# Has the soybean oil supplementation effect on milk production, feed efficiency, and grazing behavior of Girolando cows under moderate heat stress in western Amazonia?

A suplementação com óleo de soja afeta a produção de leite, eficiência alimentar e comportamento de pastejo de vacas Girolando sob estresse térmico moderado na Amazônia Ocidental? ¿Hay efecto de la suplementación con aceite de soya sobre la producción de leche, la eficiencia alimenticia y el comportamiento de pastoreo de vacas Girolando bajo estrés por calor moderado en la Amazonía occidental?

Received: 09/22/2022 | Revised: 10/19/2022 | Accepted: 10/22/2022 | Published: 10/27/2022

### Ana Karina Dias Salman

ORCID: https://orcid.org/0000-0001-6594-0667 Empresa Brasileira de Pesquisa Agropecuária, Brasil E-mail: ana.salman@embrapa.br Elaine Coimbra de Souza ORCID: https://orcid.org/0000-0002-4839-9298 Fundação Universidade Federal de Rondônia, Brasil E-mail: lainezootec@gmail.com

Giovanna Araújo de Carvalho ORCID: https://orcid.org/0000-0001-5475-0569 Fundação Universidade Federal de Rondônia, Brasil E-mail: giovanna.carvalhozootec@gmail.com

Francyelle Ruana Faria da Silva

ORCID: https://orcid.org/0000-0002-6385-9651 Fundação Universidade Federal de Rondônia, Brasil E-mail: franruana@gmail.com

### Jéssica Halfen

ORCID: https://orcid.org/0000-0002-1317-1322 Postdoctoral Research Associate at Virginia Tech, Estados Unidos E-mail: halfenzootecnista@gmail.com

Edilena Adalgiza Artiaga de Santiago

ORCID: https://orcid.org/0000-0001-9343-8885 Empresa de Assistência Técnica e Extensão Rural do Estado de Rondônia, Brasil E-mail: edilenasantiago@globomail.com

Pedro Gomes da Cruz

ORCID: https://orcid.org/0000-0002-6491-9900 Empresa Brasileira de Pesquisa Agropecuária, Brasil E-mail: pedro-gomes.cruz@embrapa.com

Eduardo Schmitt

ORCID: https://orcid.org/0000-0003-1900-3968 Federal University of Pelotas, Brasil E-mail: schmittedu@gmail.com

### Abstract

The effect of soybean oil supplementation in the diet of early lactating Girolando cows under moderate heat stress were studied in a grazing trial. Dry matter intake (DMI), milk yield, feed efficiency, and grazing behavior (during daytime and nighttime) were evaluated in two groups of eight crossbred Girolando (Holstein × Gyr) cows grazing palisade grass (*Urochloa brizantha* syn. *Brachiaria brizantha*) with initial means of  $53\pm33$  days in lactation and  $22.6\pm3$  kg of milk day<sup>-1</sup>. They were arranged in a  $2\times2$  crossover trial with two supplements (with or without soybean oil), two genetic groups and two periods. During the experimental period, the mean of temperature and humidity index (THI) was 75.11, suggesting moderate heat stress. Total DMI was based on fecal production estimated by LIPE® and the diet *in vitro* DM digestibility (IVDMD). Grazing behavior was assessed for 48 hours by acoustic data collected with MP3 recorders. Soybean oil supplementation had no significant effects on all variables evaluated in this study. Averages of individual milk yield and total DMI were 19.26 kg day<sup>-1</sup> and 15.32 kg DM day<sup>-1</sup>, respectively.

Under moderate heat stress, oil supplementation did not change grazing behavior, dry matter intake, or milk yield in early lactating Girolando cows.

Keywords: Feeding behavior; Consumption; Lipid supplementation.

# Resumo

O efeito da inclusão do óleo de soja na dieta de vacas Girolando no início da lactação sob estresse calórico moderado foram estudados em um ensaio de pastejo. A ingestão de matéria seca (IMS), produção de leite (PL), eficiência alimentar e comportamento em pastejo (durante o dia e à noite) foram avaliados em oito vacas Girolando (Holandês x Gir) pastejando capim-Marandu (*Urochloa brizantha* syn. *Brachiaria brizantha*) com médias iniciais de 53±33 dias em lactação e 22,6±3 kg de leite dia<sup>-1</sup>. Os animais foram distribuídos em delineamento *crossover* 2×2 duas suplementações (com e sem óleo de soja) e dois períodos. Durante o período experimental, o índice de temperatura e umidade (ITU) médio foi de 75,11, sugerindo que os animais estavam sob estresse moderado. A IMS total foi calculada pela relação entre a produção fecal estimada com LIPE<sup>®</sup> e a digestibilidade *in vitro* da matéria seca (DIVMS) da dieta. O comportamento em pastejo foi avaliado por 48 horas a partir de áudios obtidos com gravadores MP3. Não foi observado efeito da inclusão do óleo de soja sobre as variáveis consideradas nesse estudo. Médias da PL e da IMS total foram 19,26 kg day<sup>-1</sup> e 15,32 kg DM day<sup>-1</sup>, respectivamente. Sob estresse calórico moderado, a suplementação com óleo de soja não altera a ingestão de matéria seca (IMS), a produção de leite (PL), a eficiência alimentar e o comportamento em pastejo de vacas Girolando lactantes no terço inicial da lactação. **Palavras-chave:** Comportamento alimentar; Consumo; Suplementação lipídica.

# Resumen

El efecto de la suplementación con aceite de soja en la dieta de vacas lactantes bajo estrés por calor moderado se estudió en un ensayo de pastoreo. Se evaluó el consumo de materia seca (CMS), la producción de leche, la eficiencia alimenticia y el comportamiento de pastoreo (durante el día y la noche) en dos grupos de ocho vacas cruzadas Girolando (Holstein × Gyr) en pastoreo de pasto empalizada (*Urochloa brizantha* syn. *Brachiaria brizantha*) con medias iniciales de 53±33 días en lactancia y 22,6±3 kg de leche día<sup>-1</sup>. Se organizaron en un ensayo cruzado 2×2 con dos suplementos (con o sin aceite de soja) y dos períodos. Durante el período experimental, la media del índice de temperatura y humedad (ITH) fue de 75,11, lo que sugiere un estrés por calor moderado. El CMS total se basó en la producción fecal estimada por LIPE® y la digestibilidad *in vitro* de la MS de la dieta (DIVMS). El comportamiento de pastoreo se evaluó durante 48 horas mediante datos acústicos recopilados con grabadoras MP3. La suplementación con aceite de soya no tuvo efectos significativos en todas las variables evaluadas en este estudio. Los promedios de producción de leche individual y CMS total fueron 19,26 kg día<sup>-1</sup> y 15,32 kg MS día<sup>-1</sup>, respectivamente. Bajo estrés por calor moderado, la suplementación con aceite no cambió el comportamiento de pastoreo, el consumo de materia seca o la producción de leche en vacas Girolando lactantes en el periodo inicial de la lactación. **Palabras clave:** Conducta alimentaria; Consumo; Suplementación lipídica.

r alabras clave: Conducta annientaria, Consumo, Suplementación nj

# 1. Introduction

In Rondônia, as throughout the Brazilian Amazon, there are areas characterized by a predominance of smallholders, where dairy farms using crossbred herd in pasture-based systems play an important role in the strengthening of dairy market chain (Soler *et al.*, 2014). In this region, the climate is characterized by high air temperature coupled with high relative humidity and solar radiation (Alvares *et al.*, 2014), which causes heat stress in livestock, especially dairy cows. Extended periods of high air temperature compromise the ability of dairy animals to dissipate excess body heat, which affects feed intake, milk production, and reproductive efficiency (Correa-Calderón *et al.*, 2022).

Heat stress directly and indirectly affects the productive, physiological, and behavioral parameters of livestock. Changes in grazing and rumination patterns, such as excessive dislocation and frequent water intake, can be signs of heat stress (Correa-Calderón *et al.*, 2022). When DMI is reduced, management interventions that modify the diet composition to either promote a greater consumption or compensate for the low DMI may be possible. Nutritional recommendations to mitigate negative effects of heat stress on intake and metabolism have focused on ensuring constant availability of feed and adjustments to ration formulation to maintain energy density without lowering effective fiber. Supplemental fat has been advocated as a possible means of reducing heat stress on lactating dairy cows due to high energy content and low heat increment of fat relative

to other nutrients. It reduces thermogenesis because fat generates less heat increment than dietary carbohydrate or protein (Wang et al., 2010).

Supplementing corn oil to grazing dairy cows has been shown to increases milk yield without affecting voluntary intake or diet digestibility (Girón *et al.*, 2016), and Holstein cows supplemented with fish oil also have an increased milk yield (Pirondini *et al.*, 2015). However, studies on the effect of oil supplementation on grazing crossbred dairy cows are limited.

The strategy of crossbreeding *Bos taurus*  $\times$  *Bos indicus* has been used widely as a method to improve milk production under grazing conditions in the tropical and subtropical areas of Latin America. Brazilian breeders have sought to combine the desirable characteristics of Gyr, a *Bos indicus* breed which is resistant to hot temperatures and tropical diseases, with Holstein cows, a *Bos taurus* breed, this crossing originates the Girolando cattle (Dalcin *et al.*, 2016).

In the present study, our hypothesis was that soybean oil supplementation reduces the negative effects of heat stress on forage intake patterns, including decrease in grazing and rumination time, which strongly affects milk production. The intake, milk yield, grazing behavior (during nighttime and daytime) and feed efficiency were accessed using lactating cows grazing palisade grass with or without soybean oil supplementation.

# 2. Materials and Methods

All management practices applied to the experimental animals were approved by the Ethics Committee on Animal Use (CEUA) of Embrapa Rondônia (process number 03-2014).

The trial was carried out on an experimental field of Empresa Brasileira de Pesquisa Agropecuária (Embrapa) located in Porto Velho, Rondônia state, Brazil (8°47'38''S, 63°50'46''W). The predominant climate in this region is Am, which is characterized by a dry season from June to August and annual rainfall exceeding 2,250 mm (Alvares *et al.*, 2014).

The trial was carried out from February to March 2015, when the daily average air temperature and precipitation were 25.75 °C and 275.1 mm, respectively. The daily means of the environmental parameters registered by the weather station located 500 m from the experimental field were used for the estimation of the temperature-humidity index (THI) with the following equation by Thom (1959): THI= Tdb+ 0.36Tdp + 41.2, where Tdb is the dry bulb temperature (°C) and Tdp is the dew point temperature (°C).

The average THI during the experimental period was 75.11; during the nighttime (from 18:00 to 5:59) and daytime (from 6:00 to 17:59), the average THIs were 73.69 and 76.52, respectively.

Eight  $\frac{1}{2}$ H×G (n = 4) and  $\frac{3}{4}$ H×G (n = 4) lactating multiparous cows were used for this experiment. At the start of the experiment, these cows had an average live weight (LW) of 471±53 kg, spent an average of 53±33 days in lactation, and produced an average of 22.6±3 kg of milk day<sup>-1</sup>. Cows were distributed in a 2×2 crossover trial with two supplementation (with or without oil supplementation) and two 20-day experimental periods, with 15 days for diet adaptation and 5 days for data collection, totaling a 40-day trial period.

Cows were assigned to a treatment according to their genetic group and their average daily milk yield at the beginning of the experimental period, to control for these variation factors within the experimental design. During the total experimental period, cows grazed on palisade grass (*Urochloa brizantha* cv. Marandu), managed with an intermittent stocking with 2 days of occupation and 28 days of resting. The mean stocking rate was 2.5 animal unity (AU; 1 AU = 450 kg of body weight), and the forage available for grazing was 68 kg DM.AU<sup>-1</sup>. Cows had free access to water and mineral mixture.

The animals were mechanically milked twice daily (at 7:30 and 13:30), when they received the experimental rations. During the last five days of each experimental period, individual milk yield (kg cow<sup>-1</sup> day<sup>-1</sup>) was calculated as a sum of the total milk from the morning and afternoon milking, weighed by a portable electronic scale (Zarna Instruments Company©, India) with a 40 kg capacity and 10 grams accuracy.

In the soybean oil supplementation group (+SO), the concentrate ration contained 7% of soybean oil (based on the DM), unlike the concentrate ration without soybean oil (-SO). The proportion of ingredients in the experimental rations (Table 1) were balanced for 25 kg of daily milk production and similar levels of metabolizable energy (ME) using the Cornell Net Carbohydrate and Protein System (CNCPS) software v6.5 (Cornell University, Ithaca, NY, USA).

The individual amount of concentrate ration offered to each cow was adjusted before starting each experimental period based on their last mean of milk yield. One kilogram of ration was given to the cow for every three kilograms of milk produced above the daily production amount of eight kilograms. The experimental rations were offered individually in two daily equal meals during the milking. Soybean oil was added and manually mixed to the +SO ration just before each meal.

Marandu palisade grass pasture samples were obtained by hand-plucking at the grazing for intake measurements. At each meal, 10% of the experimental rations offered to each animal were taken as samples. The palisade grass samples were oven dried at 65 °C until a constant weight was obtained. Dried *1-mm* samples of grass and rations were analyzed following the methodologies of National Institute of Science and Technology of Animal Science (INCT-CA) reported by Detmann et al. (2012): DM (INCT - CA G-003/1), mineral matter (MM, INCT–CA M-001/1), crude protein (CP, INCT - CA N-001/1), neutral detergent fiber (NDF, INCT - CA F-001/1), acid detergent fiber (ADF, INCT - CA F-001/1), lignin (INCT-CA F-005/1). The hemicellulose and cellulose were found by the difference between NDF and ADF; and ADF and lignin, respectively (Detmann et al., 2012). The diet *in vitro* DM digestibility (IVDMD) was performed in rumen incubator (Tecnal<sup>®</sup> TE 150, Piracicaba, SP, Brazil). Total carbohydrates (TC), digestible energy (DE), metabolizable energy (ME), net energy (NE), and total digestible nutrients (TDN) were estimated by equations from Sniffen *et al.* (1992), Cappelle *et al.* (2001), and NRC (2001) (Table 1). Total DMI (DMI<sub>total</sub>, kg day<sup>-1</sup>) was estimated by: DMI<sub>total</sub> = TFP(1-IVDMD)<sup>-1</sup>, where TFP is the total fecal production (kg fecal DM day<sup>-1</sup>) of each cow estimated by the LIPE<sup>®</sup> (Produtos de Pesquisas Simões Saliba, Florestal City, MG, Brazil) marker supplied to animals. Pasture DMI (DMI<sub>pasture</sub>, kg day<sup>-1</sup>) was calculated from the difference between DMI<sub>total</sub> and the intake of the DM concentrate ration (DMI<sub>ration</sub>, kg day<sup>-1</sup>).

Grazing behavior was accessed by bioacoustic methodology (Souza *et al.*, 2019) using MP3 audio recorders adapted to the cow's halters to register the sounds of grazing, ruminating, and resting on the 17<sup>th</sup> and 18<sup>th</sup> day of the experimental period. The audio was continuously recorded for 48 hours, and the time spent for each activity during the nighttime (from 18:00 to 5:59) and the daytime (from 6:00 to 17:59) was quantified by the Audacity<sup>®</sup> software (Free Software Foundation, Boston, MA, USA).

Statistical analysis of the data was performed using the MIXED procedure in *Statistical Analysis System* (SAS) with the restricted maximum likelihood method (RELM). The model used for the grazing behavior data (time spent grazing, rumination, and resting) was:  $y_{ijklmn} = \mu + \tau_{ij} + \beta_j + \rho_k + \sigma_l + \delta_m + \sigma\delta_{lm} + \sigma\delta_{lm} + e_{ijklm}$ ; where  $\mu$  is the general mean,  $\tau$  is the random effect of each individual animal,  $\beta$  is the effect of the sequence,  $\rho$  is the period (experimental period 1 and 2),  $\sigma$  is the ration type (soybean oil and control),  $\delta$  is the day period (repeated measures corresponded to the daytime and nighttime), and *e* is the unexplained random error.

The model used for the parameters of the DMI (total and pasture), milk yield, and feed efficiency was:  $y_{ijklm} = \mu + \tau_i + \beta_j + \rho_k + \sigma_l + e_{ijkl}$ ; where  $\mu$  is the general mean,  $\tau$  is the random effect of each individual animal,  $\beta$  is the effect of the sequence,  $\rho$  is the period (experimental period 1 and 2),  $\sigma$  is the ration type (soybean oil and control), and *e* is the unexplained random error. The means were compared by Tukey's test at 5% significance.

# 3. Results and Discussion

The mean THI during the experimental period was 75.11. According to Armstrong's classification, a THI between 72 and 78 suggests that cows are subjected to mild or moderate heat stress (Armstrong, 1994). Under heat stress, animals reduce

their feed intake and time spent grazing and ruminating. It is well reported that heat stress limits animal feed intake, which directly and indirectly affects the production performance and feed behavior in bovines (Sammad *et al.*, 2020).

DM available in pasture during the experimental period was 3,830 kg DM ha<sup>-1</sup>, and the post-grazing residue was 2,688 kg DM ha<sup>-1</sup>, which is above the 2,250 kg DM ha<sup>-1</sup> considered the minimum for not limiting DMI by grazing cattle (NRC 2001). The mean of DMI<sub>pasture</sub> (in %LW) was 2.13%, which is within the variation found in previous studies; in studies by Fukumoto *et al.* (2010) and Sousa *et al.* (2008), the means of pasture DMI (in %LW) were 2.0% and 2.4% for *Brachiaria* pasture grazed by lactating Holstein × Gyr cows daily supplemented with eight and two kilograms of concentrate rations, respectively.

Supplementation with soybean oil had no significant effect on milk yield, DMI, and feed efficiency (Table 1). During the experimental period, averages of individual milk yield and  $DMI_{total}$  were 19.26 kg day<sup>-1</sup> and 15.32 kg DM day<sup>-1</sup>, respectively.

The daily intake of TDN did not differ between cows supplemented with or without soybean oil.

The addition of soybean oil in the diet of lactating cows did not change DMI<sub>total</sub> or DMI<sub>pasture</sub>, and also the time spent with grazing and ruminating. This was likely due to the level of EE (6.7%, DM basis) in the diet (Table 1) that was lower than that of 8% suggested as an intake limitation for lactating dairy cows (NRC, 2001). The reduction of DMI after dietary fat inclusion (above the level of 8%, DM basis) may be a result of an increase in ruminating time due to negative effects on rumen digestion; a slowdown of rumen emptying due to a metabolic effect of long chain fat acid. In both situations, a satiety effect due to rumen replenishment could occur (Martín *et al.*, 2013).

	Treat	Pr		
-	-SO	+SO	11	
Milk yield (kg day <sup>-1</sup> )	19.40±0.57A	19.12±0.57A	0.5504	
Dry matter intake (DMI)				
Ration (kg DM day <sup>-1</sup> )	5.35±0.31A	4.80±0.31A	0.2603	
Pasture (kg DM day <sup>-1</sup> )	10.33±0.27A	10.15±0.27A	0.3807	
Pasture (%LW)	2.15±0.10A	2.11±0.10A	0.3725	
Total (kg DM day <sup>-1</sup> )	15.69±0.23A	14.95±0.23A	0.0706	
Nutrient intake (DM basis)				
Ash (kg day <sup>-1</sup> )	1.10±0.02A	0.94±0.02B	0.0063	
CP (kg day <sup>-1</sup> )	2.51±0.08A	2.33±0.08A	0.2042	
EE (kg day <sup>-1</sup> )	0.22±0.01B	0.41±0.01A	0.0001	
NDF (kg day <sup>-1</sup> )	6.72±0.14A	6.55±0.14A	0.1044	
ADF (kg day-1)	3.37±0.07A	3.21±0.07B	0.0191	
Lignin (kg day-1)	0.43±0.01A	0.42±0.01A	0.2054	
Cellulose (kg day-1)	2.94±0.06A	2.88±0.06A	0.1702	
Hemicellulose (kg day <sup>-1</sup> )	3.35±0.07A	3.28±0.07A	0.1429	
TC <sup>1</sup> (kg day <sup>-1</sup> )	11.86±0.17A	11.27±0.17B	0.0177	
DE <sup>2</sup> (Mcal kg <sup>-1</sup> )	0.48±0.01A	0.45±0.01A	0.0592	
ME <sup>2</sup> (Mcal kg <sup>-1</sup> )	0.42±0.01A	0.39±0.01A	0.0566	
NE <sup>2</sup> (Mcal kg <sup>-1</sup> )	0.25±0.00A	0.23±0.00A	0.0503	
TDN <sup>3</sup> (kg)	10.96±0.19A	10.24±0.19A	0.0592	
Feed efficiency				
DMI <sub>total</sub> (kg milk kg <sup>-1</sup> )	1.46±0.05A	1.54±0.05A	0.0665	
DMI <sub>ration</sub> (kg milk kg <sup>-1</sup> )	3.70±0.17A	4.05±0.17A	0.2523	

Table 1 - Means (±SE)	of daily milk yield	, dry matter,	nutrient	intakes,	and feed	efficiency	within	treatment	(with -	+SO or
without -SO soybean oil	supplementation).									

(CP) crude protein, (EE) ether extract, (NDF) neutral detergent fiber, (ADF) acid detergent fiber

<sup>1</sup>Total Carbohydrates = 100 - Ash - EE - CP, according to Sniffen et al. (1992)

<sup>2</sup>Digestible Energy = (TDN 100-1)\*4.409; Metabolizable Energy = (DE\*1.01)-0.45; Net Energy = (0.0245\*ME)-0.12, according to NRC (2001)

<sup>3</sup>Total Digestible Nutrients = 9.6134+0.8294\*IVDMD, according to Cappelle et al. (2001)

(SE) Standard Error, (Pr) F-test probability

Means followed by the same upper-case letters on the line are not significantly different by Tukey's test ( $P \le 0.05$ ). Source: Authors.

Cows supplemented with soybean oil had a significantly higher intake of ether extract (EE) and a significantly lower intake of ADF and TC (Table 2). This difference was expected due to the proportion of these nutrients in the SO+ ration (Table 2). The daily intake of DE, ME, NE, and TDN did not differ between cows supplemented with and without soybean oil.

	Rations	Pasture	
	-SO	+SO	
Feed ingredients*			
Soybean meal	43.75	48.78	-
Corn	50.00	37.28	-
Mineralized salt	4.69	5.23	-
Urea	1.56	1.74	-
Soybean oil	-	6.97	-
Total	100.00	100.00	-
Chemical composition			
Dry matter (%)	88.29	88.11	29.51
Ash (%DM)	9.94	8.05	5.49
Crude Protein, CP (% DM)	31.32	31.50	8.08
Ether Extract, EE (%DM)	0.89	5.07	1.63
NDF (%DM)	10.20	10.70	59.77
ADF (%DM)	5.01	3.31	30.04
Lignin (%DM)	0.83	0.92	3.75
Cellulose (%DM)	4.18	4.39	26.29
Hemicellulose (%DM)	5.19	5.39	29.73
<sup>1</sup> TC (%DM)	57.85	55.38	84.80
<sup>2</sup> DE (Mcal day <sup>-1</sup> )	3.66	3.53	2.77
<sup>2</sup> ME (Mcal day <sup>-1</sup> )	3.25	3.12	2.34
<sup>2</sup> NE (Mcal day <sup>-1</sup> )	1.91	1.84	1.38
<sup>3</sup> TDN (%DM)	83.10	80.18	62.86
IVDMD (%DM)	88.6	85.08	66.67

**Table 2** - Centesimal composition of the ingredients, chemical composition, and *in vitro* digestibility of the dry matter (IVDMD) of the rations with (+SO) or without (-SO) soybean oil and the palisade grass pasture.

\*Requirements for a dairy cow producing 15 kg milk day<sup>-1</sup> (NRC 2001)

NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, DE: Digestible Energy, TC: Total Carbohydrates, ME: Metabolizable Energy, NE: Net Energy, TDN: Total Digestible Nutrients

 $^{1}\text{TC} = 100 - \text{Ash} - \text{EE} - \text{CP}$ , according to Sniffen *et al.* (1992)

 $^{2}\text{DE} = (\text{TDN } 100^{-1})*4.409; \text{ME} = (\text{DE}*1.01)-0.45; \text{NE} = (0.0245*\text{ME})-0.12, \text{ according to NRC } (2001)$ 

 $^{3}$ TDN = 9.6134+0.8294\*IVDMD, according to Cappelle *et al.* (2001).

Source: Authors.

Soybean oil supplementation had no significant effects on grazing behavior parameters; however, the cows spent more time grazing and ruminating at night than during the day (Table 3).

The cows in our study spent more time grazing and ruminating at night and resting during the day (Tables 3); during the daytime, the pasture was sunny with a THI of 76.52, compared to 73.69 at nighttime. Under these conditions, animals may prefer to feed at night when the air temperature is mild (lower THI) and avoid grazing during the warmer times of the day (Schmeling *et al.*, 2022).

In contrast, a study by Oliveira *et al.* (2011) found that lactating Holstein vs. Zebu cows grazing *Brachiaria decumbens* in Santo Antônio da Alegria City (Bahia State, Brazil) allocated a greater percentage of the daytime grazing compared to other behavioral activities, as ruminating and resting. These authors suggest that this behavior was caused by once-a-day milking before dawn, which intensified grazing. Additionally, the cows in that study had access to a shaded area, which would affect the preference for grazing during the daytime. In another study, we also found that, during the daytime, dairy heifers spend more time grazing in a silvopastoral when compared to heifers in pasture with full sun condition (Souza *et al.,* 2019).

After 24 hours of visual observations, the Oliveira *et al.* (2011) found that lactating Holstein×Zebu cows spent 718.59, 388.09, and 333.32 minutes grazing, ruminating, and resting, respectively. Cows spent 50% more time ruminating and resting (Table 3) in this study, because we assessed behavioral parameters by a bioacoustics method for 48 hours. Adjusting for a 24 hour period, the cows in this study spent 371.34 minutes grazing, which was 50% lower than that found in Oliveira *et al.* (2011) and below the 480 to 540 minute range considered as the minimum required for feed intake (Phillips and Rind, 2001).

Time spent by animals in grazing activities, such as grazing, rumination, and resting, is related to climate conditions, pasture traits (forage allowance and sward structure), and the physiological state of the animal, and thus relate to animal performance (Pérez-Prieto *et al.*, 2011).

**Table 3** - Mean ( $\pm$ SE) amount of time (minutes) grazing, rumination, and resting considering treatment (with +SO or without -SO soybean oil supplementation) and period of the day (measured for 48 hours).

Activity	Treatments		Pr	Period of	Total	Pr	
Activity	-SO	+80		Daytime	Nighttime	(48 h)	
Grazing	373.54±23.76A	369.16±24.76A	0.9808	283.43±27.01B	459.26±27.01A	742.69	< 0.0001
Ruminating	380.48±17.68A	392.94±18.72A	0.6303	$202.08{\pm}18.19B$	571.33±18.19A	773.41	< 0.0001
Resting	327.72±14.96A	325.60±15.81A	0.9185	370.78±15.32A	282.54±15.32B	653.32	< 0.0001

Nighttime from 18:00 to 5:59 and daytime from 6:00 to 17:59;

(SE) Standard Error, (Pr) F-test probability;

Means followed by the same upper-case letters on the line are not significantly different by F test (P < 0.05); Source: Authors

# 4. Conclusion

Under moderate heat stress, soybean oil supplementation to a diet of grazed palisade grass did not change milk yield, pasture intake, or grazing behavior of lactating crossbred cows.

We suggest carrying out further studies with a larger number of animals and testing different levels of inclusion of soybean oil in the diet of crossbred Holstein x Gir cows in lactation under grazing and heat stress.

# Acknowledgments

To CNPq, Conselho Nacional de Desenvolvimento Científico, for financial support (process number 478318/2013-8).

# References

Alvares, C.A., Stape, J.L., Sentelhas, P.C., De Moraes, G., Leonardo, J. & Sparovek, G. (2014). Koppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, 22, 711-728.

Armstrong, D.V. (1994). Heat stress interaction with shade and cooling. Journal of Dairy Science, 77, 2044-2050.

Correa-Calderon, A., Avendano-Reyes, L., Lopez-Baca, M. A. & Macias-Cruz, U. (2022). Heat stress in dairy cattle with emphasis on milk production and feed and water intake habits. Review. *Revista Mexicana de Ciencias Pecuarias*, 13(2), 488-509.

Cappelle, E.R., Valadares Filho, S.C., Silva, J.S.C. & Cecon, P.R. (2001). Estimativas do valor energético a partir de características químicas e bromatológicas dos alimentos. *Revista Brasileira de Zootecnia*, 30, 1837-1856.

Dalcin, V.C., Fischer, V., Daltro, D.D.S., Alfonzo, E.P.M., Stumpf, M.T., Kolling, G.J. & McManus, C. (2016). Physiological parameters for thermal stress in dairy cattle. *Revista Brasileira de Zootecnia* 35, 211-218.

Detmann, E., Souza, M.A., Valadares Filho, S.C., Queiroz, A.C., Berchielli, T.T., Saliba, E.O.S., Cabral, L.S., Pina, D.S., Ladeira, M. & Azevedo, J.A.G. (2012). Métodos para análise de alimentos. Visconde do Rio Branco-MG: Suprema. 214 p.

Fukumoto, N.M., Damasceno, J.C., Deresz, F., Martins, C.E., Cóser, A.C. & Santos, G.T. (2010). Produção e composição do leite, consumo de matéria seca e taxa de lotação em pastagens de gramíneas tropicais manejadas sob lotação rotacionada. *Revista Brasileira de Zootecnia*, 39, 1548-1557.

Girón, J.E.P., Restrepo, M.L.P. & Fornaguera, J.E.C. (2016). Supplementation with corn oil and palm kernel oil to grazing cows: ruminal fermentation, milk yield, and fatty acid profile. *Revista Brasileira de Zootecnia*, 45, 693-703.

Martínez Marín, A.L., Pérez, M., Pérez, L.M., Carrión, D., Garzón, A.I. & Gómez, G. (2013). Effect of dietary fat on the productive results of dairy ruminants. Revista Colombiana de Ciencias Pecuarias, 26, 69-78.

NRC. (2001). Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001. Washington, DC: The National Academies Press. https://doi.org/10.17226/9825.

Oliveira, P.A., Marques, J.A., Barbosa, L.P., Oliveira, G.J.C., Pedreira, J.C. & Silva, L.L. (2011). Aspectos metodológicos do comportamento ingestivo de vacas lactantes em pastejo de *Brachiaria decumbens*. *Revista Brasileira de Saúde e Produção Animal*, 12, 166-175.

Pérez-Prieto, L., Peyraud, J.L. & Delagarde, R. (2011). Pasture intake, milk production and grazing behavior of dairy cows grazing low-mass pastures at three daily allowances in winter. *Livestock Science*, 137,151-160.

Phillips, C.J. & Rind, M.I. (2001). The effects of social dominance on the production and behavior of grazing dairy cows offered forage supplements. *Journal of Dairy Science*, 85, 51-59.

Pirondini, M., Colombini, S., Mele, M., Malagutti, L., Rapetti, L., Galassi, G. & Crovetto, G.M. (2015). Effect of dietary starch concentration and fish oil supplementation on milk yield and composition, diet digestibility, and methane emissions in lactating dairy cows. *Journal of Dairy Science*, 98, 357-372.

Sammad, A., Wang, Y. J., Umer, S., Lirong, H., Khan, I., Khan, A., & Wang, Y. (2020). Nutritional physiology and biochemistry of dairy cattle under the influence of heat stress: Consequences and opportunities. *Animals*, *10*, 793.

Schmeling, L., Thurner, S., Erhard, M., & Rauch, E. (2022). Physiological and Behavioral Reactions of Simmental Dairy Cows to Increasing Heat Load on Pasture. *Ruminants*, 2, 157-172.

Sniffen, C. J., O'connor, J. D., Van Soest, P. J., Fox, D. G. & Russell, J. B. (1992). A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *Journal of Animal Science*, 70, 3562-3577.

Soler, L.S., Verburg, P.H. & Alves, D.S. (2014). Evolution of land use in the Brazilian Amazon: From frontier expansion to market chain dynamics. Land, 3, 981-1014.

Sousa, B.M., Saturnino, H.M., Borges, A.L.C, Lopes, F.C., Silva, R.R. & Campos, M.M. (2008). Estimativas de consumo de matéria seca e de fibra em detergente neutro por vacas leiteiras sob pastejo, suplementadas com diferentes quantidades de alimento concentrado. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 60, 890-895.

Souza, E. C., Salman, A. K. D., Cruz, P. G., Veit, H. M., Carvalho, G. A., Silva, F. R. F. & Schmitt, E. Thermal comfort and grazing behavior of Girolando heifers in integrated crop-livestock (ICL) and crop-livestock-forest (ICLF) systems. (2019). *Acta Scientiarum. Animal Sciences*, 41, e46483.

Thom, E.C. 1959. The discomfort index. Weatherwise, 12:57-6.

Wang, J.P., Bu, D.P., Wang, J.Q., Huo, X.K., Guo, T.J., Wei, H.Y., Zhou, L.Y., Rastani, R.R., Baumgard, L.H. & Li, F.D. (2010). Effect of saturated fatty acid supplementation on production and metabolism indices in heat stressed mid-lactation dairy cows. *Journal of Dairy Science*, 93, 4121–4127.