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## DRY MATTER INTAKE, METHANE EMISSIONS AND PERFORMANCE BY BEEF HEIFERS GRAZING TEMPERATE PASTURES IN TWO INTEGRATED CROP-LIVESTOCK SYSTEMS

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### ABSTRACT

Dry matter intake (DMI) is a key driver of animal production in pasture-based systems. Sward structure is related to DMI. When forage plants are cultivated under trees, they may change their morphology as a means of avoiding shade and optimizing light interception. Therefore, our aim was to compare the DMI of grass-only temperate forage, animal performance and methane emissions in two integrated crop-livestock systems (ICLS), that is: crop-livestock (CL) and crop-livestock-trees (CLT), crossed with two nitrogen fertilization levels (90 and 180 kg de N ha<sup>-1</sup>). The experimental design was randomized blocks with three replicates. Two tester animals per paddock were utilized to determine DMI by heifers, using the n-alkanes technique. No significant difference between treatments were observed for methane emissions (34 g kg<sup>-1</sup> DMI) and sward height (SH), which means were close to the target (20 cm). Despite similar SH, the daily herbage accumulation rate, tiller density and herbage allowance were significantly reduced in CLT compared with CL. An increase in N availability was not enough to overcome these differences. Consequently, DMI was also reduced in CLT (1.5±0.04 % live weight, LW) compared to CL (1.7±0.05 % LW), resulting in decreased animal production (-38%) in ICLS with 7-years old trees.

**Key words:** agroforestry; alkanes; beef cattle

### INTRODUCTION

The interest in integrated crop-livestock systems (ICLS) has increased globally, as they are aligned with principles of a cleaner production (GARRETT et al., 2017). When introducing trees in ICLS systems, understory plants can exhibit alterations in anatomy and physiology to compensate for low light quantity and distinct quality (GOBBI et al., 2009). Any morphological change in sward structure affects the animals' intake behavior (GEREMIA et al., 2018). Therefore, in ICLS typical of southern Brazil (summer cash crop/grazing cattle rotations), dry matter intake (DMI) of winter annual forages and consequently the animal performance and methane emissions, could be affected by the resultant alterations in structure and quality of herbage mass caused by N availability and association with trees. However, little information is available to test this hypothesis. By understanding these factors, ICLS managers can better balance the advantages (e.g. soil amelioration, shade, wood and a habitat for fauna) and disadvantages (e.g. competition for water and nutrients between trees and forage components) of trees incorporation into ICLS in order to maintain an economically and biologically sustainable system that meets production goals. The objective of this research was to quantify DMI, methane emission and animal performance of heifers in two ICLS (with and without trees) with two N levels.

### MATERIAL AND METHODS

A field experiment was conducted at the Rural Development Institute of Paraná – IAPAR–Emater, Ponta Grossa-PR (25°07'22''S, 50°03'01''W), in southern Brazil. The local climate is humid subtropical, or Cfb in the Köppen classification system, with frequent occurrence of frosts and a mean annual temperature of 17.6° C ranging from 14° C in July to 21° C in January. The mean annual

rainfall is 1400 mm. The soil is a transition from Typic Distrudept to Rhodic Hapludox (SOIL SURVEY STAFF, 1999), with a 4 to 9% slope and 19%, 3% and 78% of clay, silt, and sand in the upper 20 cm, respectively.

The experimental area of 13.1 ha was divided into 12 paddocks (i.e. experimental units) ranging from 0.77 to 1.22 ha. In October 2006, three tree species (eucalyptus, *Eucalyptus dunnii* Maiden; pink pepper, *Schinus terebinthifolius* Raddi; and silver oak, *Grevillea robusta* A. Cunn. ex R.Br.) were planted in 6 of the 12 paddocks, at 3×14 m spacing (238 trees ha<sup>-1</sup>). During the summer of 2013, the experimental area was thinned to 159 trees ha<sup>-1</sup> by removing pink pepper trees, many of which had been damaged by cattle activity. Beginning with the winter season in 2010, the production system was integrated cattle grazing on cool-season pasture (black oat + annual ryegrass, *Avena strigosa* Schreb cv. IAPAR 61 + *Lolium multiflorum* Lam.) during the winter followed by warm-season maize or soybean crops during the summer. For the current study, i.e. in 2013, the black oat + ryegrass mixture was sown in rows with a seeding density of 45 and 15 kg ha<sup>-1</sup>, respectively, in April and 400 kg ha<sup>-1</sup> of commercial formula 4-30-10 (N-P-K) was applied. The previous culture (i.e. summer 2012-2013) was soybean. Soybean (BRS 232) was sown (55 kg of seeds ha<sup>-1</sup>, 40 cm row spacing) after the pasture was desiccated with glyphosate (2.5 l ha<sup>-1</sup>) using 400 kg ha<sup>-1</sup> of commercial 00-20-20 (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O).

Each experimental unit received three tester animals and a variable number of animals periodically adjusted to maintain the desired sward height of 20 cm (“put-and-take” method, Mott and Lucas, 1952). The experimental animals were Purunã (¼ Aberdeen Angus, ¼ Canchim, ¼ Caracu, and ¼ Charolais) rearing beef heifers, with an average age of 10 months and weighing 256 ± 5.1 kg. Grazing started on July 3rd, i.e., when the sward height in all paddocks reached at least 20 cm. Heifers grazed until October 8<sup>th</sup> in 2013 add up to 97 grazing days for 2013.

The experimental design was randomized blocks with three replicates. Two N fertilization treatments (90 and 180 kg N ha<sup>-1</sup>, or N90 and N180, respectively) were crossed with two ICLS: crop-livestock only (CL) and crop-livestock with trees (CLT). Nitrogen fertilizer (as urea) was applied in a single procedure 40 days after the pasture was sown.

Sward height (SH) was measured at 100 points per paddock every 15 days using a sward stick (BARTHAM, 1985). Herbage mass (HM, t DM ha<sup>-1</sup>) and daily herbage accumulation rate (HAR, kg DM ha<sup>-1</sup>day<sup>-1</sup>) were estimated from samples collected every 28 days. In each paddock, three cuts were done at ground level (0.25 m<sup>2</sup>). From these samples, sub-samples were manually separated into fractions containing leaf blade, stem + sheath, dead material and other species. Five SH measurements were performed inside each sample area, to adjust the HM as a function of the average SH of the paddock, according to Kunrath et al. (2020). HAR was monitored each 28 days using three grazing exclusion cages per paddock (KLINGMAN et al., 1943) and the difference in the amount of dry matter (DM) between sampling dates was considered as the accumulated herbage. Herbage DM content was obtained by oven drying the samples at 60 °C until a constant weight. The herbage allowance (HA, %LW) was calculated according to the following equation: HA (%LW) = ((HM/n + HAR)/SR) × 100, where LW = live weight, HM = average herbage mass of each stocking period (kg DM ha<sup>-1</sup>), n = number of days of the stocking period and SR = stocking rate of each stocking period (kg LW ha<sup>-1</sup>).

The samples harvested on August 8 and September 3, 2013 for HM measurements were also milled through a 1-mm screen and analyzed for crude protein (CP), dry-matter digestibility (DMD), neutral detergent fiber (NDF) and acid detergent fiber (ADF) via near-infrared reflectance spectroscopy (FOSS-NIRSystems 5000, scanning over the spectral range of 1100–2500 nm; CEPA laboratory, Passo Fundo-RS, Brazil). Tiller density was estimated by counting tillers on a 50 cm line transect in three random areas inside each paddock.

Stocking rate (kg LW ha<sup>-1</sup>) was calculated by adding the average LW of the tester animals to the average LW of each ‘put and take’ animal multiplied by the number of days they remained in each paddock and then divided by the number of grazing days. Average daily gain (ADG, kg animal<sup>-1</sup> day<sup>-1</sup>) was calculated as the difference between final and initial live weight of each tester animal, divided by the number of grazing days. Live weight gain per hectare (Gha, kg LW ha<sup>-1</sup> day<sup>-1</sup>) was obtained by multiplying the number of animals per hectare and per day by the average ADG of tester animals. Animals were weighed after fasting from solids for approximately 15 h.

To determine daily dry matter intake (DMI) by heifers, two tester animals from each paddock were utilized, using the double n-alkane approach (DOVE & MAYES, 1996). The evaluation period started on August 21, 2013. Twenty-two animals’ testers were dosed twice daily (at 8 AM and 4PM) with cellulose pellets containing 164.87± 0.32 mg of dotriacontane (C<sub>32</sub>) and it was administered for ten days. From the 5<sup>th</sup> to the 10<sup>th</sup> day of dosing, fecal samples *per rectum* were collected simultaneously to the pellet’s administration of C<sub>32</sub>. To estimate the amount of forage consumed by the animals, the simulated grazing technique was applied between the 7<sup>th</sup> and 10<sup>th</sup> day of C<sub>32</sub> administration period in each paddock. The determination of n-alkanes in forage and in feces samples followed the methodology proposed by Dove and Mayes (2006) in the range of C-chain between 27 and 35. The dry matter intake (DMI) was then estimated according to the equation number 4 proposed by De-Stefani Aguiar et al. (2013). Please, see Pontes et al. (2018) for estimates of methane emission in the current protocol.

Shading was measured using two ceptometers (Decagon LP-80 AccuPAR, Pullman, WA, USA) with one placed in full sun and the other placed between the rows of trees (1.4, 4.2, 7, 9.8 and 12.6 m from the row). Shading percentage measurements were taken every 30 min from 9:00 to 15:00 hrs at 1 m above ground level. The decrease in light availability for the understory vegetation was calculated as the difference in the values from these two ceptometers.

Analysis of variance were performed for animal- and pasture-related variables using Statgraphics Centurion 19 to test statistical significance of the following main factors and their interactions: block (GL = 2), system (GL = 1) and N supply (GL = 1). Interactions were checked for each variable (except for block) and were removed from the model if they had a p-value > 0.05. All response variables were analyzed using a generalized linear model with block as a random effect, and system and N supply as fixed effects. Where necessary the data were transformed via log, box-cox or arcsine of the square root to normalize residuals. Pasture variables used in the current study were obtained from samples collected between August and September 2013, i.e. close to the period of DMI evaluation.

## RESULTS AND DISCUSSIONS

Figure 1 shows the n-alkane contents (mg kg<sup>-1</sup> DM) for herbage samples at the four treatments. The length of C-chain measured ranged from C<sub>23</sub> to C<sub>35</sub> and there were no interactions nor statistic differences between systems. This finding is very important because shadow and N levels could affect the amount of n-alkanes in the different systems (Dove & Mayes, 1996), which could undermine DMI assessments. The odd n- alkanes C<sub>33</sub>, C<sub>31</sub> and C<sub>29</sub> were the most abundant n-alkanes in this kind of pasture (black oat + annual ryegrass).

Pasture shading by 7-year-old trees in the CLT system led to a reduction in the mean percentage of light under the tree canopy compared to the treeless system of 41 ± 1.10%.

The severe drought in August 2013 (precipitation of 29 mm, a value even lower than the historical mean, i.e., 74 mm), which was the main period of the current study, limited forage production (e.g. low HAR values, Table 1) and the herbage nutritive value in both systems, decreased carrying capacity and, consequently, the gain per hectare. However, it was possible to maintain the SH close to the target (20 cm) in both systems without significant differences between them (Table 1).

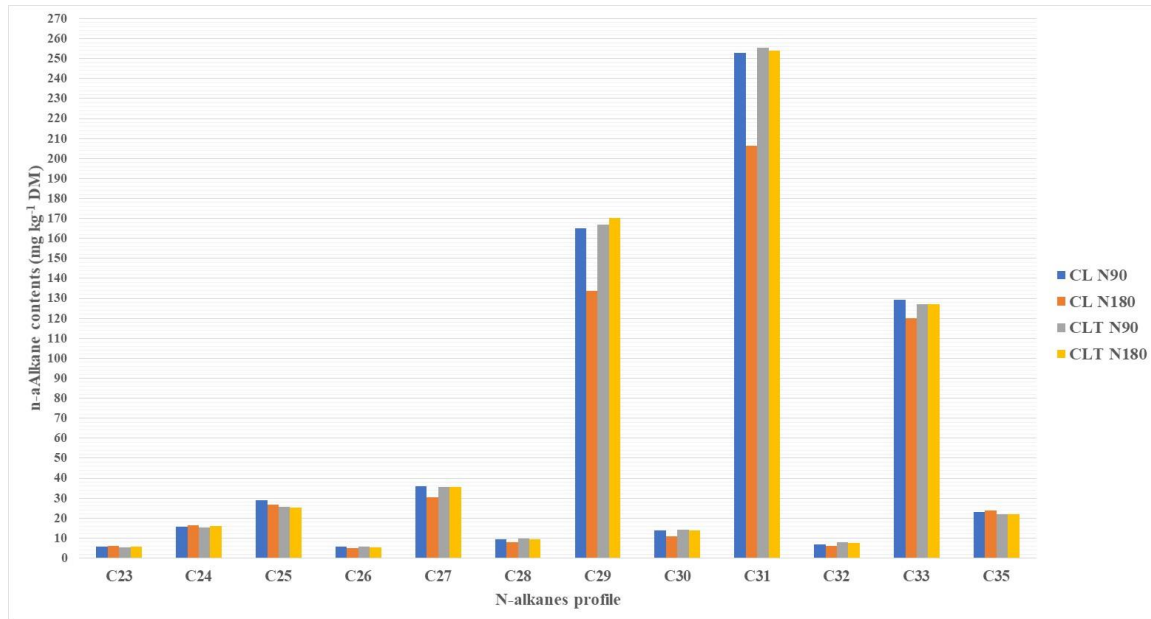


Figure 1. N-alkane (C<sub>23</sub> – C<sub>35</sub>) contents in herbage samples in a mixed *Lolium multiflorum* × *Avena strigosa* pasture under different integration systems: CL, crop-livestock and CLT, crop-livestock-trees systems, and two nitrogen levels (90 and 180 kg N ha<sup>-1</sup>).

Table 1. Pasture and animal performance variables (mean ± standard error) for beef heifers grazing on mixed *Lolium multiflorum* × *Avena strigosa* under different integration systems: CL, crop-livestock and CLT, crop-livestock-trees systems.

	CL	CLT	P
SH (cm)	20±1.13	18±0.79	0.1057
DMI (% LW)	1.7±0.05	1.5±0.04	0.0191
HM (kg DM ha <sup>-1</sup> )	2766±199.6	1449±90.7	<0.001
HAR (kg DM ha <sup>-1</sup> day <sup>-1</sup> )	44±10.5	17±4.6	0.0497
HA (%)	15±1.8	9±0.80	0.0205
SR (kg LW ha <sup>-1</sup> )	945±54.8	713±25.5	0.0121
ADG (g animal <sup>-1</sup> day <sup>-1</sup> )	826±58.3	620±61.4	0.0295
ADG kg DMI <sup>-1</sup>	0.12±0.03	0.11±0.02	0.7272
Gha (kg LW ha <sup>-1</sup> day <sup>-1</sup> )	296±16.8	185±15.6	0.0019
TD (50 cm linear)	220±11.4	146±8.5	0.0017
LP (%)	24±2.1	26±1.9	0.4774
CP (%)	16±0.40	15±0.38	0.0298
DMD (%)	64±1.31	61±0.74	0.0555
ADF (%)	34±0.69	35±0.94	0.2384
g CH <sub>4</sub> kg DMI <sup>-1</sup>	33±2.2	36±3.0	0.3217

P = probability for the system effect; DMI = dry matter intake; LW = live weight; SH = sward height; HM = herbage mass; HAR = herbage accumulation rate; HA = herbage allowance; SR = stocking rate; ADG = average daily gain; Gha = gain per hectare; TD = tiller density; LP = leaf proportion; CP = crude protein; DMD = dry-matter digestibility; NDF = neutral detergent fiber; ADF = acid detergent fiber.

Analysis of variance for sward and animal data showed no effect of N levels, except for CP ( $P < 0.05$ ,  $15 \pm 0.37$  and  $16 \pm 0.42\%$  at N90 and N180, respectively). The interaction system  $\times$  N level was only significant for FDN ( $P < 0.05$ ). While an increase in N level increased the FDN in CL systems ( $63 \pm 1.05$  and  $65 \pm 0.56\%$  at N90 and N180, respectively), the opposite was observed in CLT systems ( $65 \pm 0.72$  and  $63 \pm 1.28$  at N90 and N180, respectively).

Lin et al. (1999) stated that  $C_3$  forage grasses grown at 50% sunlight, can sustain productivity at rates comparable to those obtained in full-sun conditions. However, in the current study, important differences were observed in the HM and consequently in the HAR (Table 1) for the two systems during the experimental period, even with increasing N availability. This result indicates that shade provided by trees in the CLT – as high as 41% in relation to the open field – affected pasture growth, mainly by a reduction in the tiller density (Table 1). A reduction in tiller density is one of the main causes of a lower herbage production in shaded areas (PONTES et al., 2017). Further, the drought effect may have been more intense in CLT systems, in contrast to Ford et al. (2017) findings. Ford et al. (2017) observed greater forage production in silvopasture compared to open pasture during times of drought because of increased evapotranspiration rates in the latter system.

Higher herbage mass allowed greater DMI, resulting in increased animal performance in CL systems compared to CLT (Table 1). The greater level of intake of the CL treatments is likely to have been observed due to a combined effect of some sward factors, including increased tiller density and nutritive value (e.g. crude protein). However, for European cattle, most data of daily DMI ranges between 2 and 3% of LW (ZUBIETA et al., 2021). Herbage intake is affected by rates of digestion and passage, which are closely related to the NDF contents (PINARES-PATIÑO et al., 2003). Therefore, the high NDF (63-65%) and low CP content at the current maturity stage seems to have restricted DMI in both systems.

The 7-year-old trees in our experiment reduced beef heifer gains (ADG) on black oat + ryegrass mixture by 25% compared to pastures without trees, regardless of N level (Table 1). Thus, it seems that the possible positive effect of trees on environmental conditions, such as the protection of animals from wind and extreme temperatures (LOPES et al., 2016), was not enough to mitigate the effects of lower forage production. Furthermore, animal performance and SR used to maintain the targeted SH were the determinants of differences in Gha between treatments (Table 1). The presence of trees in our experiment reduced beef heifers Gha by 38% compared to pastures without trees. The higher SR and Gha in CL, compared to CLT systems, can be explained by the higher HM and HAR increasing the pasture carrying capacity.

ADG  $\text{kg DMI}^{-1}$  was lower when compared to the work of Souza Filho et al. (2019), who used the same type of pasture and four heights as a management goal. In addition, when we compared methane emission per kg of DMI, to the abovementioned author's data, it was higher in the CLT system but close to G40 treatment ( $30.6 \text{ g CH}_4 \text{ kg DMI}^{-1}$ ) in CL system. These differences can be partly explained by the drought that occurred in our work, which reduced quality and quantity of forage and resulted in less DMI, lower animal performance and higher methane emission per kg of DMI. Indeed, the ADG and Gha observed in 2013, in both systems (Table 1), were far below the potential of annual cool-season pastures in ICLS, probably due to the drought. For instance, Kunrath et al. (2020) observed values of around  $1.08 \text{ kg LW animal}^{-1} \text{ day}^{-1}$  and  $423 \text{ kg LW ha}^{-1}$  for a mixture of black oats and ryegrass in ICLS when the targeted SH was 20 cm.

## CONCLUSIONS

The 7-year-old trees, with a planting density of  $159 \text{ trees ha}^{-1}$ , reduced beef heifer gains per hectare on a black oat + ryegrass mixture by 38% compared to pastures without trees, despite maintaining similar sward heights in both systems. Pasture productivity was reduced due to a reduction in tiller density because of a reduction in light availability in a hydric stress period. An increase in the N level

did not compensate for these losses. Therefore, silvicultural interventions need to be intensified to reduce the shading level to below 41% and avoid losses to animal intake.

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