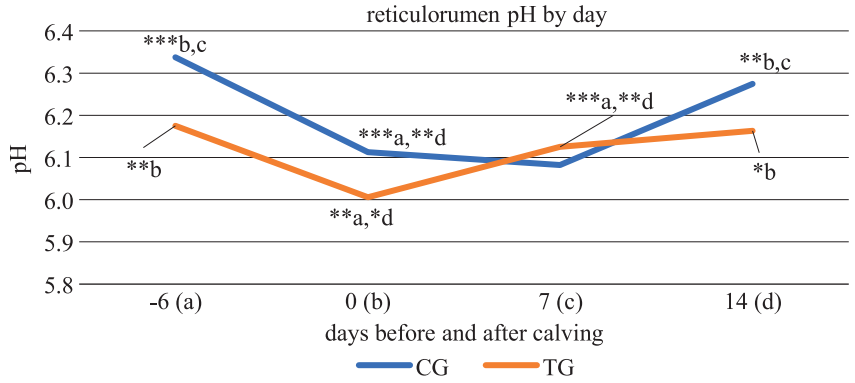

Fig. 5. Comparison of pH of reticulorumen content between day before and after calving according to group of cows; CG - control group; TG - treatment group
Different letters a, b, c and d indicate statistically significant differences between days before and after calving * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

relation was determined in both TG and CG $p<0.001$); howevers the rumination time in both groups of cows (Fig. 3) showed a dependence in the opposite direction and was negatively related to eating time and eating chews ($p<0.05$).

Impact of Optipartum C+ 200 feed supplement on ingestive-related behaviour biomarkers registered using the SmaXtec system

Analysis of the investigated traits by group of cows showed that the highest average value of pH was estimated in cows of the CG group, which was higher compared to the TG; temperature was higher in the CG group compared to the TG, while for walking activity the higher average value was determined in the TG group compared to CG. According to multiple comparisons of means, all differences between the groups of cows were statistically not significant ($p>0.05$), (Fig. 4).

The lowest pH in the CG group was detected at 7 days after calving ($p<0.001$), while in the TG group the lowest pH was at 0 days before and after calving (Fig. 5). Analysis of reticulorumen pH during the days before and after calving showed statistically significant differences between the groups only in the interval of 6 days before calving. The higher average value of reticulorumen pH was found in the CG group of cow's ($p<0.001$).

In the CG group we received statistically significant differences before and after calving: 6 days before calving with 0 days and 6 days before calving with 7 days after calving ($p<0.001$); also, statistically significant differences were detected between 0 days with 14 days after calving and 7 days with 14 days after calving ($p<0.01$).

In the TG group we received the highest statistically significant difference of mean between 6 days and 0 days before and after calving ($p<0.01$) and 0 days with 14 days after calving ($p<0.05$).

The lowest and similar temperature in the CG and in the TG groups was detected in class 0 days after calving. The higher average values of temperature were found in the CG group, compared to the TG group, $p>0.05$, (Fig. 6). In the CG group we received statistically significant differences between classes of 6 days compared to 0 days before and after calving ($p<0.01$). In the TG group statistically significant differences

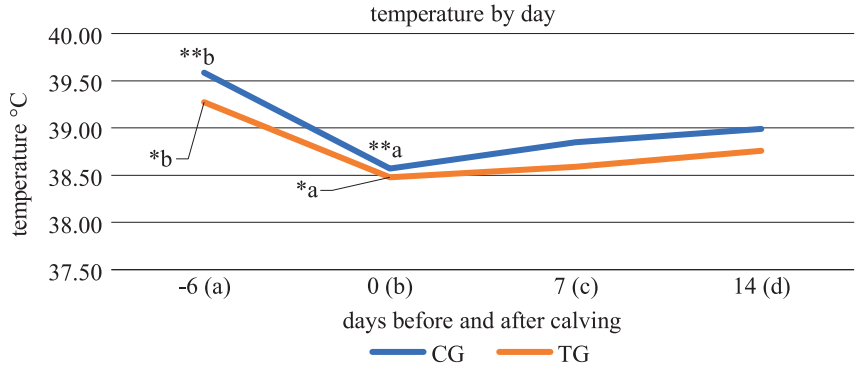

Fig. 6. Comparison of temperature of reticulorumen content between days before and after calving according to group of cows; CG - control group; TG - treatment group.
Different letters a, b, c and d indicate statistically significant differences between days before and after calving * p<0.05, ** p<0.01, *** p<0.001.

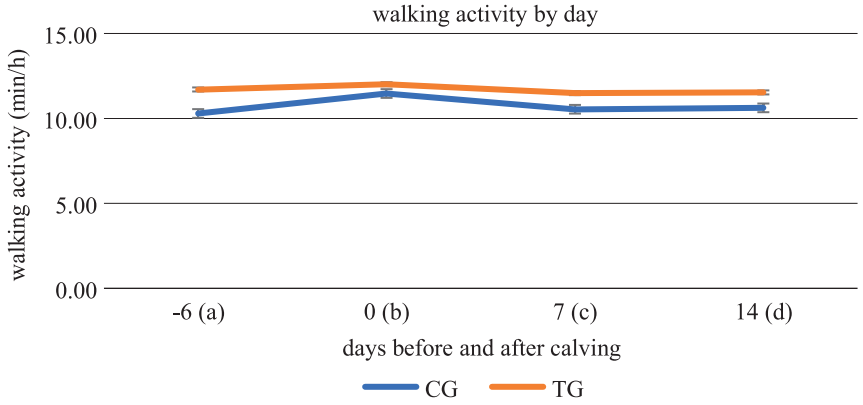

Fig. 7. Comparison of walking activity of cows by days before and after calving according to group of cows; CG- control group; TG - treatment group.
Different letters a, b, c and d indicate statistically significant differences between days before and after calving * p<0.05, ** p<0.01, *** p<0.001.

were noted before and after calving, also just between 6 and 0 days before calving (p<0.05).

The results for walking activity were similar in both groups. The lower average values for, walking activity were detected in the CG group; from 0.54 to 1.42 lower compared to the TG (p>0.05). Also no statistically significant mean differences between days before and after calving were detected in the CG and TG (p>0.05), (Fig. 7).

In class 0, we found the lowest temperature without drink cycles (39.06 - 39.04) in both groups of cows. Analysis of temperature without drink cycles showed statistically not significant differences between the CG and TG groups. The higher average values of temperature without drink cycles were found in group the CG (except off class 7 days after calving, where the results for both groups was almost similar 39.42-39.44). In the CG and TG groups of cows we received statistically significant differences before and after calving (p<0.001 to p<0.05), (Fig. 8).

The data in Fig. 9 show that the pH of the TG group changed from 6.179 to 6.305, while in the CG group it changed from 6.028 to 6.359 during the day. The range of changes was 2.63 times higher compared to cows in the TG group. The higher mean value of reticulorumen pH during the day was noted in the CG group of cows (6.234±0.002); in the TG it was (6.151±0.002) (p>0.05).

Discussion

According to our results, in TG group we found an increase in: 9% rumination time and eating time, 19% drinking time, 16% eating chews, 11% rumination chews, 13% number of boluses per rumination, 5% chews per minute and 16% chews per bolus.

Kovács et al. (2017), considered that rumination is one of the main indicators of animal welfare. Also, according to the same authors, rumination is a very important indicator of ruminant physiological health. Pinos-Rodriguez et al. (2008) found that feeding with enzyme additives improved dry matter intake and digestibility of fiber. Yang et al. (2002) reported a positive effect of exogenous enzymes on milk productivity. Ac-

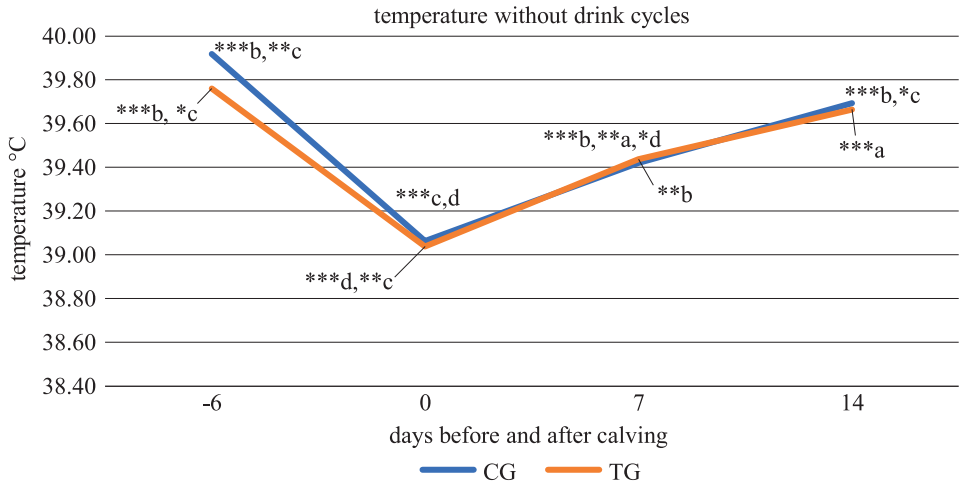

Fig. 8. Comparison of temperature of content of reticulorumen without drink cycles between days before and after calving according to group of cows; CG - control group; TG - treatment group.
Different letters a, b, c and d indicate statistically significant differences between days before and after calving * p<0.05, ** p<0.01, *** p<0.001.

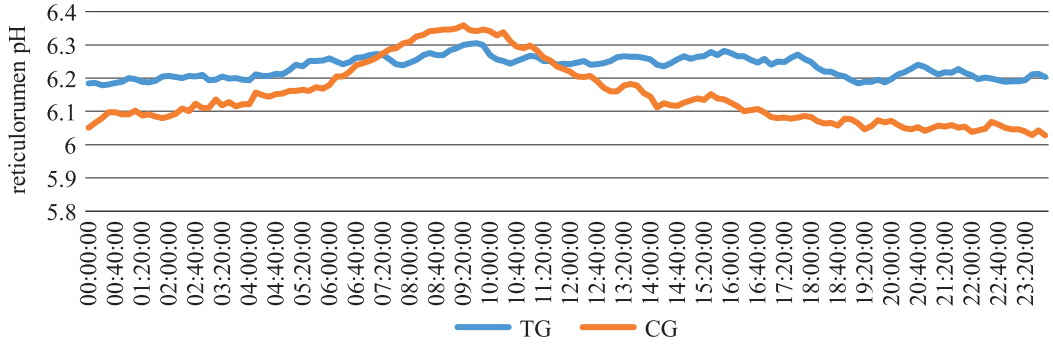

Fig. 9. Changes of reticulorumen pH during the day (24 hours per day).

cording to the literature, the degree of the use of starch is determined by the nutritional and chemical composition of the diet, adaptation of ruminal microorganisms to the new substrate and exogenous enzyme complex that can be used to degrade forage fiber, improving the digestion of forage-based diets (Kovacs et al. 2017). The addition of enzymes may increase nutrient digestibility (Rodriguez et al. 2008). The same results were found by Yang et al. (2002), who reported a variable effect of feed additives with exogenous enzymes on animal performance and feed digestion. Yang et al. (2002) reported that feed supplementation with exogenous enzymes can increase digestion and nutrient flow from the rumen and increase feed digestibility or maintain and increase intake (Alsaaod et al. 2017).

Exogenous fibrolytic enzymes are used in low quality feeds to improve carbohydrate and the degradation of the cell wall (Zehner et al. 2017). On the other hand, Bowman et al. (2003) found that enzyme supplementation had no effect on the feeding and ruminating behaviour of cows. However, the effectiveness of the diet with enzymatic additives in order to promote chewing was not reduced (Bowman et al. 2003).

According to the literature shorter rumination times may be indicative of reduced DMI in the pre-partum phase (Guatteo et al. 2014), however several farm-level and management-related factors vary and have a significant impact on rumination time (Kaufman et al. 2016). Rumination behaviour may be a promising biomarker for tracking metabolic circumstances linked to a decrease in DMI (Kaufman et al. 2016). When compared mong with different experiments, the amount of time dairy cows spend eating can be very different. White et al. (2017) found a mean eating time of 284 minutes per day, ranging from 141 to 507 minutes per day. Part of the variation may be attributed to slightly different criteria used to determine eating time among studies, but eating time is also heavily influenced by feed management, DMI, the physical and chemical makeup of the diet, and natural diversity across animals (White et al. 2017). Ruminating and eating time appear to have a compensating relationship. According to Dado and Allen (1994), the correlation coefficient between eating time and ruminating time for dairy cows with unlimited feed access was 0.62, indicating that cows who spend less time eating tend to ruminate longer. Predicting the

amount of time cows spend chewing or ruminating can be a useful management tool for improving cow health, but the accuracy can be low due to the many interacting components (Bauchemin et al. 2018). Furthermore, numerous factors can influence water intake, including changes in ambient temperature, increased water loss due to increased milk production, amount of feed consumed, salt and potassium consumption, diet dry matter, and physiological conditions and diseases (Brandstetter et al. 2019).

According to the results of our study, we found a 1.28% lower reticulorumen pH and a 0.64% lower reticulorumen temperature in cows fed with the supplement compared with the control group.

Our results coincide with Elghandour et al. (2016), where the mean ruminal fluid pH was lowered with exogenous enzymes compared with the control group. This may be due to greater enzymatic hydrolysis of feeds into readily fermentable substrates that depressed the pH. Elghandour et al. (2016) also found reduced ruminal pH values when four fibrous substrates were incubated with different levels of an exogenous enzyme. On the other hand, Vallejo et al. (2016) stated that maize treated with enzymes had no effect on final pH values in vitro. Šilinskas et al. (2020) found that supplementing diets exclusively with exogenous fibrolytic enzymes for a prolonged period could reduce reticulorumen pH and increase the risk of health problems associated with lower reticulorumen pH. In another study, the addition of an exogenous fibrolytic enzyme to the diet of lactating Holstein cows did not alter ruminal fermentation or rumen pH (Soliman 2006). Lewis et al. (1996) in their study on beef steers, reported a decrease in ruminal pH for the group consuming a forage-based ration supplemented with enzymes. Barley grain enriched with enzyme products also decreased the ruminal pH of the studied animals (Adesogan et al. 2014). The higher rate of fermentation of barley grain in comparison with other grain sources may have contributed to the depressed rumen pH (Bowman et al. 2003). In addition, Yang et al. (2002) also reported a lower ruminal pH for diets containing barley grain compared with those using corn grain. By increasing the rate of fermentation within the rumen, the ruminal pH is decreased, which in turn decreases fiber digestion (Valdes et al. 2015). Supplementing the feed with fibrolytic enzymes has been shown to increase fiber digestion, and ruminal pH has been lowered in some cases (Lewis et al. 1996), but not always (Valdes et al. 2015). New studies indicate that exogenous enzymes have the potential to increase forage degradation in the rumen (Šilinskas et al. 2020). Yang et al. (2002) stated that the reason for the inconsistencies in the responses to supplemental enzyme additives are probably due to the characteristics of the enzymes, including enzymatic activities in ruminal conditions (temperature and pH) and the composition of the target forage. Šilinskas et al. (2020) found that TMR supplementation with exoenzymes and active yeast could affect the daily time of the occurrence maximum pH values, but had no effect on the time for the minimum pH values.

The higher mean (pH) value was estimated in the control group (6.234±0.002) in comparison to the treatment group (6.151±0.002), ($p>0.05$). The range of changes was 2.63 times higher compared to cows in the TG group.

Higher average values of walking activity were detected in all classes of treatment group of cows, but the mean differences compared to the control group of cows was not statistically significant (due to a small sample size, suggesting that walking activity was not affected by treatment).

According to Edwards and Tozer (2004), daily changes in walking activity have proven to be useful for identifying potential disorders during the pre-breeding and stage of lactation. Furthermore, daily exercise has been suggested to improve the well-being of dairy cows during the transition period (Gado et al. 2007). Increasing movement opportunity can benefit cow health, behaviour, and welfare (Benaissa et al. 2019)

Higher average values of reticulorumen pH were found in the CG group and higher average values of temperature were also detected in all classes of the CG group. According to a literature review, a decreased reticulorumen pH in SARA-susceptible cows in early postpartum could indicate faster ruminal fermentation compared to SARA-tolerant cows, resulting in a higher rate of VFA generation in the rumen fluid (Aschenbach et al. 2011). Increased DMI and, in particular, higher intake of easily fermentable carbohydrate sources such as rapidly degradable starch and sugars are known to stimulate VFA formation in the rumen (Zebeli et al. 2008). Because reduced chewing time reduces daily saliva secretion (Allen et al. 1997), this may result in lower rumen buffering and reduces ability to regulate pH in SARA-susceptible cows' rumens, despite the fact that a high NDF consumption is known to enhance chewing activity (Zebeli et al. 2008). Furthermore, past research has indicated that increased chewing activity is not always associated with increased ruminal pH (Gao et al. 2014). Individual cows differ greatly in their feeding and rumination activities, as well as their total eating and ruminating time (Christensen et al. 2000), and recently it has been demonstrated that dairy cows tolerant to high-grain diets select less against long particles than vulnerable cows (Gao et al. 2014). Aside from temperature fluctuations associated with calving,

it has been demonstrated that rumen temperature is inversely connected with rumen pH in nursing cows suffering from SARA (AlZahal et al. 2008) These researchers discovered that rumen temperature between 39 and 41°C is crucial for SARA diagnosis since it has a negative connection with pH. Furthermore, a poor relation (r = 0.24) was established between the time duration of reticulorumen temperature >39.5°C and pH 5.8. As a result, ruminal temperature data do not appear to be a helpful management tool for diagnosing SARA in the field (Humer et al. 2015).

Conclusions

We found that supplementing the feed of cows, from 21 days before calving to 30 days after calving, with alfa-amylase and beta-glucanase (Optipartum C+ 200) (with a dose of 200g/cow/day) had a positive effect on ingestive-related biomarkers registered with real-time sensors such as rumination time, eating time, drinking time, rumination chews, eating chews, number of boluses per rumination, chews per minute and chews per bolus. On the practical side, for improvement of ingestive-related biomarkers, we recommend using a feed supplement with alfa-amylase and beta-glucanase (Optipartum C+ 200) (with a dose of 200g/cow/ /day).

Due to the small sample size (n=42), it was difficult to obtain a statistical significance in some cases, so further studies are therefore needed with a larger number of cows.

References

Adesogan AT, Ma ZX, Romero JJ, Arriola KG **(2014)** Ruminant Nutrition Symposium: Improving cell wall digestion and animal performance with fibrolytic enzymes. J Anim Sci 92: 1317-1330.

Allen MS **(1997)** Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J Dairy Sci 80: 1447-1462.

Alsaaod M, Niederhauser JJ, Beer G, Zehner N, Schuepbach-Regula G, Steiner A **(2017)** Development and validation of a novel pedometer algorithm to quantify extended characteristics of the locomotor behavior of dairy cows. J Dairy Sci 98: 6236-6242.

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

Antanaitis R, Juozaitienė V, Malašauskienė D, Televičius M **(2020)** Can reticulorumen ph, temperature and cow activity registered before calving act as biomarkers of diseases after calving? Pol J Vet Sci 23(2): 221-227.

Aschenbach JR, Penner GB, Stumpff F, Gäbel G **(2011)** Ruminant nutrition symposium: Role of fermentation acid absorption in the regulation of ruminal pH. J Anim Sci 89: 1092-1107.

Bauchemin KA **(2018)** Invited review: Current perspectives on eating and rumination activity in dairy cows. J Dairy Sci 101(6): 4762-4784.

Benaissa S, Tuyttens FA, Plets D, Cattrysse H, Martens L, Vandaele L, Joseph W, Sonck B **(2019)** Classification of ingestive-related cow behaviours using RumiWatch halter and neck-mounted accelerometers. Appl Anim Behav Sci 211: 9-16.

Bowman GR, Beauchemin KA, Shelford JA **(2003)** Fibrolytic enzymes and parity effects on feeding behavior, salivation, and ruminal pH of lactating dairy cows. J Dairy Sci 86: 565-575.

Brandstetter V, Neubauer V, Humer E, Kröger I, Zebeli Q **(2019)** Chewing and drinking activity during transition period and lactation in dairy cows fed partial mixed rations. Anim 9(12): 1088.

Christensen DA, Fehr M **(2000)** Eating and feeding behavior of dairy cows: Dietary influences and impact on production. Department of Animal and Poultry Science, University of Saskatchewan, Canada, Alberta Proceedings.

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.

Clement P, Guatteo R, Delaby L, Rouillé B, Chanvallon A, Philipot JM, Bareille N **(2014)** Short Communication: Added value of rumination time for the prediction of dry matter intake in lactating dairy cows. J Dairy Sci 97: 6531-6535

Dado RG, Allen M **(1994)** Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy cows. J Dairy Sci 77: 132-144

Edwards JL, Tozer PR **(2004)** Using activity and milk yield as predictors of fresh cow disorders. J Dairy Sci 87: 524-531.

Elghandour MMY, Kholif AE, Hernández J, Mariezcurrena MD, López S, Camacho LM, Márquez O, Salem AZM **(2016)** Influence of the addition of exogenous xylanase with or without pre-incubation on the in vitro ruminal fermentation of three fibrous feeds. Czech J Anim Sci 61: 262-272.

Gado HM, Metwally HM, El-Basiony AA, Soliman HS, Abd El-Galil ER **(2007)** Enzymatic treatments of sugarcane bagasse by different sources of cellulase enzymes. Egyp J Nutri Feeds 10: 607-620.

Gao X, Oba M **(2014)** Relationship of severity of subacute ruminal acidosis to rumen fermentation, chewing activities, sorting behavior, and milk production in lactating dairy cows fed a high-grain diet. J Dairy Sci 97: 3006-3016.

Grant RJ, Albright JL **(1995)** Feeding behavior and management factors during the transition period in dairy cattle. J Anim Sci 73: 2791-2803.

Humer E, Ghareeb K, Harder H, Mickdam E, Khol-Parisini A, Zebeli Q **(2015)** Peripartal changes in reticuloruminal pH and temperature in dairy cows differing in the susceptibility to subacute rumen acidosis. J Dairy Sci 98: 8788-8799.

Kaufman EI, LeBlanc SJ, McBride BW, Duffield TF, DeVries TJ **(2016)** Short communication: Association of lying behavior and subclinical ketosis in transition dairy cows. J Dairy Sci 99: 7473-7480.

Kovács L, Kézér FL, Ruff F, Szenci O **(2017)** Rumination time and reticuloruminal temperature as possible predictors of dystocia in dairy cows. J Dairy Sci 100: 1568-1579.

Lewis GE, Hunt CW, Sanchez WK, Treacher R, Pritchard GT, Feng P **(1996)** Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers. J Anim Sci 74: 3020-3028.

NRC (2001) Nutrient requirements of dairy cattle. Seventh Revised Edition.

Pinos-Rodríguez JM, Moreno R, González SS, Robinson PH, Mendoza G, Álvarez G **(2008)** Effects of exogenous fibrolytic enzymes on ruminal fermentation and digestibility of total mixed rations fed to lambs. Anim Feed Sci Technol 142: 210-219.

Silinskas I, Monkeviciene I, Dovydaitienė G, Tapio I, Musayeva K, Japertas S, Sedereviuius A, Zelvyte R **(2020)** Impact of a diet supplemented with exoenzymes and yeast on predicted circadian rhythm of reticulorumen pH. Veterinaria in Zootechnika 77(99): 45-50.

Shepley E, Lensink J, Vasseur E **(2020)** Cow in motion: a review of the impact of housing systems on movement opportunity of dairy cows and implications on locomotor activity. Appl Anim Behav Sci 230: 105026.

Silinskas I, Monkeviciene I, Dovydaitienė G, Tapio I, Musayeva K, Japertas S, Pereira GM, Sharpe KT, Heins BJ **(2021)** Evaluation of the RumiWatch system as a benchmark to monitor feeding and locomotion behaviors of grazing dairy cows. J Dairy Sci 104: 3736-3750

Soliman MS (**2006**) Utilization of peanut hay in ruminant feeding. PhD Thesis. Alexandria University, Alexandria, Egypt.

Valdes KI, Salem AZM, López S, Alonso MU, Rivero N, Elghandour MMY, Domínguez IA, Ronquillo MG, Kholif AE **(2015)** Influence of exogenous enzymes in presence of Salix babylonica extract on digestibility, microbial protein synthesis and performance of lambs fed maize silage. J Agri Sci 153: 732-742.

Vallejo LH, Salem AZM, Camacho LM, Kholif AM, Mariezcurrena MD, Cipriano M, Alonso MU, Olivares J, López S (**2016**) Effects of xylanase supplementation on feed intake, digestibility and ruminal fermentation in Rambouillet sheep. J Agric Sci 154: 1110-1117.

White RR, Hall MB, Firkins JL, Kononoff PJ **(2017)** Physically adjusted neutral detergent fiber system for lactating dairy cow rations I: Deriving equations that identify factors that influence effectiveness of fiber. J Dairy Sci 100: 9551-9568.

Yang WZ, Beauchemin KA **(2006)** Effects of physically effective fiber on chewing activity and ruminal pH of dairy cows fed diets based on barley silage. J Dairy Sci 89: 217-228.

Yang WZ, Beauchemin KA, Vedres DD **(2002)** Effects of pH and fibrolytic enzymes on digestibility, bacterial protein synthesis, and fermentation in continuous culture. Anim Feed Sci Technol 102: 137-150.

Zebeli Q, Dijkstra J, Tafaj M, Steingass H, Ametaj BN, Drochner W **(2008)** Modeling the adequacy of dietary fiber in dairy cows based on the responses of ruminal pH and milk fat production to composition of the diet. J Dairy Sci 91: 2046-2066.

Zehner N, Umstätter C, Niederhauser JJ, Schick M **(2017)** System specification and validation of a noseband pressure sensor for measurement of ruminating and eating behavior in stable-fed cows. Comput Electron Agric 136: 31-41.