



Ingestive behavior of dairy goats fed diets containing increasing levels of neutral detergent fiber and particle size using multivariate analysis

Erica Beatriz Schultz^{1*}, Rafael Marzall do Amaral², Leonardo Siqueira Glória³, Fabyano Fonseca e Silva¹, Marcelo Teixeira Rodrigues¹ and Ricardo Augusto Mendonça Vieira³

¹Departamento de Zootecnia, Universidade Federal de Viçosa, Avenida Peter Henry Rolfs, s/n, 36570-900, Viçosa, Minas Gerais, Brasil. ²EARTH University, San José, Costa Rica. ³Laboratório de Zootecnia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, Brasil.
*Author for correspondence. E-mail: ericabeatrizschultz@gmail.com

ABSTRACT. The purpose of this study was to evaluate the effect of interaction between increasing neutral detergent fiber content and particle sizes on ingestive behavior of dairy goats. Forty-eight lactating, multiparous Saanen and Alpine goats, with average milk production of 1.4 ± 0.57 kg d⁻¹, around 60th ± 12 day of lactation were distributed in a 3 x 4 factorial completely randomized design. The diets consisted: three particles sizes (02, 05 or 15 cm) and four levels of neutral detergent fiber (34, 41, 49 or 57% NDF_f) from forage (Tifton 85 hay). The ingestive behavior was monitored during 24 hours. A regression and a multivariate time series cluster analysis were performed. No interaction was found ($p > 0.05$) between treatments. Feeding time was different according to the particle size, having an increasing linear effect. Rumination and idle times were not affected ($p > 0.05$). The temporal feeding behavior was clustered into two groups according to the profile of particle size of the diet. Rumination peaks were randomly distributed with more intense activity before morning and afternoon meals. The increase in NDF_f content in the diet did not change the ingestive behavior. The multivariate cluster analysis in a time series data is useful to interpret animal feeding behavior.

Keywords: animal behavior; feed intake; rumination.

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Introduction

Large amounts of fiber limits the feed intake and, at the same time, the energy intake (Mertens, 1987), however minimum amounts of physically effective fiber are necessary to stimulate rumination and prevent metabolic disorders (i.e. ruminal acidosis) (Mertens, 1997). Fiber is one of the main components that is directly related to diet digestibility and passage rates, which has influence on the gastrointestinal tract filling and feed intake capacity. Thus, affecting the overall use of feed nutrients and animal performance.

Studies have been proposed to evaluate ingestive behavior, including feeding, rumination and idle related to chemical and physical properties of fiber, for example, the effect of different fiber particle sizes NDF_{pe} in small ruminants (Gomes et al., 2012; Jang et al., 2017) and different NDF concentrations in heifers (Oh et al., 2016), and both variables evaluated in dairy cows (Yang & Beauchemin, 2007).

However, analysis of variance and regression are commonly used to evaluate ingestive behavior; however, understanding a time series data on a mean implies loss of information about behavior pattern belonging to a circadian cycle. Thus, another method has been proposed to assess feeding behavior on ruminants with observations taken sequentially during a 24-hour period composing a circadian pattern (Roland et al., 2018; Ruuska, Kajava, Mughal, Zehner, & Mononen, 2016). This approach using time series data have been used to understand the relationship between different types of activities, states and causes of ingestive behavior (Fischer, Dutilleul, Deswysen, Dèspres, & Lobato, 2000) and their relationship with animal performance.

In view of the knowledge and relevance of the concentration effect and the physical action of the fiber on ruminant nutrition, this study aimed to investigate the ingestive behavior of dairy goats receiving diets with increasing levels of neutral detergent fiber and particle sizes from forage, using a multivariate time series cluster analysis.

Material and methods

Forty-eight adult lactating goats, 22 Saanen animals and 26 Alpine animals, multiparous, with 51.1 ± 9 kg body weight (BW), and average milk production of $1.4 \text{ kg d}^{-1} \pm 0.57 \text{ kg d}^{-1}$, around $60 \pm 12^{\text{th}}$ days on milk were used in this trial. There was sixteen days for adaptation, during a 96-day evaluation. The animals were randomly distributed and kept in individual stalls of 3 m^2 , with slatted wooden floor, feeder and water fountain. The goats were fed twice a day (07:00 and 16:00 h) and had access to feed and water ad libitum.

A 3×4 factorial completely randomized design was used, with the combination of three lengths of particle size (2, 5 or 15 cm), four levels of neutral detergent fiber (NDFf; 34, 41, 49 or 57%) from forage (Tifton 85 hay), with four replicates per treatment.

Forage: concentrate ratio varied according to the level of NDFf required for the experimental diets, thus, influencing the chemical composition of the experimental diets (Table 1). The dry matter, crude protein, ether extract contents were determined using the methodology described by Silva and Queiroz (2002), and fiber fraction analysis according to Van Soest, Robertson, and Lewis (1991). Estimated metabolizable and net energy values were made according to Agricultural and Food Research Council (AFRC, 1993).

To obtain the desired hay particle sizes, different grinding processes were conducted. Large-particle hay (15 cm) was obtained in a silage chopper with knives and counter-knives system (Nogueira – Model EM 6600®). Medium-particle hay (5 cm) was obtained using a hay disintegrator (Massey Ferguson – Model MF 4970®), without sieves. Small-particle hay (2 cm) was obtained with the same equipment used to obtain the large-particle hay and then passed on a hammer mill (Nogueira – Model DPM-4®).

In order to characterize the particle size profile of the supplied diet and leftovers, standard sieves (Smith & Waldo, 1969) were used with openings of 4.76, 2.38, 1.19, 1.00, 0.71 and 0.297 mm. It was calculated the particle size percentages of the fiber retained ≥ 1.19 mm as the sum of fiber retained in the sieve 4.76, 2.38 and 1.19 mm, these fractions were called physically effective fiber (≥ 1.19 mm). The particle size percentages of the fiber retained < 1.19 mm were calculated as: $100\% - \geq 1.19\text{mm}$ (%), these fractions were called no physically effective fiber fractions (< 1.19 mm). The peNDF (physically effective neutral detergent fiber) was calculated by multiplying neutral detergent fiber (NDF) content of the diet by $\text{pef}_{1.18\text{mm}}$ (Mertens, 1997).

To evaluate the feeding behavior without interference of human presence, a monitoring system with camcorders connected to a computer software (Geovision, model GV800®) was installed to uninterruptedly record animal behavior (two animals per camera). Through the video images, it was observed and counted: feeding, ruminating and idle every five minutes through 24 hours during one day at the end of experimental period. In order to characterize the animal behavior of the circadian period, average of the repetitions was made at each observation, which is, the average time spent in the activities, in seconds. One goat, from particle size 2 cm and 34% NDFf treatment, was identified as outlier, presenting an atypical behavior during the experiment, and it was removed from dataset.

Table 1. Proportion of the ingredients and chemical composition of the experimental diets.

Item	NDFf Content (%DM)			
	34	41	49	57
Ingredients (g kg ⁻¹)				
Tifton 85 hay	55.74	67.21	80.32	93.44
Ground corn	34.13	24.94	14.70	4.39
Soybean meal	8.24	5.97	3.20	0.44
Dicalcium phosphate	0.70	0.75	0.71	0.75
Limestone	0.49	0.38	0.36	0.23
Mineral mix ^a	0.70	0.75	0.71	0.75
Diet composition (g kg ⁻¹ DM)				
Dry matter	882.66	884.33	884.40	883.48
Crude protein	153.00	151.04	155.81	148.52
Ether extract	21.59	15.39	13.94	12.72
Total carbohydrates	746.38	746.69	730.71	732.81
Non-fiber carbohydrates	339.86	287.03	201.66	145.75
Neutral detergent fiber	461.85	523.56	605.63	670.31
Neutral detergent fiber from forage	347.05	418.50	500.16	581.82
Estimated energy values				
Metabolizable energy (Mcal.kg ⁻¹)	2.50	2.46	2.24	2.09
Net energy (Mcal.kg ⁻¹)	1.50	1.48	1.36	1.28

^a Mineral mix: 0.32% ferrous sulfate; 0.48% copper sulfate; 0.71% manganese sulfate; 2.67% zinc sulfate; 0.02% cobalt sulfate; 0.0125% potassium iodate; 0.006% sodium selenite; 95.78% sodium chloride.

Total time spent on ingestive activities were assessed using regression analysis, according to the statistic model: $Y_{ij} = \mu + P_i + F_j + P \times F_{ij} + \varepsilon_{ij}$, where: Y_{ij} = observation; μ = overall mean; P_i = fixed effect of particle size i ; F_j = levels of neutral detergent fiber from forage j ; $P \times F_{ij}$ = interaction effect of particle size i and levels of neutral detergent fiber j ; ε_{ij} = random error.

To assess behavioral patterns, a multivariate time series cluster analysis was applied. Time series were sequential observations over time, which form an object. The objects were used to describe temporal distribution of activities containing more information than usual methods with cumulative average time. The clustering analysis of time series objects allow group formation based on their dissimilarities. The cluster method used was the Euclidean distance to dissimilarity measures and agglomerative hierarchical average to create the groups, using *pvc* package (Suzuki & Shimodaira, 2006). The number of clusters were determined according to resampling technique (Suzuki & Shimodaira, 2006) (bootstrap) in 10,000 times, generating unbiased approximation p-values (AU) with the *nboot* function. These p-values were used to determine the accuracy of the clusters within the database adopting for dissimilarity between the clusters $\alpha \leq 0.05$. Statistical analyses were performed using the R software (R-Core-Team, 2016).

Results and discussion

The percentages of physically effective fiber (≥ 1.19 mm) in the offered feed and leftovers presented different proportions (Figure 1), suggesting feed selection.

As particle sizes increased, the physically effective fiber fractions (≥ 1.19 mm) in the offered diets increased as well, moreover, as particle sizes increased in leftovers, the percentages of physically effective fiber also increased (Figure 1). Treatments with the particle size of 2 cm presented a higher proportion of no physically effective fiber (< 1.19 mm) in leftovers than in the offered feed. Treatments with particle size of 15 cm, with 34, 41 and 49% of NDFf had the opposite situation, showing that animals select small particles. The goats are classified as intermediate selective feeders that can distinguish between bitter, sweet, salty and sour tastes (Hofmann, 1989). In a free-choice situation when a more concentrated diet is supplied, selection does not always maximize energy; ruminants eat some straw to prevent rumen disorders. Goats seek herbage species relatively low in protein but rich in fiber, it is likely that animals select vegetation to reduce the variation on ingesting composition, as far as possible, in the face of large seasonal variations or diet composition (Baumont, Prache, Meuret, & Morand-Fehr, 2000).

There was no interaction between particle size and NDFf level on ingestive activities. For ingestive behavior, only particle size for the feeding activity was significant ($p \leq 0.05$) (Table 2).

As particle sizes increased, the total feeding time (min day^{-1}) increased with a linear effect, besides, there were no effects when NDFf increased in the diet (Table 3). Yang and Beauchemin (2007) working with four particle sizes for dairy cows and found an increase in feeding time with increasing particle size; the authors justify this behavior as a result of chewing before swallowing to promote particle reduction.

The increase in feeding time can also be related to increasing peNDF content of the diets. The study showed that peNDF content raised, as the particle size and the NDFf content increased (Table 4). According to Li et al. (2014), the increase from low peNDF (18.7%) to high peNDF (24.3%) in the diet for goats led to an increase from 76 to 196 (min day^{-1}) time spent in feeding. Although values of feeding time were lower than found in this study, the amount of peNDF in the treatments and the change in the feeding behavior is explained by raising the dietary forage particle length that decreases surface area available for microbial attachment and forage digestibility, consequently, animals spend more time feeding to meet nutrient requirements.

The rumination and idle time did not differ between different particle sizes and NDFf levels (Table 3). Differently, the increase in NDFf contents was significant on idle and rumination behavior for some authors (Branco et al., 2011; Sousa et al., 2018). According to Branco et al. (2011) and Sousa et al. (2018), increased NDFf contents caused no change in the feeding activity, but increased rumination, because this behavior is related in the processing of dietary fiber to pass through the digestive tract of ruminants. However, we demonstrated that there was a selective behavior in the feeding activity, and this may have removed, among the treatments, the need to increase fiber processing through rumination when the NDFf content and the particle size is increased.

The clustering analysis for feeding activity was significant ($p \leq 0.05$); generating two clusters. The first grouping consisted of 34, 41 and 49% NDFf levels, with 2 cm particle size and 34% NDFf, with 5 cm particle size, these groups spent less time on meals. The second group was composed of the other treatments: 57% NDFf, with 2 cm particle size, 41, 49 and 57% NDFf, with 5 cm particle size and 34, 41, 49 and 57% NDFf, with 15 cm particle size, which had longstanding meals.

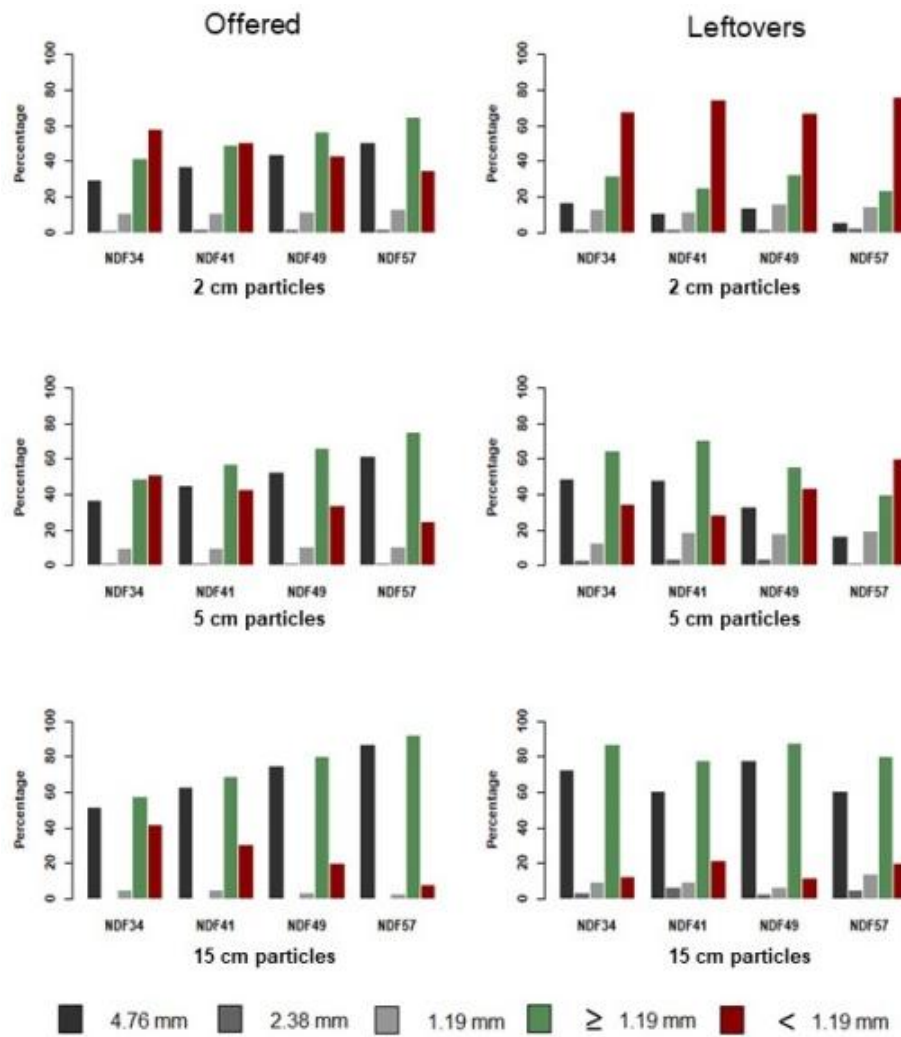


Figure 1. Particle size profile of offered feed and leftovers of the experimental diets.

Table 2. P-values of analyzed variables in relation to particle size and NDFf content (%DM) and their respective interaction.

Item	Factors			Interactions (Particle size x NDFf Content (%DM))
	Particle size	NDFf Content (%DM)		
Feeding (min day ⁻¹)	<.005	0.809		0.783
Rumination (min day ⁻¹)	0.259	0.504		0.792
Idle (min day ⁻¹)	0.978	0.531		0.762

NDFf: neutral detergent fiber from forage; DM: dry matter.

Table 3. Time spent in ingestive behavior with different particle sizes and concentrations of neutral detergent fiber from forage.

Item	Particle size			SEM	p-value	
	2 cm	5 cm	15 cm		L	Q
Time (min day ⁻¹)						
Feeding ¹	250.49	296.44	312.41	74.42	<0.023	0.526
Rumination	451.49	400.65	395.56	99.24	0.118	0.467
Idle	734.77	739.27	727.39	152.19	0.891	0.865
	NDFf Content (%DM)					
	34%	41%	49%	57%		
Time (min day ⁻¹)						
Feeding	270.07	279.53	294.09	299.91	74.42	0.316
Rumination	379.73	409.46	437.62	434.54	99.24	0.154
Idle	787.40	746.11	704.70	701.04	152.19	0.134

NDFf: neutral detergent forage from fiber; DM: dry matter; SEM: Standard error of the mean; L: linear effect; Q: quadratic effect; P – value with 0.05 significance ($\alpha \leq 0.05$). ¹Y = 256.729 + 3.997x.

Table 4. peNDF (%) content on the experimental diets with increasing levels of neutral detergent fiber and particle size.

Item	NDFf Content (%DM)			
	34%	41%	49%	57%
Particle size				
2cm	19.32	25.48	34.2	43.55
5cm	22.54	29.64	39.6	50.25
15cm	26.68	35.88	48	61.64

peNDF: physically effective neutral detergent fiber; NDFf: neutral detergent fiber from forage; DM: dry matter.

The feeding pattern (Figure 2) was consistent with the description of Baumont et al. (2000). These authors described that 60 to 80% of daily intake occurs during the two main meals, with highest intake at the beginning of the meal then starts to decrease continuously until satiation. The control of short-term feeding behavior, within a day, is influenced by the stimuli of the center of hunger and satiety and the physical effects of filling, generating the reduction in intake (Allen, 2000). The stimulus at the beginning of meal times, 07:00 and 16:00 h, present a sustained peak of feeding pattern on treatments composed of medium and large particles (5 and 15 cm) and acute peaks in animals consuming small particles (2mm), as a result of selective and chewing actions, these behaviors were clustered into two groups ($p < 0.05$).

Meal frequency reduces approximately two hours after the feeding time, however, short time meals are conducted during the day. According to Baumont, Malbert, and Ruckebusch (1990), when rumen fill is increased with indigestible material, animals increase the number of meals.

Rumination pattern followed a random distribution between the meals. This result can be justified by the NDFpe content of the diets (Table 4). According to Mertens (1997), a minimum requirement of NDFpe for dairy cows was determined to be 22% of ration on a DM basis to maintain ruminal health within a 6.0 pH. For dairy goats, diets with 20.4% to 20.8% did not present an apparent risk of causing subcutaneous ruminal acidosis (Zhao, Zhang, Xu, & Yao, 2011). However, in the experimental diet with smaller particles and NDFf, the peNDF accounted for 19.3%, which means, in this study, the minimum NDFpe fiber to maintain the same rumination patterns between treatments.

The distribution of rumination activity was uniform between treatments ($p > 0.05$) and showed peaks few hours before the next meal and not just after the meal (Figure 3). This effect is related to solubilization and lag phenomenon, which is the time required for comminution and microbial colonization (Mertens, 1977; Sauvant & Noziere, 2016).

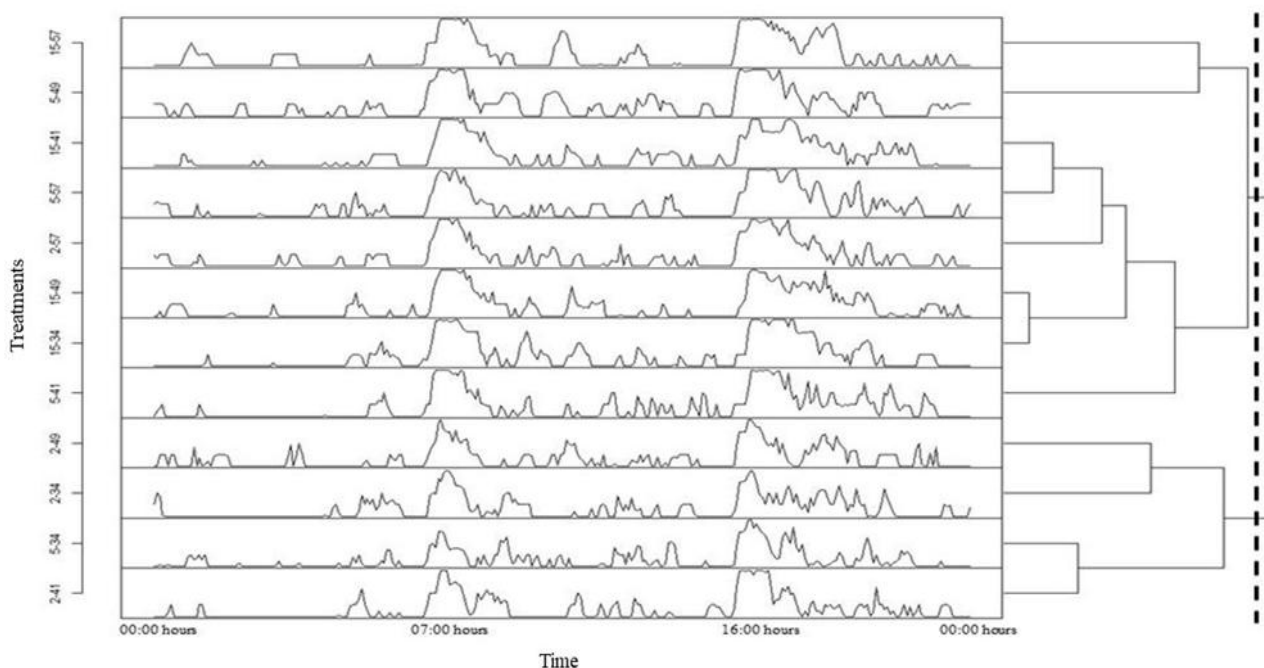


Figure 2. Dendrogram of temporal distribution patterns of feeding activity for 34%, 41%, 49% and 57% levels of NDFf combined with 2, 5 and 15 cm of particle size. The dashed line represents the dendrogram cutoff level of significance with 0.05 P – value (AU).

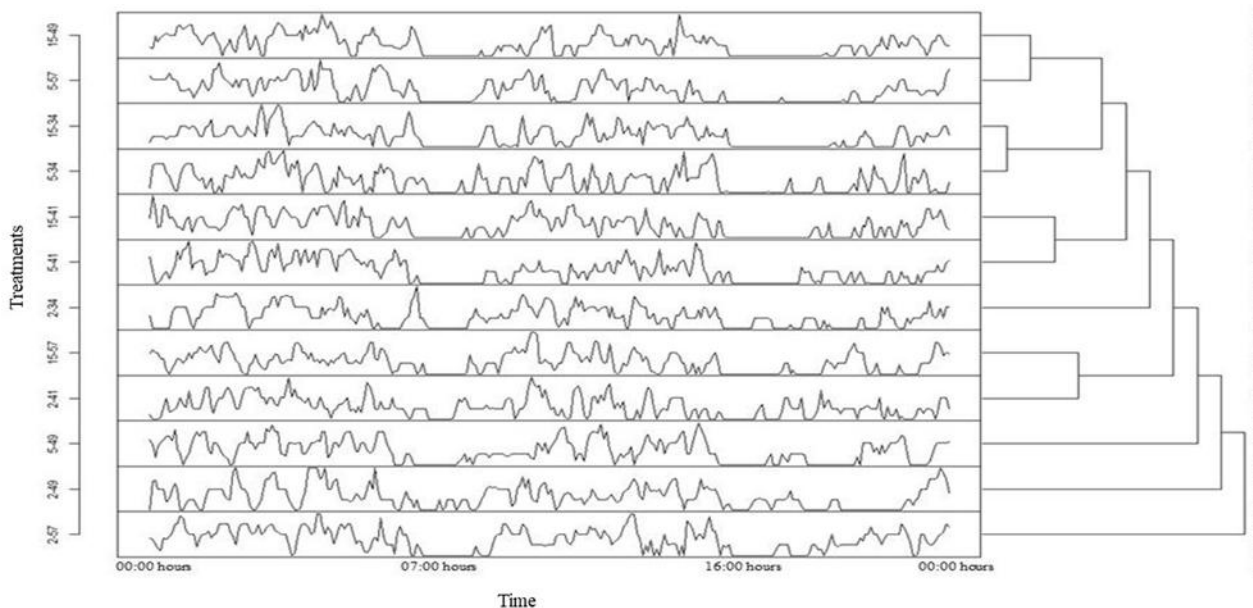


Figure 3. Dendrograms of temporal distribution patterns of rumination activity for 34%, 41%, 49% and 57% levels of NDFf combined with 2, 5 and 15 cm of particle size. The dashed line represents the dendrogram cutoff level of significance with 0.05 P – value (AU).

Temporal analysis of idle activity presented peaks just before the meals and it is a direct antagonist of feeding activity (Figure 4). This random time distribution for idle activity was also observed in dairy cows regardless of the frequency of offered feed (DeVries, von Keyserlingk, & Beauchemin, 2005).

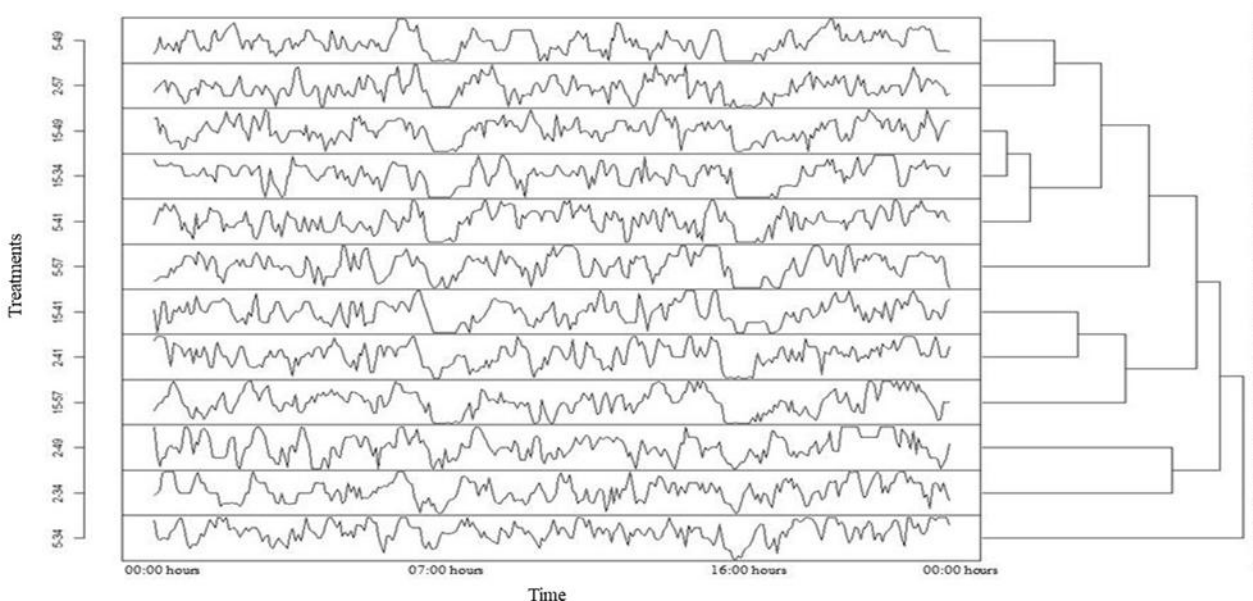


Figure 4. Dendrograms of temporal distribution patterns of idle activity for 34%, 41%, 49% and 57% levels of NDFf combined with 2, 5 and 15 cm of particle size. The dashed line represents the dendrogram cutoff level of significance with 0.05 P – value (AU).

The time series cluster analysis has been demonstrated as a tool to help animal science researchers to identify patterns in the ingestive behavior of dairy goats. Time series clustering is the most used approach as an exploratory technique, the clustering of complex objects is particularly advantageous because it leads to the discovery of interesting patterns in time series datasets (Aghabozorgi, Shirkhorshidi, & Wah, 2015).

Conclusion

As particle sizes increased from 2 cm to 15 cm in the goat diet, mean of feeding time and feeding distribution patterns also increased, nevertheless rumination and idle activities did not change. The increase in NDFf content from 34% to 57% did not influence feeding, ruminating and idle activities.

Temporal distribution utilizing clustering analysis of time series showed consistent results and can be used in other feeding behavior researches.

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