***Animal Journal***

**Greenhouse gas balance and carbon footprint** **of** **pasture-based beef cattle production systems in the tropical region (Atlantic Forest biome).**

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**Supplementary material**

Supplementary Table S1. Composition of mineral supplement used in finishing beef cattle (Nelore steers) pasture-based production systems.

|  |  |  |
| --- | --- | --- |
| Item | Mineral supplement of rainy season (%)\* | Mineral supplement of dry season (%) |
| CP | 0.0 | 40.0 |
| P | 13.0 | 1.2 |
| Na | 0.0 | 3.5 |
| Ca | 21.4 | 8.0 |
| Mg | 2.3 | 0.2 |
| S | 4.0 | 1.5 |
| Zn | 1 | 0.064 |
| Cu | 0.35 | 0.017 |
| Mn | 0.17 | 0.013 |
| Co | 0.03 | 0.0010 |
| I | 0.03 | 0.012 |
| Se | 0.003 | 0.0003 |
| F | 0.13 | 0.02 |

\*Mineral supplement of rainy season was diluted in sodium chloride (25/50 kg)

CP – Crude Protein.

Supplementary Table S2.Characteristics of pastures according to different levels of intensification in finishing beef cattle (Nelore steers) pasture-based production systems and native vegetation.

|  |  |  |
| --- | --- | --- |
|  | Treatments |  |
| Item | IHS | RHS | RMS | DP | Forest |
| Vegetation | *Megathyrsus (Panicum) maximum* Jacques (cv. Tanzânia and Mombaça) | *Megathyrsus (Panicum) maximum* Jacques (cv. Tanzânia and Mombaça) | *Urochloa (Brachiaria) decumbens* Stapf (cv. Basilisk) | *Urochloa* (*Brachiaria*) *decumbens* Stapf (cv. Basilisk) with *Paspalum* infestation | Native vegetation (“seasonal semi-deciduous forest”) |
|  |  |  |  |  |  |
| Management time (years)\* | 9 | 9 | 15 | 15 | - |
|  |  |  |  |  |  |
| Grazing Management | Rotational(3 days of occupation and 33 days of rest) | Rotational (3 days of occupation and 33 days of rest) | Rotational (6 days of occupation and 30 days of rest) | Continuous grazing | - |
|  |  |  |  |  |  |
| Stocking rate | High | High | Medium | Low | - |
| Liming | Yes | Yes | Yes | No | - |
| Fertilization | Yes | Yes | Yes | No | - |
| N fertilization (kg/ha.year) | 600 | 400 | 200 | 0 | - |
| Irrigation | Yes | No | No | No | - |
| Overseeding | Yes | No | No | No | - |
| Applications number per year (N top-dressing) | 10 | 5 | 5 | 0 | - |
| Applications number per year (liming top-dressing) | 1 | 1 | 1 | 0 | - |
| P2O5 fertilization (kg/ha) | 18 first year 99 sec. year | 13.5 first year 103.5 sec. year | 81 first year 108 sec. year | 0 | - |
| K2O fertilization (kg/ha) | 0 first year 0 sec. year | 0 first year 0 sec. year | 0 first year 145 sec. year | 0 | - |
| Irrigation electricity consumed (Mwh/ ano) | 23.7723first year25.7207 second year | 0 | 0 | 0 | - |
| Irrigation (mm/ha) | 352.6mmfirst year381.5 mmsecond year | 0 | 0 | 0 | - |

IHS - Irrigated pasture with High Stocking rate, RHS - Rainfed pasture with High Stocking rate, RMS - Rainfed pasture with Medium Stocking rate, DP - Degraded Pasture. \*relative to the date that soil samples were collected.

Supplementary Table S3. Equations used to calculate the greenhouse gases and carbon footprint of finishing beef cattle (Nelore steers) pasture-based production systems.

|  |  |  |
| --- | --- | --- |
| **C stocks (t/ha):**t = 1 ton = 1 000 kgha = hectare = 10 000 m2

|  |  |
| --- | --- |
|  | (1) |

Cs = total C stock (t/ha), corrected based on the soil mass of a reference area; = sum of C stocks from the first to the next-to-last deepest layer sampled in the treatment (Mg/ha);Mtn = soil mass of the deepest layer in the treatment (t/ha); = sum of total soil mass in the treatment (t/ha); = sum of total soil mass in the reference area (t/ha);Ctn = soil C content in the deepest layer (t/t of soil).Before the correction using soil mass, C stocks of each layer were calculated using Equation 2 (Veldkamp, 1994):Cst = (CO x Ds x e)/10 (2)Cst = C stock in a certain layer (t/ha) CO = total organic C content in the layer (g/kg) Ds = soil density in the layer (kg/dm)e = layer thickness (cm) |
| **Annual C accumulation rate, 0-100 cm layers (t/ha)** =(C stocks in the pasture systems – C stocks the native forest (reference) / number of years passed since the implementation of the pasture systems until the soil sampling date), according to Fernandes et al. (2014). (3) |
| **Carbon balance (t of CO2e. /ha per year)** = ((annual C accumulation rates 0-100 cm layers t/ha \* 3.67) – (annual emissions of CO2e. t/ha)). (4) |
| **Annual emissions of CO2e. (t of CO2e. /ha per year)** = (CH4 emissions from enteric fermentation + N2O emissions from N fertilization and animal wastes + CH4 emissions from N fertilization and animal wastes), using AR4 IP CC 2007 (GWP CH4=21, N2O=310) or AR5 IPCC 2013 (GWP CH4=27.75, N2O=265) and the conversion factor of C to CO2e. = 3.67 (5) |
| **CH4 emissions from enteric fermentation (t CO2e./ha per year)** = (annual average of animal’s number per ha \* annual average emission of individual animal) \* GWP \* 365 days. (6) |
| **Annual average of animal´s number per ha** = (stocking rate daily spring average + stocking ratedaily summer average + stocking rate daily autumn average + stocking ratedaily winter average)/4, considering “put and take” method. (7) |
| **Annual average emission (CH4) of individual animal (t /animal)** = (CH4 daily spring average + CH4 daily summer average + CH4 daily autumn average + CH4 daily winter average)/4 (8) |
| **N2O emissions from N fertilization and animal wastes (t CO2e./ha per year)** = (annual average of emission N2O per ha \* GWP). (9) |
| **CH4 emissions from N fertilization and animal wastes (t CO2e./ha per year)** = (annual average of emission CH4 per ha \* GWP). (10) |
| **Annual average GHG emission from fertilization and animal wastes (t/ha per year)** = (daily spring average GHG emission\*91.25 days) + (daily summer average GHG emission\*91.25 days) + (daily autumn average GHG emission\*91.25 days) + (daily winter average emission\*91.25 days). (11) |
| **Intensities of GHG emissions considering only GHG emissions** = ((Annual emissions of CO2e. /ha per year) / (product output: stocking rate (steers/ha) or live body weight (kg/ha per year) or carcass (kg/ha per year) or CEP (carcass edible portion; kg/ha per year)). (12) |
| **Intensities of GHG emissions considering C balance** = ((Carbon balance in CO2e./ha per year) / (product output: stocking rate (steers/ha) or live body weight (kg/ha per year) or carcass (kg/ha per year) or CEP (carcass edible portion; kg/ha per year)). (13) |
| **Carbon footprint/ha per year (t of CO2e./ha per year)** = ((annual C accumulation rates 0-100 cm layers t/ha \* 3.67) – (annual emissions of CO2e t/ha + annual emission of CO2e t/ha from inputs)). (14) |
| **Annual emission of CO2e. t/ha from inputs** = (emissions from the diesel fuel used by machinery during liming and fertilization, according to Hachuy, 2008 and CETESB, 2017 + electricity consumed during the irrigation of pastures, according MCTI, 2019 + nitrogen, according Nemecek et al., 2007, phosphorus and potassium, according Macedo, 2008, fertilizer production). (15) |
| **Emissions from the diesel fuel used by machinery during liming and fertilization (tCO2e./ha per year)** = ((annual number of application \* 5 \* 2.603) /1000)), considering the consumption of 5L / ha for each application of lime or fertilizer (top-dressing) (according Hachuy, 2008) and 2.603 kg CO2e./L diesel (according Cetesb, 2017). (16) |
| **Emission of electricity consumed during the irrigation of pastures (tCO2e./ha per year)** = (electricity consumed in each year in Mwh/year \* tCO2e./Mwh). (According MCTI, 2019 the emissions were 0.1022 and 0.11645 tCO2e./ Mwh produced in the first and second year, respectively.) Obs: These values ​​consider the weighting of the different sources of energy used in each year (hydroelectric is the main source in Brazil, followed by thermoelectric). (17) |
| **Emission of N fertilizer production** = ((kg N-urea applicated \* kg CO2e./kgN)/1000)). (According to Nemecek et al., 2007, the emissions were 3.05 and 3.02 tCO2e./ Mwh per kg N-fertilizer produced in the first and second year, respectively). (18) |
| **Emission of P2O5 fertilizer production** = ((kg P2O5 applicated \* kg CO2e./kg P2O5)/1000)). (According to Macedo, 2008, the emission was 1.3 tCO2e./Mwh per kg P2O5-fertilizer produced). (20) |
| **Emission of K2O fertilizer production** = ((Kg K2Oapplicated \* kg CO2e./kg K2O)/1000)). (According to Macedo, 2008, the emission was 0.71 tCO2e/ Mwh per kg K2O-fertilizer produced). (21)  |
| **Land-saving effect (ha)** =((HIS or RMS or RHS stocking rate (steers/ha) - DP stocking rate (steers/ha)) / DP stocking rate (steers/ha). (22) |

GHG – greenhouse gases, GWP – global warming potential.

IHS - Irrigated pasture with High Stocking rate, RHS - Rainfed pasture with High Stocking rate, RMS - Rainfed pasture with Medium Stocking rate, DP - Degraded Pasture.

Supplementary Table S4. Evaluation periods of greenhouse gases fluxes from finishing beef cattle (Nelore steers) pasture-based production systems.

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| --- |
| Evaluation periods of ruminal methane |
| Year | Season | Period |
| First | Spring | 01/10/2012 to 07/10/2012 |
| First | Summer | 21/01/2013 to 27/01/2013 |
| First | Autumn | 22/04/2013 to 28/04/2013 |
| First | Winter | 29/07/2013 to 04/08/2013 |
| Second | Spring | 02/12/2013 to 08/12/2013 |
| Second | Summer | 02/04/2014 to 08/04/2014 |
| Second | Autumn | 09/06/2014 to 15/06/2014 |
| Second | Winter | 11/08/2014 to 17/08/2014 |

Evaluation periods of nitrous oxide and methane fluxes from pasture

|  |  |  |  |
| --- | --- | --- | --- |
| Year | Season | Period |  |
| First | Spring | 13/10/2012 to 01/11/2012 |  |
| First | Summer | 29/01/2013 to 17/02/2013 |  |
| First | Autumn | 08/04/2013 to 29/04/2013 |  |
| First | Winter | 22/07/2013 to 12/08/2013 |  |
| Second | Spring | 07/10/2013 to 28/10/2013 |  |
| Second | Summer | 24/02/2014 to 17/03/2014 |  |
| Second | Autumn | 19/05/2014 to 10/06/2014 |  |
| Second | Winter | 25/08/2014 to 15/09/2014 |  |

Supplementary Material M1. Assurance quality of CHN/S 2400ii elemental analyzer (Perkin Elmer).

External calibration was employed in order to quantify the analytes. According to the equipment manufacturer’s instructions, the elemental analyzer was calibrated with acetanilide purchased from Sigma-Aldrich (HPLC grade purity). The precision of the instrument is ±0.4% (weight) for each element – carbon, hydrogen and nitrogen.

Supplementary Material M2. Assurance quality of “Greenhouse” GC-2014 gas chromatograph (Shimadzu).

External calibration was employed in order to quantify the analytes. A calibration curve was generated with 5 different certified reference gas mixtures, each one containing (SF6, CH4 + N2 balance) with increasingly quantities. Certified reference gas mixtures were purchased from Praxair with the following concentrations (molar based) and relative uncertainty: [SF6] = 10ppt ± 10%, [CH4] = 2.26ppm ± 5%; [SF6] = 60ppt ± 10%, [CH4] = 7.59ppm ± 5%; [SF6] = 106ppt ± 10%, [CH4] = 9.92ppm ± 5%; [SF6] = 305 ± 10%, [CH4] = 15.86ppm ± 5%; [SF6] = 1076ppt ± 10%, [CH4] = 19.96ppm ± 5%. All mixtures are traceable to standard masses following RBC-INMETRO nºM-40370/12 calibration certificate. All the necessary calculation was carried out following the IUPAC guidelines (Compendium of Chemical Terminology, 2nd ed, 1997).

Supplementary Material M3. Assurance quality of Thermo Scientific™ TRACE™ 1310 GC.

External calibration was employed in order to quantify the analytes. A calibration curve was generated with 4 different certified reference gas mixtures, each one containing (N2O, CH4, CO2 + N2 balance) with increasingly quantities. Certified reference gas mixtures were purchased from Praxair with the following concentrations (molar based) and relative uncertainty: [N2O] = 257.3ppb ± 5%, [CH4] = 0.5309ppm ± 5%, [CO2] = 260.2ppm ± 5%; [N2O] = 502.8ppb ± 5%, [CH4] = 1.037ppm ± 5%, [CO2] = 508.3ppm ± 5%; [N2O] = 999.5ppb ± 5%, [CH4] = 2.914ppm ± 5%, [CO2] = 1058ppm ± 5%; [N2O] = 2328ppb ± 5%, [CH4] = 5.174ppm ± 5%, [CO2] = 1995ppm ± 5%. All mixtures are traceable to standard masses following RBC-INMETRO nºM-40370/12 calibration certificate. All of the necessary calculation was carried out following the IUPAC guidelines (Compendium of Chemical Terminology, 2nd ed, 1997).

Supplementary Material M4. Statistic Description

Data were analysed by the MIXED procedure of SAS software (SAS Inst. Inc., Cary, NC) with repeated measures (Littell et al., 1998), after verifying the residue normality by the Shapiro-Wilk test (PROC UNIVARIATE). The model included the fixed effects of treatment (four types of pasture) and year (1 and 2) and their interactions (treatments x year) for average C balance and intensity. For tree number simulation the effects considered were the same. The effect of block (area repetition) was considered as random factors. The matrix that fitted best to the data was the autoregressive covariance structure. Effects were considered significant at P ≤ 0.05. All means are presented as least squaremeans, and effects were separated by the PDIFF option of SAS and Tukey average test. The level effect was evaluated by the use orthogonal polynomials, separating the effect in linear, quadratic and quadratic deviation. The statistical model used was described according to the equation below:

Yijk =µ + Ti + Cj + Ti \* Cj + Bk + eijk

Where:

Yijk = observationconcerning Treatments (i) + Years (j) + Treatments (i) \* Years (j) + Blocks (k) + random error association with each observation (eijk);

µ = overall mean;

Ti = Treatment effect (fixed effect);

Cj = Years effect (fixed effect);

Ti \* Cj = interaction between treatment (i) and years (j) (fixed effect);

Bk = Blocks effect (aleatory effect);

eijk = Random error associated with each observation. A significance level of 5% was adopted.

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