Milk production and feeding behavior of lactating cows supplemented with a dry fungus fermentation product that expresses residual fibrolytic enzyme activity

Produção de leite e comportamento alimentar de vacas em lactação suplementadas com um produto de fermentação de fungo seco que expressa atividade de enzima fibrolítica residual

DOI:10.34117/bjdv7n1-174

Recebimento dos originais: 10/12/2020
Aceitação para publicação: 10/01/2021

Joana Piagetti Noschang
Mestre em Zootecnia
Universidade Federal Pelotas – UFPEL
Eliseu Maciel Road, SN, CEP: 96160-000, Pelotas, RS, Brasil
E-mail: joana.piagetti@hotmail.com

Magna Fabrícia Brasil Savela
Médica Veterinária
Universidade Federal de Pelotas – UFPEL
Eliseu Maciel Road, SN, CEP: 96160-000, Pelotas, RS, Brasil
E-mail: fabibrasil93@gmail.com

Edgard Gonçalves Malaguez
Mestre em Ciência Animal
Universidade Federal de Pelotas – UFPEL
Eliseu Maciel Road, SN, CEP: 96160-000, Pelotas, RS, Brasil
E-mail: edgardgmalaguez@gmail.com

Antônio Amaral Barbosa
Doutor em Medicina Veterinária
Universidade Federal de Pelotas – UFPEL
Eliseu Maciel Road, SN, CEP: 96160-000, Pelotas, RS, Brasil
E-mail: antoniobarbosa.vet@hotmail.com

Francisco Augusto Burkert DelPino
Doutor em Ciências Biológicas
Universidade Federal de Pelotas – UFPEL
Eliseu Maciel Road, SN, CEP: 96160-000, Pelotas, RS, Brasil
E-mail: fabdelpino@gmail.com

Viviane Rohrig Rabassa
Doutora em Medicina Veterinária
Universidade Federal de Pelotas – UFPEL
Eliseu Maciel Road, SN, CEP: 96160-000, Pelotas, RS, Brasil
E-mail: vivianerabassa@gmail.com

Marcio Nunes Corrêa
Doutor em Biotecnologia
Universidade Federal de Pelotas – UFPEL
Eliseu Maciel Road, SN, CEP: 96160-000, Pelotas, RS, Brasil
E-mail: marcio.nunescorrea@gmail.com

Cássio Cassal Brauner
Doutor em Zootecnia
Universidade Federal d ePelotas – UFPEL
Rua Eliseu Maciel, SN, CEP: 96160-000, Pelotas, RS, Brasil
E-mail: cassiocb@gmail.com

ABSTRACT
The aim of the study was to evaluate the effect of supplementation with a product of fermentation of dry fungi expressing residual enzymatic fibrolytic activity on the productive and behavioral performance of lactating cows. The study was carried out on a commercial dairy farm. 36 Holstein cows divided into two groups (control and supplemented) were evaluated for 23 days. The product came from a commercial source MAXFIBER® (Provita Supplements, Pinneberg / Germany) and was supplied in the amount of 10g / animal / day. The total digestibility of the mixed feed, the individual milk production, the feeding behavior in intelligent feeders (Intergado®), and the behavior of the animals were verified using cowMed animal monitoring collars (ChipInside® Technology / Santa Maria / RS / Brazil). Supplementation affected the animals' behavior, with an increase in activity time (P = 0.002) and a tendency to decrease in rumination time (P = 0.08). There was an increase in the frequency of ingestion (P = 0.001) and the duration of the meal (P = 0.001). Milk production was higher in the supplemented group (P = 0.05). The use of this additive alters the animals' behavior, reflecting an increase in milk production.

Keywords: dairy cattle, digestibility, precision nutrition, rumination, solid state fermentation.

RESUMO
O objetivo do estudo foi avaliar o efeito da suplementação com um produto da fermentação de fungos secos expressando atividade fibrolítica enzimática residual sobre o desempenho produtivo e comportamental de vacas em lactação. O estudo foi realizado em uma fazenda comercial de laticínios. 36 vacas Holandesas divididas em dois grupos (controle e suplementadas) foram avaliadas por 23 dias. O produto é de origem comercial MAXFIBER® (Provita Supplements, Pinneberg / Alemanha) e foi fornecido na quantidade de 10g / animal / dia. A digestibilidade total da ração mista, a produção individual de leite, o comportamento alimentar em comedouros inteligentes (Intergado®) e o comportamento dos animais foram verificados por meio de coleiras de monitoramento de animais cowMed (ChipInside® Technology / Santa Maria / RS / Brasil). A suplementação afetou o comportamento dos animais, com aumento do tempo de atividade (P = 0.002) e tendência de diminuição do tempo de ruminação (P = 0.08). Houve aumento na frequência de ingestão (P = 0.001) e na duração da refeição (P = 0.001). A produção de leite foi maior no grupo suplementado (P = 0.05). O uso desse aditivo altera o comportamento dos animais, refletindo no aumento da produção de leite.

Palavras-chave: Bovinocultura de leite, digestibilidade, fermentação em estado sólido, nutrição de precisão, ruminação.

1 INTRODUCTION

The forages are characterized by being a feeding base in ruminant's diets and represent 40 to 100% in the diet costs dairy cows, playing a fundamental role in animal production, health, and well-being (Adesogan et al., 2019). Ruminants are able of transforming low-quality fibers into proteins of high biological value, due to the synthesis and secretion of microorganism enzymes present in the ruminal fluid that act by promoting the hydrolysis of the plant cell wall (Refat et al., 2018).

Such wall is structured by cellulose, hemicellulose, and lignin, constituents that can be resistant to an enzymatic attack carried out by ruminal bacteria, limiting fiber digestibility and the energy available to the animal (Beauchemin, et al., 2003) which, in optimal conditions, can reach up to 70% of digestibility (Sujani and Seresinhe, 2015). Therefore, maximizing the use of fodder is essential to maintain the sustainability of the system, taking into account that food represents the largest proportion of production costs (Peters et al., 2015).

Therefore, exogenous fibrolytic enzymes (EFE), which have the synergistic action of hydrolysis of specific fiber bonds together with ruminal bacteria, become an interesting alternative for the greater use of the fibrous fraction of the diet (Arriola et al., 2017). This synergism of endogenous enzymes and EFE can reduce the levels of neutral detergent fiber (NDF) and acid detergent fiber (FDA), increasing the digestibility of the diet (Zilio et al., 2019). This is because EFE can remove structural barriers of feed, facilitating the access and adhesion of bacteria, releasing monosaccharides for the bacteria to convert to short-chain fatty acid (SCFA) (Arriola et al., 2017).

The improvement in feed efficiency provided by the inclusion of additives has shown effects on metabolic and behavioral since changes in rumination, leisure, and serum markers are evident in these cases. In this context, the observation of feeding behavior is configured as another instrument of control of cows diets that makes it possible to effectively adjust feed management (Borchers et al, 2016; Johnston and Devries, 2018). However, up to now, few studies are describing such results (Silva et al, 2016; Peters et al., 2015).

Thus, the efficacy of enzyme products is still unclear, due to the great variability of results. Therefore, the objective of this study was to evaluate the effect of including a fermentation product of dry fungi on animal behavior and consumption and its reflex on the productive performance of dairy cows.

2 METHOD

This study was approved by the Animal Ethics and Experimentation Committee of the Federal University of Pelotas (46050-2019).

The study was carried out on a commercial dairy farm in the municipality of Rio Grande, at Rio Grande do Sul, at geographic coordinates 32º16'S, 52 ° 32'L, during the period from September 23 to November 11, 2019.

Thirty-six Holstein cows were allocated in a compost barn system and milked twice a day. The cows were segregated into two groups, the control group (CG) and the supplemented group (SG), both with 18 animals, followed for 23 days. For SG there was the inclusion of 10g / animal / day of a dry fungus fermentation product that expresses residual fibrolytic enzymatic activity produced from the strains of Aspergillus niger, *Aspergillus tubingensis*, *Aspergillus orzyae*, *Aspergillusoyae* and *Neurospora intermedia* (MAXFIBER®, Provitta Supplements GmbH, Pinneberg / Germany), containing xylanase, endoglucanase, and exoglucanase activities, added to the total mixed feed (TMR) before the morning feed. The CG was fed the same diet without the inclusion of the dried fungus fermentation product.

Both groups were kept together during the experimental period and received the same diet in a roughage proportion: 70:30 concentrate (Table 1), twice a day, with water ad libitum. To maintain the homogeneity of the groups, some lactations were considered, between two and four, days in milk (DIM) from 40 to 70 days, average milk yield of 38.0 ± 4.10 kg, the average body weight of 700 ± 20 kg and 3 ± 0.44 body condition score (BCS). The individual and daily milk yields were recorded automatically by the mechanized milking system (DeLaval®).

Table 1: Ingredient and composition bromatological of the animals' TMR (n = 36) of the control group (CG) and supplemented group (SG) with 10g / cow / day of product resulting from the solid-state fermentation of fungi, with residual enzymatic activity (MAXFIBER®, Provita Supplements GmbH, Pinneberg/Germany).

Diet composition (%DM)						
Ingredient (TMR)			%DM		Kg/animal/day	
water			0.01		8.0	
Corn grain, ground fine			86.14		1.0	
bran soy meal			87.84		2.9	
Soy bark			89.86		2.6	
Rice bran			88.65		1.9	
Moist grain corn			70.00		2.0	
Corn Silage			44.85		27	
Pre dried ryegrass			44.37		2.0	
Rice residue			89.20		1.0	
Bromatological composition of TMR (%)						
DM[1]	MM[2]	CP[3]	EE[4]	NDF[5]	ADF[6]	LIG[7]
91.74	9.37	12.71	5.54	35.04	18.89	1.59

TMR: total mixed ration; DM[1]: Dry matter, MM[2]: matter mineral, CP[3]: Crude protein, EE[4]: Ether extract**;** NDF[5]: Neutral detergent fiber, ADF[6]: Acid detergent fiber, LIG[7]: Lignin

Feeding behavior was assessed daily through the use of intelligent feeders (INTERGADO®) in an automatic and individualized way (Robles et al., 2007). Animal behavior was assessed using CowMed monitoring collars (ChipInside® Tecnologia - Santa Maria / RS / Brazil /) which indicated the time in minutes that the animal remained active (social, search for food, water consumption), rumination or leisure daily for 24 hours, for 23 days.

Daily samples of the diet were collected for analysis of dry matter (DM) content and weekly collected and sent to the Laboratory of Bromatology and Nutrition of Ruminants, at the University of Santa Maria, to proceed with in vitro digestibility analysis by the method described by Tilley & Terry (1963). A daily sample of the total ration was also taken to evaluate the particle size using a sieve system (Pen State Particle Separator - Nasco, Fort Atkinson, WI) as described by Kononoff et al., (2003).

All cows were weighed after the morning milking on days 0, 14, 19, and 23 of the experimental period, using a weighing tape and the BCE was measured through visual assessment using parameters 1 to 5, with intervals 0.25 (Edmonson et al., 1989).

Statistical analysis of the data obtained was performed using the Statistical Analysis System program (SAS Institute Inc. Cary, NC, USA). For this, analysis of variance with Mixed Model was used to compare the groups (supplemented and control), collections, and their interaction (group x collection) through the Tukey HS test, with significant values of $P \leq 0.05$ and biased values of $P < 0.1$.

3 RESULTS

In the assessment of behavior, the rumination time showed a tendency (P = 0.08; Figure 1) being lower in the supplemented group (619.78 min ± 5.42 vs 632.47 min ± 4.8). There was a longer activity time (P = 0.002; Figure 2) in the supplemented group compared to the control group (199.60 min ± 4.31 vs 183.79 min ± 2.96 P = 0.02) while the leisure time showed no difference (P = 0.33), being 615.56 min ± 6.07 for the CG and 623.73 min ± 5.97 for the SG.

Figure 1. Effect of supplementation with 10g / cow / day of dry fungus fermentation product that expresses residual fibrolytic enzymatic activity (MAXFIBER®, Provita Supplements GmbH, Pinneberg / Germany) on the rumination time of animals in the supplemented group (SG) and Control (CG) (n = 36) evaluated daily using monitoring collars (ChipInside®).

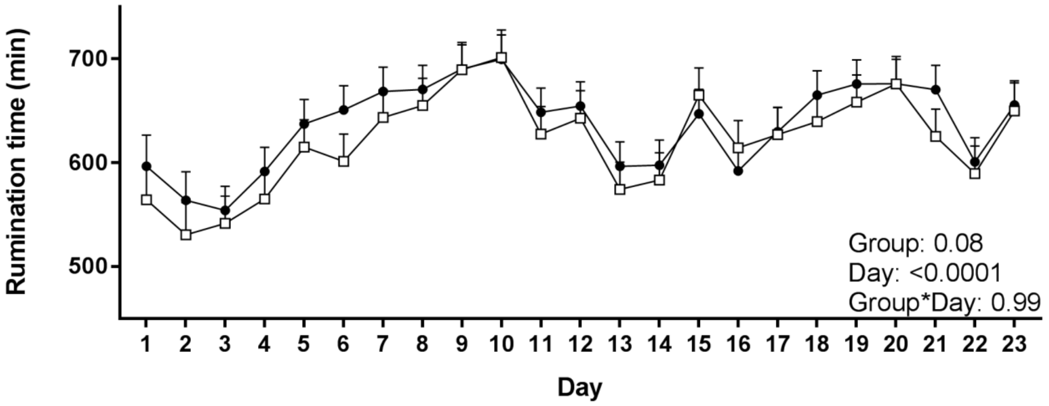
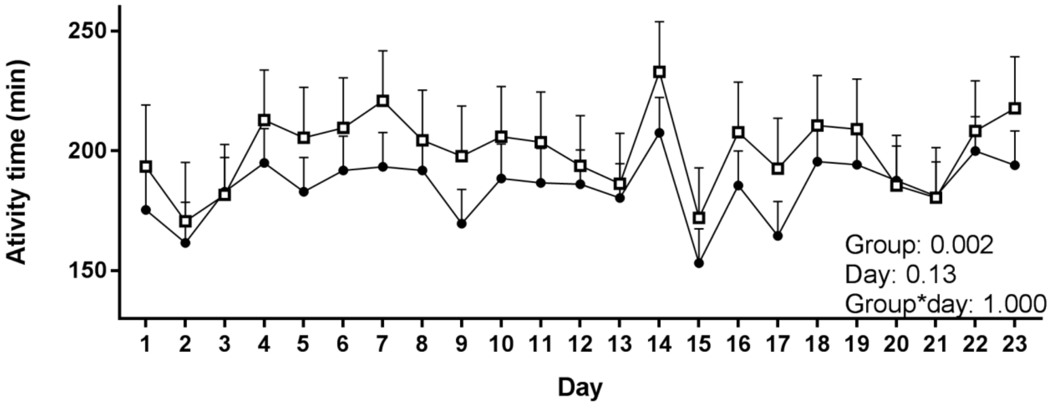


Figure 2. Effect of supplementation with 10g / cow / day of dry fungus fermentation product that expresses residual fibrolytic enzymatic activity (MAXFIBER®, Provita Supplements GmbH, Pinneberg / Germany) on the ativity time of animals in the supplemented group (SG) and Control (CG) (n = 36) evaluated daily using monitoring collars (ChipInside®).



In the results of feeding behavior, there was a higher intake frequency (P = 0.001; Figure 3) in the SG when compared to the CG (26.45 n° / day ± 0.53 vs 23.66 n° / day ± 0.53), as well as an increase in the frequency of accesses to feeders in the supplemented group (67.62 ± 1.40 vs 60.71 ± 1.12) (P = 0.001). In contrast, the meal size (P = 0.005; Figure 4) and the meal intake duration (5.83 ± 015 vs 5.92 ± 0.18 min/meal) (P = 0.05; Figure 5) were higher for the CG compared to the SG. There was no difference between the groups in the intake of DM obtaining averages of 20.67 ± 0.31 vs 20.25 ± 0.31 (P = 0.32) and in the intake time 122.18 ± 2.41 vs 118.59 ± 2.23 (P = 0.32) in the SG and CG respectively.

Figure 3: Effect of supplementation with 10g / cow / day of dry fungus fermentation product that expresses residual fibrolytic enzyme activity on the intake frequency of animals in the supplemented group (SG) (MAXFIBER®, Provita Supplements GmbH, Pinneberg / Germany) and Control (CG) (n = 36) assessed daily using Smart Feeders (Intergado®).

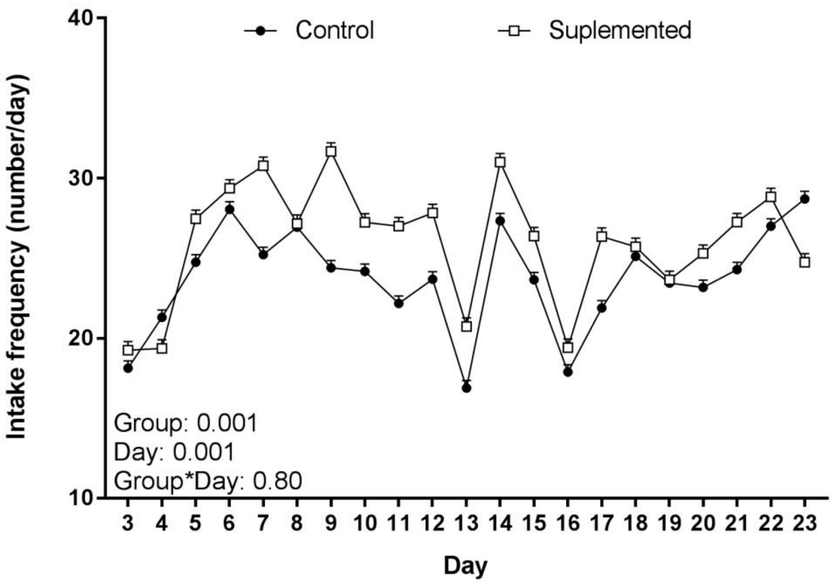


Figure 4: Effect of supplementation with 10g / cow / day of dry fungus fermentation product that expresses residual fibrolytic enzyme activity on the meal size of animals in the supplemented group (SG) (MAXFIBER®, Provita Supplements GmbH, Pinneberg / Germany) and Control (CG) (n = 36) assessed daily using Smart Feeders (Intergado®).

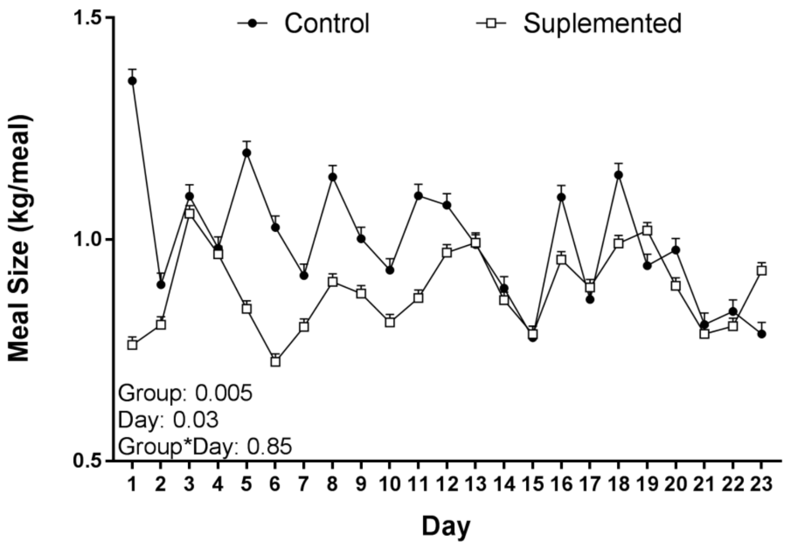
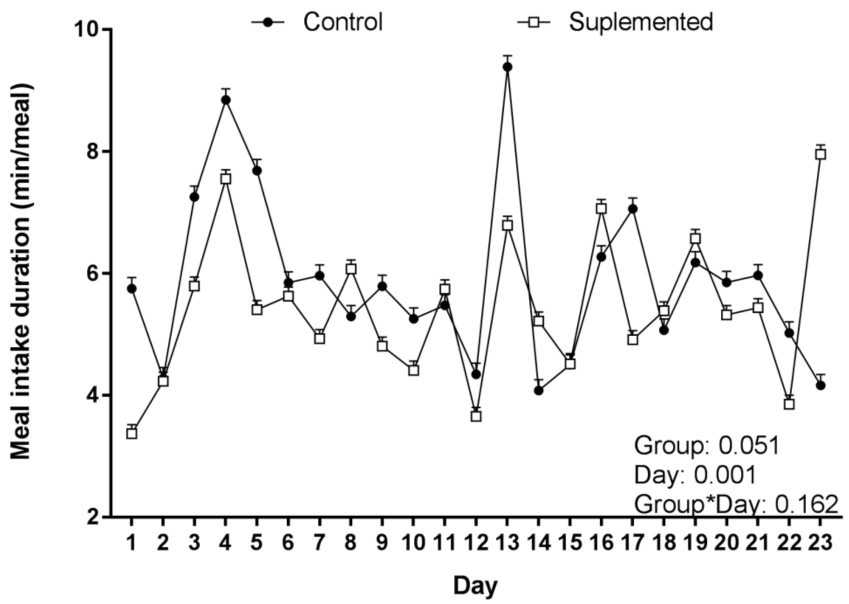
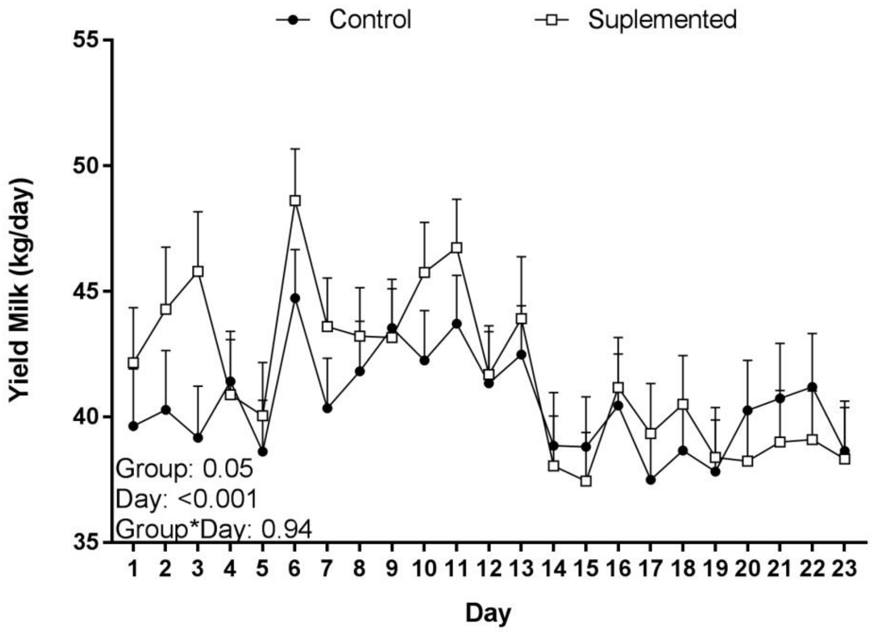


Figure 5: Effect of supplementation with 10g / cow / day of dry fungus fermentation product that expresses residual fibrolytic enzymatic activity on the meal intake duration of animals in the supplemented group (SG) (MAXFIBER®, Provita Supplements GmbH, Pinneberg / Germany) and Control (CG) (n = 36) assessed daily using Smart Feeders (Intergado®).



The yield milk was greater than SG when compared to CG (P = 0.05; Figure 6). The digestibility analysis was similar between the groups (P = 0.501), obtaining averages of 60,270 ± 4.99 and 62,014 ± 4.54 for the CG and SG, respectively. There was no difference between the groups regarding weight and BCS (P> 0.05).

Figure 6: Milk Yield of cows (n = 36) of the control group (GC) and of the supplementing group (GS) with 10g / cow / day of dry fungus fermentation product that expresses residual fibrolytic enzymatic activity (MAXFIBER®, Provita Supplements GmbH, Pinneberg / Germany).



4 DISCUSSION

When assessing animal behavior in the present study, we observed a tendency to reduce the rumination time (RT) of animals in SG and this may be related to a smaller size of diet particles, caused by the increase in endogenous microbiota and its enzymatic activity. The average percentage of long fiber (> 19mm) in this study was 11%. According to Kononoff et al (2003), the guidelines for TMR of high production dairy cows are from 2 to 8% of the particles in the upper sieve. Thus, this greater amount of long particles should have increased the RT, which did not happen in the current study, suggesting that the tendency to decrease rumination may have been affected by an effect of the dry fungus fermentation product with residual fibrolytic enzymatic activity on endogenous enzyme activity. Silva et al. (2016) found an increase in RT of cows that received fibrolytic enzymes in the diet due to the higher consumption of long fibers observed in the supplemented group. According to Mertens (1997) rumination is stimulated by the intake of physically effective fiber (feFDN) defined by a particle size greater than 8 mm. Krause et al. (2002) found a positive relationship between dry matter intake of long particles and RT, since more rumination time is needed to process long fiber as well as an increasing amount of food. The TR observed in the present study (SG = 619.78 min ± 5.42 and CG = 632.47 min ± 4.83) are within the standards verified by Soriani et al (2012) who found a range of 400 to 685 min / day of lactating cows.

Activity time (AT) indicates how much the animal has moved, but without specifying the type of action, some authors report that the decline in AT is associated with the presence of possible diseases, being used as an aid for early diagnosis (King et al, 2017). The average time spent on activity in the SG was 199.60 min, of which 121.56 min was for intake, with 78 min remaining for other activities. While in the CG, values of 183.70 minutes were found, where 50.77 min spent on activities when excluding intake time. It is important to note that this subdivision in leisure time into intake time and other activities is only possible due to the simultaneous use of troughs and collars, given that this has been little described in the literature until then.

The increase in activity time in the SG corroborates the higher intake frequency observed for this group when evaluated in the intelligent feeder systems. Kondratovich et al (2019) found no difference in the activity rate of beef cattle supplemented with EFE but observed an increase in rumination time justified by the increase in consumption verified in the experiment.

Idle time (IT) is defined by the time that the animal is not eating feed, ruminating, or drinking water. In the present study, IT was similar between groups and both remained within the average values reported by Carvalho (2009) of 600 min / animal/day for lactating cows in confinement systems, showing that the animals did not show signs of stress during the experimental period.

The increase in the intake frequency in the SG may be related to a faster rate of passage of the digest through the gastrointestinal tract, since we noticed an increase in the AT and a trend in the RT, suggesting that the animals needed less time to fractionate the particles of the diet and moved more often to the smart feeders, as there was also an increase in the frequency of access to the troughs for the SG. Also, the meal size was smaller for the SG, demonstrating that these animals ate more frequently and in smaller proportions, which corroborates with Devries (2019) when he emphasized that smaller and more frequent meals are beneficial to maximize fermentation ruminal since the higher frequency of feeding favors the buffering of the rumen and the activity of fibrolytic bacteria.

In the present study, supplementation with the fungal fermentation product with residual fibrolytic enzyme activity did not alter intake (19.34 ± 0.28 and 19.24 ± 0.30 for the CG and SG, respectively), which corroborates with Refat et al. (2018) that verified more access to the feeding troughs without changing intake. With EFE supplementation, an increase in DMI was expected, since the enzymes can increase the speed of fiber hydrolysis and digestion of nutrients, which in turn feeds the dry matter intake by allowing a higher rate of passage of digested nutrients through their beneficial effects on ruminal fermentation kinetics parameters (Elghandour et al., 2013; Kondratovich et al., 2019).

Contrary to the results of the present study, Clément et al (2014) found a higher intake, as well as a longer intake time in cows with high yield milk. In contrast, Holstshausen et al (2011) found a decrease in the DMI with the inclusion of enzyme products in the diets, speculating that it is due to the increased efficiency in the use of nutrients leading to the animals' satiety. He et al. (2015) found that feeding time increased when fibrolytic enzymes were added to the diet of beef heifers. In the current study, the use of the dry fungus fermentation product with residual fibrolytic enzyme activity increased the intake frequency as well as the frequency of access to troughs, demonstrating that the animals in the supplemented group became more active. Such findings are following the study by SILVA (2014), which found that the animals had more visits to the feeding troughs.

The DMI in ruminants can be regulated by the animal's energetic state on stimulation of the brain's satiety center (Allen et al., 2009). Although greater digestibility of nutrients can increase the rate of passage of food and promote the feed consumption , it also can provide greater amounts of oxidizable fuels for hepatocytes, resulting in an intake depression (GRANDA, et al. , 2017). Corroborating this, Allen (2000) when infusing propionate or acetate in the reticulum-rumen of lactating cows observed a reduction in DMI by 33% and 14%, respectively when compared to the control group.

With the possible increase in the microbiota and/or its enzymatic activity, we suggest that there may have been an increase in the synthesis of short-chain fatty acids (AGCC), providing greater energy available to the animal. This greater available energy sends signals to the satiety center in the hypothalamus, ceasing intake. This possible increase in energy availability caused by supplementation may have caused an increase in yield milk, a fact observed in the GS of the current study. Ratifying this speculation Oh et al. (2019) and Abid et al. (2019) observed an increase in AGCC synthesis with EFE supplementation, also suggesting that the occurrence of this fact is due to greater rumen enzymatic activity.

Besides, Gado (2009) found an increase of 3 kg of milk in cows supplemented with fibrolytic enzymes, speculating that it was due to increased digestibility, intake, as well as changes in the acetate/propionate ratio in the rumen, which increased the energy available for the production of milk.

The use of fibrolytic enzymes resulting from fungal fermentation in the solid-state (SSF), which has been used in human nutrition for many years, to increase its nutritional value, has the potential to reduce the level of concentrate in the diet of ruminants by reducing feed costs. due to the greater availability of nutrients, resulting from the improvement in rumen bacterial fermentation, as suggested by the general results found in the present study with supplementation with MAXFIBER®. However, there is still great variability in the answers about the effectiveness of enzymatic products that can be attributed to the animal's nutritional needs, lactation stage, diet composition, enzymatic activity, dose, and method of using the products (Ran et al., 2019).

5 CONCLUSIONS

Supplementation of a dry fungus fermentation product that expresses residual fibrolytic enzymatic activity for lactating cows increases the time spent for activity time, increases the intake frequency, as well as the frequency of access to feeders by cows, decreasing the size of meals and tending to decrease rumination time with positive effects on milk yield.

REFERENCES

ABID, K.; JABRIL, J.; BECKERS, Y.; YAICH, H.; MALEK, A.; REKHIS, J.; KAMOUN, M. Influence of adding fibrolytic enzymes on the ruminal fermentation of date palm by-products. **Archives Animal Breeding**, v.62, p.1-8, 2019. DOI: http://dx.doi.org/10.5194/aab-62-1-2019

ADESOGAN, A. T.; ARRIOLA, K.G.; JIANG, Y.; OYEBADE, A.; PAULA, E.M.; PECH-CERVANTES, A.A.; ROMERO, J.J. Symposium review: Technologies for improving fiber utilization. **Journal of Dairy Science**, v.102, p.1–30, 2019. DOI: http://dx.doi.org/10.3168/jds.2018-15334

ALLEN, M.S. Effects of diet on short-term regulation of feed intake by lactating dairy cattle**. Journal of Dairy Science**, v.83, p.1598–1624, 2000. DOI: http://dx.doi.org/10.3168/jds.S0022-0302(00)75030-2

ALLEN, M.S.; BRAFORD, B.J.; OBA, M. The hepatic oxidation theory of the control of feed intake and its application to ruminants**. Journal of Animal Science**, v.87, p.3317–3334, 2009. DOi: http://dx.doi.org/10.2527/jas.2009-1779

ARRIOLA, K.G.; KIM, S.C.; STAPLES, C.R.; ADESOGAN, A.T. Effect of fibrolytic enzyme application to low and high concentrate diets on the performance of lactating dairy cattle**. Journal of Dairy Science**, v.94, p.832–841, 2011. DOi: http://dx.doi.org/10.3168/jds.2010-3424

ARRIOLA, K.G.; OLIVEIRA, A.S.; MA, Z.X.; LEAN, I.J.; GIURCANU, M.C.; ADESOGAN, A.T. A meta-analysis on the effect os dietary application of exogenous fibrolitic enzymes on the performance of dairy cow. **Journal of Dairy Science**, v.100, p.1-1, 2016. DOI: http://dx.doi.org/10 .3168/jds .2016 -12103.

BEAUCHEMIN, K.A.; COLOMBATTO, D.; MORGAVI, D.P. Use of exogenous fibrolytic enzymes to improve animal feed utilization by ruminants. **Journal Animal of Science**, v.81, p.37-47, 2003. DOI: http://dx.doi.org/10.2527/2003.8114

BORCHERS, M.R.; CHANG, Y.M.; TSAI, I.C.; WADSWORTH, B.A.; BERLEY, J.M. A validation of technologies monitoring dairy cow feeding, ruminating, and lying behaviors. **Journal of Dairy Science**, v.99, p.7458–7466, 2016. DOI: http://dx.doi.org/10.3168/jds.2015-10843

CARVALHO, P.C.F.; ANGUINONI, I.; MORAES, A; SOUZA, E.D.; SULC, R.M.; LANG, C.R.; FLORES, J.P.C.; LOPES, M.L.T.; DILSA, J.L.S.; CONTE, O.; WESP, C.L.; LEVIEN, R.; FONTANELI, R.S.; BAYER, C. Managing grazing animals 6 to achieve nutrient cycling and soil improvement in no-till integrated systems. **Nutrient Cycling in Agroecosystems**, p.259–273, 2009. DOI: http://dx.doi.org/10.1007/s10705-010-9360-x

CLEMENT, P.; GUATTEO, R.; DELABY, L.; CHANVALLON, A.; PHILIPOT, J.M.; BAREILLE, N. Short communication: added value of rumination time for the prediction of dry matter intake in lactating dairy cows. **Journal of Dairy Science**, v.97, p.6531–6535, 2014. DOI: http://dx.doi.org/10.3168/jds.2013-7860

DEAN, D.B.; STAPLES, C.R.; LITTELL, R.C.; KIM, S.; ADESOGAN, A.T. Effect of method of adding a fibrolytic enzyme to dairy cow diets on feed intake digestibility, milk production, ruminal fermentation, and blood metabolite. **Animal Nutrition and Feed Technology**, v.13, p.337–357, 2013. ISSN: 0972-2963

DEVRIES, T. Feeding Behavior, Feed Space, and Bunk Design and Management for Adult Dairy Cattle. **Vet Clin Food Animal**, v.35, p.61–76, 2019. DOI: http://dx.doi.org/10.1016/j.cvfa.2018.10.003

ELGHANDOUR, M.M.Y.; SALEM, A.Z.M.; GONZALEZ-RONQUILLO, M.; BÓRQUEZ, J.L.; GADO, H.M.; ODONGO N.E.; PEÑUELAS, C.G. Effects of exogenous enzymes on in vitro gas production kinetics and ruminal fermentation of four fibrous feeds. **Animal Feed Science and Technology**, v.179, p.46-5, 2013. DOI: http://dx.doi.org/10.1016/j.anifeedsci.2012.11.010

GADO, H.M.; SALEM, A.Z.M.; ROBINSON, P.H.; HASSAN, M. Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk prodution and composition in dairy cows. **Animal Feed Science and Tecnology**, p.36-46, 2009. DOI: http://dx.doi.org/10.1016/j.anifeedsci.2009.07.006

HE, Z.X.; WALKER, N.D.; MCALLISTER, T.A.; YANG, W.Z. Effect of wheat dried distillers grains with solubles and fibrolytic enzymes on ruminal fermentation, digestibility, growth performance, and feeding behavior of beef cattle. **Journal of Animal Science**, v.93, p.1218–1228, 2015. DOI: http://dx.doi.org/10.2527/jas2014-8412

HOLTSHAUSEN, L.; CHUCH, Y.H.; GERARDO-CUERVO, H.; OBA, M.; BEAUCHEMIN, K.A. Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. **Journal of Dairy Science**, v.94, p.899–907, 2011. Doi: http://dx.doi.org/10.3168/jds.2010-3573

JOHNSTON, C.; DEVRIES, T.J. Short communication: Associations of feeding behavior and milk production in dairy cows. **Journal of Dairy Science**, v.101, p.3367–3373, 2018. DOI: http://dx.doi.org/10.3168/jds.2017-13743

KING, M.T.M.; DANCY, K.M.; LE BLANC, S.J.; PAJOR, E.A.; DEVRIES, T.J. Deviations in behavior and productivity data before diagnosis of health disorders in cows milked with an automated system. **Journal of Dairy Science**, v.100, p.1–14, 2017. DOI: http://dx.doi.org/10.3168/jds.2017-12723

KONONOFF, P.J.; HEINRICHS, A.J.; BUCKMASTER, D.R. Modification of the Penn State Particle Separator and the effects of moisture on its measurements. **Journal of Dairy Science**, v.86, p.1858–1863, 2003. DOI: http://dx.doi.org/10.3168/jds.S0022-0302(03)73773-4

KRAUSE, K.M.; COMBS, D.K.; BEAUCHEMIN, K.A. Effects of forage particle size and grain fermentability in midlactation cows. II. Ruminal pH and chewing activity. **Journal of Dairy Science**, v.85, p.1947–1957, 2002. DOI: http://dx.doi.org/10.3168/jds.S0022-0302(02)74271-9

KONDRATOVICH, L.B.; SARTURI, J.O.; HOFFMANN, C.A.; BALLOU, M.A.; TROJAN, S.J.; CAMPANILI, P.R.B. Effects of dietary exogenous fibrolytic enzymes on ruminal fermentation characteristics of beef steers fed high- and low-quality growing diets. **Journal of Animal Science**, v.97, p.3089–3102, 2019. DOI: http://dx.doi.org/10.1093/jas/skz165

MERTENS, D.R. Creating a system for meeting the requirements of dairy cows. **Journal of Dairy Science**, v.80, p.1463–1481, 1997. DOI: http://dx.doi.org/10.3168/jds.S0022-0302(97)76075-2

OH, J.; HARPER, M.; MELGAR, A.; COMPART, DMP.; HRISTOV, A.N. Effects of Saccharomyces cerevisiae-based direct-fed microbial and exogenous enzyme products on enteric methane emission and productivity in lactating dairy cows. **Journal of dairy science**, v.102, p.6065–6075, 2019. DOI: http://dx.doi.org/10.3168/jds.2018-15753

PETERS, A.; MEYER, U.; DANICKE, S. Effect of exogenous fibrolytic enzymes on performance and blood profile in early and mid-lactation cows. **Animal Nutrition**, v.1, p.229–238. 2015. DOI: http://dx.doi.org/ 0.1016/j.aninu.2015.09.001

RAN, T.; SALEEM, A.M.; SHEN, Y.; RIBEIRO, G.O.; BEAUCHEMIN, K.A.; TSANG, A.; YANG, W.; MCALLISTER, T.A. Effects of a recombinant fibrolytic enzyme on fiber digestion, ruminal fermentation, nitrogen balance, and total tract digestibility of heifers fed a high forage diet. **Journal of Animal Science**, v.97, p.3578-3587, 2019. DOI: http://dx.doi.org/10.1093/jas/skz216

REFAT, B.; CHRISTENSEN, D.A.; MCKINNON, J.J.; YANG, W.; BEATTIE, A.D.; MCALLISTER, T.A.; EUN, J.; ABDEL-RAHMAN, G.A.; YU, P. Effect of fibrolytic enzymes on lactational performance, feeding behavior, and digestibility in high-producing dairy cows fed a barley silage–based diet. **Journal of Dairy Science**, v.101, p.1-9, 2018. DOI: http://dx.doi/org/ 10.3168/jds.2017-14203

ROBLES, V.; GONZÁLEZ, L.A.; FERRET, A.; MANTECA, X.; CALSAMIGLIA, S. Effects of feeding frequency on intake, ruminal fermentation, and feeding behavior in heifers fed high-concentrate diets. **Journal of Animal Science**, v.85, p.2538–2547, 2007. DOI: http://dx.doi.org/10.2527/jas.2006-739

SILVA, T.H.; TAKIYA, C.S.; VENDRAMINI, T.H.A.; JESUS, E.F.; ZANFERARI, F.; RENNÓ, F.P. Effects of dietary fibrolytic enzymes on chewing time, ruminal fermentation, and performance of mid-lactating dairy cows. **Journal Animal Feed Science and Technology**, v.221, p.35-43, 2016. DOI: http://dx.doi.org/10.1016/j.anifeedsci.2016.08.013

SILVA, J.; CARRARA, T.V.B.; PEREIRA, M.C.S.; OLIVEIRA, C.A.; JÚNIOR, I.C.B.; WATANABE, D.H.M.W.; RIGUEIRO, A.L.N.; ARRIGONI, M.B.; MILLEN, D.D. Feedlot performance, feeding behavior and rumen morphometrics of Nellore cattle submitted to different feeding frequencies. **Scientia Agricola**, v.75, p.121-128, 2018. DOI: http://dx.doi.org/10.1590/1678-992x-2016-0335

SORIANI, N.; TREVISI, E.; CALAMAR, L. Relationships between rumination time, metabolic conditions, and health status in dairy cows during the transition period. **Journal**

of Dairy Science, v.90, p.4544–4554, 2012. DOI: http://dx.doi.org/10.2527/jas.2012-5064

SUJANI, S.; SERESINHE, R.T. Exogenous enzymes in ruminant nutrition: A review. **Asian Journal of Animal Science**, v.93, p.85-99, 2015. DOI: http://dx.doi.org/10 .3923/ajas .2015 .85 .99

STEENSELS, M.; MALTZ, E.; BAHR, C.; BERCKMANS, D. Towards practical application of sensors for monitoring animal health: the effect of post-calving health problems on rumination duration, activity and milk yield. **Journal of Dairy Research**, v.84, p,132–138, 2017. DOI: http://dx.doi.org/10.1017/S0022029917000176

USDA-UNITED STATES DEPARTMENT OF AGRICULTURE. Agricultural Statistics, 2017.

TILLEY, J.M.A.; TERRY, R.A. A two-stage technique for the in vitro digestion of forage crop. **Journal British Grassland Society**, v.18, p.104-111, 1963. DOI: http://dx.doi.org/10.1111/j.1365-2494.1963.tb00335

VAN SOEST, P.J.; ROBERTSON, J.B.; LEWIS, B.A. Methods for dietary fiber, neutral detergent fiber, and no starch polysaccharides in relation to animal nutrition**. Journal of Dairy Science**, v.74, p.3583-3597, 1991. DOI: http://dx.doi.org/10 .3168/ jds .S0022 -0302(91)78551 -2

ZILIO, E.M.C.; DEL VALLE, T.A.; GHIZZI, L.G.; TAKIYA, C.S.; DIAS, M.S.S.; NUNES, A.T.; SILVA, G.G.; RENNÓ, F.P. Effects of exogenous fibrolytic and amylolytic enzymes on ruminal fermentation and performance of mid-lactation dairy cows. **Journal of Dairy Science**, v.102, p.1–11, 2019. DOI: http://dx.doi.org/10.3168/jds.2018-14949