RESEARCH ARTICLE

Phenotypic association among performance, feed efficiency and methane emission traits in Nellore cattle

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Abstract

Enteric methane (CH4) emissions are a natural process in ruminants and can result in up to 12% of energy losses. Hence, decreasing enteric CH4 production constitutes an important step towards improving the feed efficiency of Brazilian cattle herds. The aim of this study was to evaluate the relationship between performance, residual feed intake (RFI), and enteric CH4 emission in growing Nellore cattle (Bos indicus). Performance, RFI and CH4 emission data were obtained from 489 animals participating in selection programs (mid-test age and body weight: 414±159 days and 356±135 kg, respectively) that were evaluated in 12 performance tests carried out in individual pens (n = 95) or collective paddocks (n = 394) equipped with electronic feed bunks. The sulfur hexafluoride tracer gas technique was used to measure daily CH4 emissions. The following variables were estimated: CH4 emission rate (g/day), residual methane emission and emission expressed per mid-test body weight, metabolic body weight, dry matter intake (CH4/DMI), average daily gain, and ingested gross energy (CH4/GE). Animals classified as negative RFI (RFI<0), i.e., more efficient animals, consumed less dry matter (P < 0.0001) and emitted less g CH4/day (P = 0.0022) than positive RFI animals (RFI>0). Nonetheless, more efficient animals emitted more CH4/DMI and CH4/GE (P < 0.0001), suggesting that the difference in daily intake between animals is a determinant factor for the difference in daily enteric CH4 emissions. In addition, animals classified as negative RFI emitted less CH4 per kg mid-test weight and metabolic weight (P = 0.0096 and P = 0.0033, respectively), i.e., most efficient animals could emit less CH4 per kg of carcass. In conclusion, more efficient animals produced less methane when expressed as g/day and per kg mid-test weight than less efficient animals, suggesting lower emissions per kg of carcass produced. However, it is not possible to state that feed efficiency has a direct effect on enteric CH4 emissions since emissions per kg of consumed dry matter and the percentage of gross energy lost as CH4 are higher for more efficient animals.
Introduction

Enteric methane (CH\(_4\)) emission is a natural process in ruminants that can result in losses of 2 to 12% of the total energy consumed by the animal [1]. The variation is the result of some factors such as chemical composition of the diet, intake level [2], and even genetic [3] and metagenomic [4].

Residual feed intake (RFI) has been used as a selection criterion in beef cattle in order to increase individual feed efficiency [5,6]. Most efficient animals (negative RFI) have a significant economic advantage as they consume less dry matter than expected for their weight and weight gain [7]. Consequently, the use of negative RFI animals has the potential to significantly reduce meat production costs.

Generally, the higher the dry matter intake (DMI), the higher the daily enteric emissions of CH\(_4\) since a larger amount of substrate will be available for fermentation in the rumen and consequently more hydrogen will be available for methanogenesis [8,9]. Therefore, the use of more efficient animals may reduce enteric CH\(_4\) emissions proportionally to the lower feed intake [10]. However, it is unclear whether the differences in enteric CH\(_4\) emissions are due to the variation in digestive efficiency between negative and positive RFI animals or simply the result of from the lower DMI associated with negative RFI animals [11,12].

Inconsistencies still exist regarding the relationship between feed efficiency (RFI and FC) and enteric CH\(_4\) emission by cattle. Studies have shown this correlation is positive and favorable in the case of highly digestible diets [10,13,14], while the phenotypic relationship between feed efficiency and enteric CH\(_4\) emission is zero or even negative and unfavorable in diets with low digestibility [14–17], suggesting that individual enteric CH\(_4\) emission may even increase with the improvement of feed efficiency. Furthermore, few studies have investigated Bos indicus animals receiving high roughage diets [16,18,19] and there is a lack of studies involving a large number of zebuine animals. The aim of the present study was to evaluate the relationship among performance, feed efficiency and enteric CH\(_4\) emission traits in Nellore cattle (Bos indicus). The hypothesis was that the use of animals with low DMI and similar ADG could be a strategy to reduce greenhouse gases emissions in the beef production system.

Materials and methods

Location and animals

The data were collected in 2011, 2012, 2018, 2019 and 2020 in Sertãozinho-SP, Brazil, as well as in Botucatu-SP, Brazil, in 2019. Performance, feed efficiency and enteric CH\(_4\) emission data were obtained from 489 Nellore animals evaluated in performance tests. This study was carried out in strict accordance with the recommendations in the Guidelines for Animal Welfare and Humane Slaughter (São Paulo State, Law Number 11.977). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Institute of Animal Science (Protocol Number 278–19), Nova Odessa-SP, Brazil.

Treatments and management

The performance tests had an average duration of 76.5 ± 12 days preceded by 28 days of adaptation [5]. The animals started the test at 376 ± 164 days of age, from June to December of each year, and were kept in individual pens (n = 95) or collective paddocks equipped with electronic feed bunks (GrowSafe®, Airdrie-AB, Canada; or Intergado®, Contagem-MG, Brazil) for automated recording of individual daily feed intake (n = 394), with ad libitum access to diet and water. The animals were weighed at the beginning and end of the test after fasting for 14 h, or at predetermined intervals without previous fasting (Table 1).
In each test, the animals were fed a single diet that differed among the years. The diets during the performance tests consisted of silage (corn or sorghum), *Brachiaria* hay, sugar cane bagasse, meal (cottonseed, soybean or peanut), corn (ground or wet grain), citrus pulp, mineral premix, salt, ammonium sulfate, and urea (Table 2).

After pre-drying (55 ± 5°C for 72 h), diet samples were ground in a Willey-type mill (R-TE-650 model, Tecnal Equipamentos Científicos, Piracicaba, São Paulo, Brazil) to pass a 1-mm screen and analyzed for dry matter (method 934.01), ash (method 942.05) and ether extract (method 920.39) contents following the AOAC [22] guidelines. The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the methodology of Mertens [23] using a Tecnal fiber analyzer (TE-149, Piracicaba, São Paulo, Brazil) using α-amylase and without sodium sulphite. The NDF and ADF were expressed exclusive of ash. The determination of crude protein (method 990.03) was performed by the Dumas method [24] based on the release of nitrogen by combustion at high temperature in pure oxygen in DUMATHERM® analyzer. Total carbohydrates were calculated according to the methodology described by Sniffen et al. [25]: 

\[
\text{CHOT} = 100 - (\text{CP} + \text{EE} + \text{MM})
\]

and non-fiber carbohydrates were obtained by subtracting the NDF. The gross energy (GE) determinations were performed in an adiabatic calorimetric pump of the brand IKA WERKE Model C5003 (Parr Instrument Company, Illinois, USA).

The amount of feed was adjusted weekly to guarantee daily leftovers of 5 to 10% of the total amount supplied in order to ensure *ad libitum* intake. The troughs were cleaned and leftovers were removed and discarded three times per week. Intake records were discarded when there were no feed leftovers and in the case of evidence of malfunctioning of the electronic measurement devices. Weekly samples of the ingredients were obtained for determination of the dry matter content of the diet.

### Animal performance and feed efficiency

The following traits were calculated as described by Grion et al. [5] and Ceacero et al. [6]: DMI, average daily gain (ADG), metabolic body weight (BW^{0.75}), RFI, and feed conversion (FC). The DMI was obtained as the mean of all valid days during the period. The ADG was

| Group | Year | Sex category | Days in test | Facility | Collector container | Capsule emission (mg SF₆/day) | No. of animals | Initial age (days) | Initial weight (kg) | No. of weight recordings |
|-------|------|--------------|-------------|----------|-------------------|---------------------------|----------------|-------------------|---------------------|------------------------|
| 1     | 2011 | Heifers      | 83          | Individual pen | Canister       | 1.623 ± 0.08             | 23             | 294 ± 26          | 219 ± 28             | 4                     |
| 2     | 2011 | Bulls        | 71          | Individual pen | Canister       | 1.405 ± 0.05             | 23             | 268 ± 24          | 254 ± 34             | 19                    |
| 3     | 2012 | Bulls        | 90          | Individual pen | Canister       | 2.334 ± 0.19             | 24             | 264 ± 23          | 229 ± 34             | 13                    |
| 4     | 2012 | Heifers      | 85          | Individual pen | Canister       | 1.938 ± 0.16             | 25             | 325 ± 26          | 261 ± 28             | 14                    |
| 5     | 2018 | Bulls        | 83          | GrowSafe® Cylinder | Canister   | 3.119 ± 0.27             | 34             | 347 ± 28          | 270 ± 46             | 6                     |
| 6     | 2018 | Bulls        | 83          | GrowSafe® Cylinder | Canister   | 3.145 ± 0.23             | 36             | 354 ± 25          | 275 ± 43             | 6                     |
| 7     | 2019 | Bulls        | 83          | GrowSafe® Cylinder | Canister   | 4.549 ± 0.30             | 60             | 249 ± 31          | 224 ± 33             | 6                     |
| 8     | 2019 | Bulls        | 56          | Intergado® Cylinder | Canister   | 3.471 ± 0.17             | 58             | 647 ± 36          | 465 ± 39             | 2                     |
| 9     | 2019 | Bulls        | 56          | Intergado® Cylinder | Canister   | 3.062 ± 0.09             | 58             | 667 ± 35          | 573 ± 48             | 2                     |
| 10    | 2019 | Bulls        | 83          | GrowSafe® Cylinder | Canister   | 2.471 ± 0.15             | 62             | 329 ± 24          | 285 ± 49             | 7                     |
| 11    | 2020 | Bulls        | 83          | GrowSafe® Cylinder | Canister   | 2.621 ± 0.35             | 42             | 237 ± 24          | 226 ± 42             | 7                     |
| 12    | 2020 | Bulls        | 83          | GrowSafe® Cylinder | Canister   | 2.656 ± 0.34             | 44             | 239 ± 22          | 221 ± 33             | 7                     |

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**Table 1. Description of test groups for evaluating the association among performance, feed efficiency and enteric methane emission traits of Nellore (Bos indicus).**
estimated by the linear regression coefficient of weights on days in test (DIT) according to the equation: \( y_i = \alpha + \beta \times \text{DIT}_i + \epsilon_i \), where \( y_i \) = weight of the animal in the \( i^{th} \) observation; \( \alpha \) = intercept representing the initial weight of the animal; \( \beta \) = linear regression coefficient representing ADG; \( \text{DIT}_i \) = days in test in the \( i^{th} \) observation; \( \epsilon_i \) = random error associated with each observation. The \( \text{BW}^{0.75} \) was obtained as follows: \( \text{BW}^{0.75} = (\text{BW}_i + (0.5 \times \text{DIT} \times \text{ADG}))^{0.75} \), where \( \text{BW}_i \) = initial body weight and \( \text{DIT} \) = days in test.

The RFI was calculated as the difference between observed and expected DMI, which was estimated by multiple regression of DMI on ADG and \( \text{BW}^{0.75} \) within the test group \([i = 1, \ldots, 12; \text{formed by year of birth, sex (48 females and 441 intact males), facility, site}]\), using the GLM procedure (SAS Inst., Inc., Cary, NC). The FC was obtained as the ratio between DMI and ADG. Mean residual gain was calculated as the difference between observed and expected ADG, which was estimated by multiple regression of ADG on DMI and \( \text{BW}^{0.75} \) within the test group, using the same procedure as described above.

### Ruminal methane measurement

The modified sulfur hexafluoride (SF₆) tracer gas technique described by Deighton et al. [26] was used for methane collection. The technique uses a permeation tube or capsule
administered to the animal and deposited in the rumen. These capsules were calibrated and prepared specifically for each sampling year. The mean SF$_6$ gas emission of the capsules used during each sampling period was similar, with minimal variation in mg/day (Table 1).

Before the beginning of the sampling period, the animals were adapted to the sampling apparatus for at least seven days. Methane gas was collected for five consecutive days, with the evacuated sampling polyvinyl chloride canisters (n = 95 animals) [16,18] or stainless-steel cylinder (n = 394 animals) being changed every 24 hours (Fig 1). The gas expelled through the mouth and nostrils of the animal was aspirated under vacuum with a capillary tube fixed in a halter and connected to the collector container, which was attached to the neck of the animal (polyvinyl chloride canister) or to a saddle on the back of the animal (stainless-steel cylinder) (Table 1). Collector tubes were kept in the same environment as the animals to measure background concentrations of CH$_4$ and SF$_6$ during the sampling period. After each sampling period, the collectors were sent for gas chromatography analysis and their content was diluted with pure nitrogen to determine the quantities of SF$_6$ and CH$_4$ gases. The background concentrations of CH$_4$ and SF$_6$ measured by chromatography were subtracted from the concentrations found in the evacuated sampling containers of the animals.

There was a total of 12 CH$_4$ sampling periods (Table 1). Of the 489 animals evaluated, samples from 481 animals could be used. The losses were due to problems with the capsules. The sampling periods were September 2011; October and December 2012; November and December 2018; June, August and October 2019, and August 2020.

A gas chromatograph (HP6890, Agilent, Wilmington, Delaware, USA) was used for the analysis of CH$_4$ (ppm, parts per million) and SF$_6$ (ppt, parts per trillion). The concentrations of CH$_4$ and SF$_6$ collected in the evacuated sampling containers were determined with a flame ionization detector at 280°C (HP-Plot Al$_2$O$_3$ M column, 30 m length × 0.53 mm i.d. × 15 μm film thickness) and an electron capture detector at 300°C (HP-Plot MoleSieve column, 30 m length × 0.53 mm i.d. × 25 μm film thickness), respectively, with two loops of 0.5 cm$^2$ maintained at 80°C attached to 2 six-way valves. Chromatography analysis was carried out immediately after the end of the field sampling periods, which allowed the reuse of the evacuated containers in the subsequent sampling period.
Methane-related variables

Daily CH$_4$ emission (g/day) of each animal was obtained as the arithmetic mean of emissions on five consecutive sampling days. Enteric methane emission was also expressed as: CH$_4$ emission expressed per DMI (CH$_4$/DMI, g/kg), ADG (CH$_4$/ADG, g/kg), mid-test body weight (CH$_4$/MBW, g/kg) and BW$^{0.75}$ (CH$_4$/BW$^{0.75}$, g/kg), residual CH$_4$ emission (observed CH$_4$ – predicted CH$_4$ by regression of CH$_4$ on DMI as described by Donoghue et al. [3]), and CH$_4$ emission expressed per gross energy intake (CH$_4$ Mcal/100 Mcal GE, as described by IPCC [27]).

Statistical analysis

The animals were classified as negative RFI (RFI<0) or positive RFI (RFI>0). The variables were analyzed using the MIXED procedure (SAS Inst., Inc., Cary, NC), fitting a model that included the fixed effect of RFI class (i = 1, 2), age of animal at the start of the performance test as covariate (linear effects), and the random effects of test group (i = 1, . . . , 12), in addition to the residual random effect. The relationships of CH$_4$ (g/day) with DMI, ADG and MBW were explored by Pearson’s correlation and regression analyses using the CORR and GML procedures (SAS Inst., Inc., Cary, NC). The regression model for CH$_4$ (g/day) included the linear effect of DMI or ADG or MBW as covariate and the random effects of test group and residual. Statistical significance was declared when $P<0.05$.

Results

The mean weights (initial, mid-test and metabolic) or ADG did not differ between animals classified as negative and positive RFI (Table 3). The mean RFI was -0.556 and 0.565 kg DM/day for negative and positive RFI animals, respectively, showing a difference in DMI of 1.16 kg/day between the most and least efficient animals. Animals classified as negative RFI

| Trait                           | N  | Negative RFI (n = 246) | Positive RFI (n = 243) | SEM   | P           |
|---------------------------------|----|------------------------|------------------------|-------|-------------|
| Initial age (days)              | 489| 390                    | 389                    | 44.0  | 0.5353      |
| Initial body weight (kg)        | 489| 317                    | 317                    | 34.2  | 0.8675      |
| Mid-test body weight (kg)       | 489| 353                    | 354                    | 11.2  | 0.8498      |
| Dry matter intake (kg/day)      | 489| 7.405                  | 8.550                  | 0.23  | <0.0001     |
| Average daily gain (kg/day)     | 489| 1.228                  | 1.237                  | 0.07  | 0.7121      |
| Metabolic body weight (kg)      | 489| 79.7                   | 79.8                   | 1.59  | 0.8937      |
| RFI (kg/day)                    | 489| -0.556                 | 0.565                  | 0.03  | <0.0001     |
| Feed conversion (kg/kg)         | 489| 6.695                  | 7.764                  | 0.453 | <0.0001     |
| Residual average daily gain (kg/day) | 489| 0.066                  | -0.064                 | 0.014 | <0.0001     |
| CH$_4$ (g/day)                  | 481| 179.7                  | 189.8                  | 10.1  | 0.0022      |
| CH$_4$/DMI (g/kg/day)           | 481| 23.46                  | 21.34                  | 1.09  | <0.0001     |
| CH$_4$/ADG (g/kg/day)           | 481| 169.3                  | 175.2                  | 16.2  | 0.0724      |
| CH$_4$/MBW (g/kg)               | 481| 0.529                  | 0.548                  | 0.03  | 0.0096      |
| CH$_4$/BW$_{0.75}$ (g/kg)       | 481| 2.259                  | 2.353                  | 0.14  | 0.0033      |
| CH$_4$Res (g/day)               | 481| 4.811                  | -4.953                 | 1.95  | 0.0004      |
| CH$_4$/GE (%GE)                | 481| 7.78                   | 7.08                   | 0.41  | <0.0001     |

RFI: Residual feed intake; SEM: Standard error of the mean; CH$_4$: Enteric methane emission; CH$_4$/DMI: CH$_4$ emission expressed per dry matter intake; CH$_4$/ADG: CH$_4$ emission expressed per average daily gain; CH$_4$/MBW: CH$_4$ emission expressed per mid-test body weight; CH$_4$/BW$_{0.75}$ = CH$_4$ emission expressed per metabolic body weight; CH$_4$Res: Residual CH$_4$ emission; CH$_4$/GE: % consumed gross energy lost as CH$_4$.  

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consumed on average 13% less DM than animals classified as positive RFI; consequently, FC and residual ADG higher for more efficient animals. There was a 5% reduction of CH$_4$ emission (g/day) in negative RFI animals compared to animals with positive RFI. In addition, despite a similar performance, more efficient animals emitted less methane expressed as g CH$_4$/kg MBW and g CH$_4$/kg BW$^{0.75}$. Conversely, lower CH$_4$ emission in relation to DMI (g CH$_4$/kg DMI), lower residual CH$_4$ emission and a lower percentage of GE lost as CH$_4$ were observed in positive RFI animals compared to animals with negative RFI (Table 3).

The simple correlation coefficients of CH$_4$ (g/day) with DMI, ADG and MBW were 0.77, 0.70, and 0.78 ($P<0.0001$), respectively. Scatter plots of CH$_4$ (g/day) with DMI, ADG and MBW and their respective regression equations are shown in Figs 2–4. For each kg of DMI the animals emitted on average 17.5 g CH$_4$/day, for each kg of ADG the animals emitted on average 58.0 g CH$_4$/day, and for each kg of MBW the animals emitted 36.0 g CH$_4$/day. The regression equations of CH$_4$ on DMI, ADG and MBW within RFI class differed from one another ($P = 0.001$), accompanying the results shown in Table 3.

**Discussion**

In general, studies investigating the relationship between feed efficiency and enteric CH$_4$ emissions in cattle did not include a large number of animals, mainly because of the difficulty in measuring individual enteric CH$_4$ emissions in the animals, and the ones that did were conducted on *Bos taurus* (Table 4). In contrast, the present study evaluated 489 *Bos indicus* animals and enteric CH$_4$ was measured individually by the SF$_6$ tracer gas technique.

Greater reductions in enteric CH$_4$ emissions (15–30%) were reported in the literature for more efficient taurine animals [10,13,32] compared to the reduction of approximately 5% in the emission of zebuine animals classified as negative RFI in the present study (Table 3).

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**Fig 2.** Relationship between enteric methane (CH$_4$) emissions and dry matter intake (DMI) of Nellore bulls and heifers classified as negative (triangle) or positive (circle) residual feed intake (RFI). The general linear regression equation of CH$_4$ on DMI was: $y = 39.0 \pm 14.6 + 17.5(\pm 1.23)x + \text{residual}$. 

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Fig 3. Relationship between enteric methane (CH$_4$) emissions and average daily gain (ADG) of Nellore bulls and heifers classified as negative (triangle) or positive (circle) residual feed intake (RFI). The general linear regression equation of CH$_4$ on ADG was: $y = 112(\pm13.7) + 58.0(\pm5.31)x + \text{residual}$. 
https://doi.org/10.1371/journal.pone.0257964.g003

Fig 4. Relationship between enteric methane (CH$_4$) emissions and mid-test body weight (MBW) of Nellore bulls and heifers classified as negative (triangle) or positive (circle) residual feed intake (RFI). The general linear regression equation of CH$_4$ on MBW was: $y = 35.7(\pm13.9) + 0.43(\pm0.030)x + \text{residual}$. 
https://doi.org/10.1371/journal.pone.0257964.g004
Table 4. Studies in the literature showing the relationship between residual feed intake classes and enteric methane emission.

| Reference          | N   | Sex category | Cattle breed          | Measurement technique         | CH₄ (g/day) | CH₄/DMI (g/kg/day) | P            |
|--------------------|-----|--------------|-----------------------|-----------------------------|-------------|--------------------|--------------|
| Nkrumah et al. [13] | 19  | Steers       | Continental x British | Indirect calorimetry        | 135         | 180                | <0.05        |
| Hegarty et al. [10] | 20  | Steers       | Angus                 | SF₆                         | 142         | 190                | 0.01         |
| Jones et al. [14]  | 25  | Pregnant cows| Angus                 | OP-FTIR                     | 133         | 125                | <0.05        |
| Manafiazar et al. [34] | 48  | Heifers      | Jersey/Holstein-Friesian | GreenFeed                  | 260         | 297                | 0.04         |
| Dini et al. [32]   | 16  | Heifers      | Crossbred             | GreenFeed Respirometry chamber | 203        | 222                | 0.02         |
| Flay et al. [33]   | 56  | Heifers      | Jersey/Holstein-Friesian | GreenFeed                   | 253         | 256                | 0.60         |
| Manafiazar et al. [34] | 139 | Heifers      | Crossbred             | GreenFeed                   | 180         | 241                | <0.001       |
| Batalha et al. [19] | 24  | Bulls        | Nellore               | SF₆                         | 235         | 249                | 0.365        |

SF₆: SF₆ tracer gas technique; OP-FTIR: Open-path Fourier transform infrared spectroscopy; CH₄: Methane emission; RFI-: Negative residual feed intake.; RFI+: Positive residual feed intake.

Results similar to those of the present study were observed in crossbred taurine heifers and cows classified as more efficient, with a daily CH₄ reduction of 2.5% and 3.7%, respectively, compared to less efficient animals [34]. Lower enteric CH₄ emissions were also reported for negative RFI Angus cows compared to positive RFI cows grazing on high-quality pasture [14]. However, there was no difference in CH₄ emissions (g/day) for animals grazing a pasture of low nutritional quality [14]. On the other hand, Freetly and Brown-Brandl [15], Velazco et al. [17], Flay et al. [33] and Batalha et al. [19] found no differences in CH₄ emissions (g/day) between more and less efficient animals.

The difference in the DMI of the animals might be responsible for the differences in daily enteric CH₄ emissions between RFI classes [9,35], which would explain the results found in the present study (Table 3). Given that they have the same body weight, same weight gain and same amount of body fat, negative RFI animals tend to emit less CH₄ per day because of lower DMI [34]. Some studies evaluating CH₄ emission in animals classified as negative and positive RFI reported differences in emissions per kg of DMI, although they found no differences in CH₄ production [30,33]. However, other studies reported lower CH₄ emissions and lower CH₄ emission per DMI in negative RFI animals [29,32]. One explanation would be a lower particle passage rate in the rumen due to differences in feeding behavior since more efficient animals spend less time feeding and therefore exhibit a higher feeding rate than positive RFI animals [19,32]. Residual feed intake is an intrinsic trait of the individual that reflects maintenance requirements [36]; thus, another explanation for the lower CH₄ emissions would be a lower energy intake of negative RFI animals compared to positive RFI animals [29], since CH₄ emission is positively associated with energy intake and differences in digestibility, CH₄ emissions, heat production and energy retention are the main factors responsible for the variation of RFI between animals [13].

Lower or equal CH₄ emission (g/day), but higher emission per kg of DMI (CH₄/DMI) and a higher percentage of gross energy lost as CH₄, in negative RFI animals compared to positive RFI were observed in the present study and have also been reported by other authors [30,33,34]. The reasons for these differences in CH₄/DMI and CH₄/GE between RFI classes...
are still unclear. Possible explanation is an increase in rumen organic matter degradation with consequent increase in H$_2$ ions availability for methanogenesis in negative RFI animals [30]. Nellore animals classified based on RFI from the same contemporary groups differed in their nutrient digestive capacity [19,37,38]. Although Magnani et al. [37] and Bonilha et al. [38] demonstrated higher digestibility of dry matter (8%), neutral detergent fiber (13 to 19%) and acid detergent fiber (11%) in positive RFI animals compared to negative RFI animals, Batalha et al. [19] found lower digestibility (4.7% to 9%) of dry matter, neutral detergent fiber and acid detergent fiber, as well as of crude protein.

The lower CH$_4$ emissions per kg of live and metabolic weight observed in animals classified as negative RFI ($P = 0.0096$ and $P = 0.0033$ for CH$_4$/MBW and CH$_4$/BW$^{0.75}$, respectively) were similar to the results reported by Nkrumah et al. [13] and Fitzsimons et al. [28]. These findings indicate that, regardless of the effects of DMI, potential selection of cattle for RFI and reduced enteric CH$_4$ emission is possible [16,28]. The strong relationship between CH$_4$ and DMI and the divergent results reported in the literature for RFI and CH$_4$ emissions underscore the lack of evidence of a direct effect of RFI on enteric methane emission [35].

Considering the number of animals evaluated in this study and the fact that negative and positive RFI animals were compared (and not only extreme animals), it is possible to state that CH$_4$ emissions per kg of live body weight were different, with lower values in more efficient animals. This confirms the hypothesis that negative RFI animals emit less CH$_4$ per day or per kg of live body weight or per kg of ADG and, furthermore, these animals have strong potential to emit less CH$_4$ per kg of carcass. Considering that live body weight in the present study is the yearling weight, and the evidence of high genetic (0.55 to 0.89) and phenotypic (0.67 to 0.72) correlation between yearling weight and carcass weight [39–41], live body weight is a real indicator of the carcass weight.

Differences in the number of methanogenics in the rumen between more and less efficient animals may explain the differences observed in the intensity of CH$_4$ emission, regardless of the diet supplied [42]. In fact, Lopes et al. [43] and Andrade et al. [44] reported differences in the microbial composition of fecal samples between Nellore animals classified as negative and positive RFI. However, these differences in microbial populations between negative and positive RFI cattle may be due to differences in the ruminal passage rate and digestion as a result of different DMI levels [45]. Another approach to explain the variation in CH$_4$ emission between more and less efficient animals independent of DMI would be to identify differences in the efficiency of feed utilization, such as nutrient absorption, appetite regulation, and cell metabolism [35].

In conclusion, more efficient animals emit less CH$_4$ expressed as g/day and per kg of live body weight than less efficient animals, suggesting lower emission per kg of carcass. However, it is not possible to state that RFI has a direct effect on enteric CH$_4$ emissions since emission per kg of consumed dry matter and the percentage of gross energy lost as CH$_4$ were greater for negative RFI animals.

**Supporting information**

S1 Table. Description of test groups for evaluating the association among performance, feed efficiency and enteric methane emission traits of Nellore (*Bos indicus*).

(DOCX)

S2 Table. Percentage of ingredients and nutrient composition of diets offered to the animals during the performance test according each test group.

(DOCX)
S3 Table. Mean values of performance, feed efficiency and enteric methane emission traits according to residual feed intake class of Nellore (Bos indicus).

(DOCX)

S4 Table. Studies in the literature showing the relationship between residual feed intake classes and enteric methane emission.

(DOCX)

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**References**

1. Johnson KA, Johnson DE. Methane emissions from cattle. J Anim Sci. 1995; 73(8):2483–92. https://doi.org/10.2527/1995.7382483x PMID: 8567486.

2. Warner D, Bannink A, Hattem B, van Laar H, Dijkstra J. Effects of grass silage quality and level of feed intake on enteric methane production in lactating dairy cows. J Anim Sci. 2017; 95(8):3687. https://doi.org/10.2527/jas.2017.1459 PMID: 28805897.

3. Donoghue KA, Bird-Gardiner T, Arthur PF, Herd RM, Hegarty RF. Genetic and phenotypic variance and covariance components for methane emission and postweaning traits in Angus cattle. J Anim Sci. 2016; 94(4):1438–45. https://doi.org/10.2527/jas.2015-0065 PMID: 27136003.

4. Roehe R, Dewhurst RJ, Duthie C-A, Rooke JA, McKain N, Ross DW, et al. Bovine host genetic variation influences rumen microbial methane production with best selection criterion for low methane emitting and efficiently feed converting hosts based on metagenomic gene abundance. PLoS Genet. 2016; 12(2):e1005846. https://doi.org/10.1371/journal.pgen.1005846 PMID: 26891056.

5. Grion AL, Mercadante MEZ, Cyrillo JNSG, Bonilha SFM, Magnani E, Branco RH. Selection for feed efficiency traits and correlated genetic responses in feed intake and weight gain of Nellore cattle. J Anim Sci. 2014; 92(3):955–65. https://doi.org/10.2527/jas.2013-6682 PMID: 24492579.
6. Ceacero TM, Mercadante MEZ, Cyrillo JNSG, Canesin RC, Bonilha SFM, de Albuquerque LG. Pheno-
typic and genetic correlations of feed efficiency traits with growth and carcass traits in Nellore cattle
selected for postweaning weight. PLoS One. 2016; 11(8):e0161366. https://doi.org/10.1371/journal.
pone.0161366 PMID: 27537268.

7. Carberry CA, Kenny DA, Han S, McCabe MS, Waters SM. Effect of phenotypic residual feed intake and
dietary forage content on the rumen microbial community of beef cattle. Appl Environ Microbiol. 2012;
78(14):4949–58. https://doi.org/10.1128/AEM.07759-11 PMID: 22562991.

8. Beauchemin KA, McGinn SM. Methane emissions from feedlot cattle fed barley or corn diets. J Anim
Sci. 2005; 83(3):631–61. https://doi.org/10.2527/2005.833633x PMID: 15705762.

9. Grainger C, Clarke T, McGinn SM, Auld DJ, Beauchemin KA, Hannah MC, et al. Methane emissions
from dairy cows measured using the sulfur hexafluoride (SF₆) tracer and chamber techniques. J Dairy
Sci. 2007; 90(6):2755–66. https://doi.org/10.3168/jds.2006-967 PMID: 17517715.

10. Hegarty RS, Goopy JP, Herd RM, McCorkell B. Cattle selected for lower residual feed intake have
reduced daily methane production. J Anim Sci. 2007; 85(6):1479–86. https://doi.org/10.2527/jas.2006-
236 PMID: 17296777.

11. Kelly AK, McGee M, Crews DH Jr, Fahey AG, Wylie AR, Kenny DA. Effect of divergence in residual
feed intake on feeding behavior, blood metabolic variables, and body composition traits in growing beef
heifers. J Anim Sci. 2010; 88(1):109–23. https://doi.org/10.2527/jas.2009-2196 PMID: 19820067.

12. Lawrence P, Kenny DA, Earley B, Crews DH Jr, McGee M. Grass silage intake, rumen and blood vari-
ables, ultrasonic and body measurements, feeding behavior, and activity in pregnant beef heifers differ-
ning in phenotypic residual feed intake. J Anim Sci. 2011; 89(10):3248–61. https://doi.org/10.2527/jas.
2010-3774 PMID: 21622881.

13. Nkrumah JD, Okine EK, Mathison GW, Schmid K, Li C, Basarab JA, et al. Relationships of feedlot feed
efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy par-
titioning in beef cattle. J Anim Sci. 2006; 84(1):145–53. https://doi.org/10.2527/2006.841145x PMID:
16361501.

14. Jones FM, Phillips FA, Naylor T, Mercer NB. Methane emissions from grazing Angus beef cows
selected for divergent residual feed intake. Anim Feed Sci Technol. 2011; 166–167:302–7. https://doi.
org/10.1016/j.anifeedsci.2011.04.020.

15. Freerly HC, Brown-Brandl TM. Enteric methane production from beef cattle that vary in feed efficiency.
J Anim Sci. 2013; 91(10):4826–31. https://doi.org/10.2527/jas.2011-4781 PMID: 23965389.

16. Mercadante MEZ, Caliman AP de M, Canesin RC, Bonilha SFM, Berndt A, Frighetto RTS, et al. Relation-
ship between residual feed intake and enteric methane emission in Nellore cattle. Rev Bras Zootec. 2015;
44(7):255–62. http://dx.doi.org/10.1590/S1806-92902015007000004.

17. Velazco JI, Herd RM, Cottle DJ, Hegarty RS. Daily methane emissions and emission intensity of grazing
beef cattle genetically divergent for residual feed intake. Anim Prod Sci. 2017; 57(4):827–35. https://doi.
org/10.1071/AN15111.

18. Oliveira LF, Ruggieri AC, Branco RH, Cotta OL, Canesin RC, Costa HJU, et al. Feed efficiency and
enteric methane production of Nellore cattle in the feedlot and on pasture. Anim Prod Sci. 2018; 58
(5):886. https://doi.org/10.1071/AN16303.

19. Batalha CDA, Morelli M, Branco RH, Cyrillo JNSG, Carrilho RC, Mercadante MEZ, et al. Associa-
tion between residual feed intake, digestion, ingestive behavior, enteric methane emission and nitrogen
metabolism in Nellore beef cattle. Anim Sci J. 2020; 91(1):e13455. https://doi.org/10.1111/ajas.13455
PMID: 33025683.

20. Weiss WP, editor. Energy prediction equations for ruminant feeds. Proceedings of the 61st Cornell
Nutrition Conference for Feed Manufactures (Cornell University, Ithaca) pp 176–85; 1999.

21. NRC. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washingto
n, DC. 2000.

22. AOAC. Association of Official Analytical Chemistry. Official methods of analysis. 15th ed. Assoc. Off.
Anal. Chem., Arlington, VA. 1990.

23. Mertens D.R. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with reflux-
ing in beakers or crucibles: collaborative study. J AOAC Int. 2002; 85(6):1217–40. PMID: 12477183.

24. Etheridge RD, Pesti GM, Foster EH. A comparison of nitrogen values obtained utilizing the Kjeldah-
l nitrogen and Dumas combustion methodologies (LECO CNS 2000) on samples typical of an animal
nutrition analytical laboratory. Anim Feed Sci Technol. 1998; 73:21–8. https://doi.org/10.1016/S0377-
8401(98)00136-9.

25. Sniffen CJ, O’Connor JD, Van Soest PJ. A net carbohydrate and protein for evaluating cattle diets. II.
Carbohydrate and protein availability. J Anim Sci. 1993; 70:3562–77. https://doi.org/10.2527/1992.
70113562x PMID: 1459919.
26. Deighton MH, Williams SRO, Hannah MC, Eckard RJ, Boland TM, Wales WJ, et al. A modified sulphur hexafluoride tracer technique enables accurate determination of enteric methane emissions from ruminants. Anim Feed Sci Technol. 2014; 197:47–63. https://doi.org/10.1016/j.anifeedsci.2014.08.003.

27. IPCC (2006) Emissions from livestock and manure management, chapter 10. In: Guidelines for National Greenhouse Gas Inventories, Vol 4, Prepared by the National Greenhouse Gas Inventories Programme (eds Eggleston HS, Buendia L, Miwa K, Ngara T, Tanabe K), 10.7–10.84. IGES, Japan.

28. Fitzsimmons C, Kenny DA, Deighton MH, Fahey AG, McGee M. Methane emissions, body composition, and rumen fermentation traits of beef heifers differing in residual feed intake. J Anim Sci. 2013; 91 (12):5789–800. https://doi.org/10.2527/jas.2013-6956 PMID: 24146149.

29. Sharma VC, Mahesh MS, Mohini M, Datt C, Nampoorthi VM. Nutrient utilisation and methane emissions in Sahiwal calves differing in residual feed intake. Arch Anim Nutr. 2014; 68(5):345–57. https://doi.org/10.1080/1745039X.2014.951193 PMID: 25156936.

30. McDonnell RP, Hart KJ, Boland TM, Kelly AK, McGee M, Kenny DA. Effect of divergence in phenotypic residual feed intake on methane emissions, ruminal fermentation, and apparent whole-tract digestibility of beef heifers across three contrasting diets. J Anim Sci. 2016; 94(3):1179–93. https://doi.org/10.2527/jas.2015-0080 PMID: 27065279.

31. Alemu AW, Vyas D, Manafiazar G, Basarab JA, Beauchemin KA. Enteric methane emissions from low–residual feed intake beef heifers measured using GreenFeed and respiration chamber techniques. J Anim Sci. 2017; 95(8):3727. https://doi.org/10.2527/jas.2017.1501 PMID: 28805902.

32. Dini Y, Cajarville C, Gere JI, Fernandez S, Fraga M, Pravia MI, et al. Association between residual feed intake and enteric methane emissions in Hereford steers. Transl Anim Sci. 2019; 3(1):239–46. https://doi.org/10.1093/lias/byx111 PMID: 32704795.

33. Flay HE, Kuhn-Sherlock B, Macdonald KA, Camara M, Lopez-Villalobos N, Donaghy DJ, et al. Hot topic: Selecting cattle for low residual feed intake did not affect daily methane production but increased methane yield. J Dairy Sci. 2019; 102(3):2708–13. https://doi.org/10.3168/jds.2018-15234 PMID: 30639015.

34. Manafiazar G, Baron VS, McKeown L, Hoffmann K, Plastow G, et al. Methane and carbon dioxide emissions from yearling beef heifers and mature cows classified for residual feed intake under drylot conditions. Can J Anim Sci. 2020; 100(3):522–35. https://doi.org/10.1139/cjas-2019-0032.

35. Kenny DA, Fitzsimmons C, Waters SM, McGee M. Invited review: Improving feed efficiency of beef cattle—the current state of the art and future challenges. Animal. 2018; 12(9):1815–26. https://doi.org/10.1017/S1751731118003976 PMID: 29779496.

36. Arthur JPF, Herd RM. Residual feed intake in beef cattle. Rev Bras Zootec. 2008; 37(special):269–79. http://dx.doi.org/10.1590/S1516-35982008001300031.

37. Magnani E, Nascimento CF, Branco RH, Bonilha SFM, Ribeiro EG, Mercadante MEZ. Relações entre consumo alimentar residual, comportamento ingestivo e digestibilidade em novilhas Nelore. Bol Ind Anim. 2013; 70(2):187–94. https://doi.org/10.17523/bia.v70n2p187.

38. Bonilha SFM, Branco RH, Mercadante MEZ, Cyrillo JNSG, Monteiro FM, Ribeiro EG. Digestion and metabolism of low and high residual feed intake Nelore bulls. Trop Anim Health Prod. 2017; 49(3):529–35. https://doi.org/10.1007/s11250-017-2244-9 PMID: 28124731.

39. Meyer K, Johnson DJ, Graser H-U. Estimates of the complete genetic covariance matrix for traits in multi-trait genetic evaluation of Australian Hereford cattle. Aust J Agric Res. 2004; 55(2):195–210. https://doi.org/10.1071/AR03164.

40. Bergen R, Miller SP, Wilton JW. Genetic correlations among indicator traits for carcass composition measured in yearling beef bulls and finished feedlot steers. Can J Anim Sci. 2005; 85(4):463–73. https://doi.org/10.4141/A05-013.

41. Tonussi RL, Espigolan R, Gordo DGM, Magalhães AFB, Venturini GC, Baldi F, et al. Raits in Nelore cattle. Genet Mol Res. 2015; 14(4):16713–19. https://doi.org/10.4238/2015.December.28.20 PMID: 26782521.

42. Carberry CA, Waters SM, Kenny DA, Creevey CJ. Rumen methanogenic genotypes differ in abundance according to host residual feed intake phenotype and diet type. Appl Environ Microbiol. 2014; 80 (2):586–94. https://doi.org/10.1128/AEM.03131-13 PMID: 24212590.

43. Lopes DRG, La Reau AJ, Duarte M de S, Detmann E, Bento CBP, Mercadante MEZ, et al. The bacterial and fungal Microbiota of Nelore steers is dynamic across the gastrointestinal tract and its fecal-associated Microbiota is correlated to feed efficiency. Front Microbiol. 2019; 10:1263. https://doi.org/10.3389/fmicb.2019.01263 PMID: 31293524.

44. Andrade BQN, Bressani FA, Cuadrat RRC, de Oliveira PSN, Mourão GB, et al. The structure of microbial populations in Nelore GIT reveals inter-dependency of methanogens in feces and rumen. J Anim Sci Biotechnol. 2020; 11(1):6. https://doi.org/10.1186/s40104-019-0422-x PMID: 32123563.
45. Freitly HC, Lindholm-Perry AK, Hales KE, Brown-Brandl TM, Kim M, Myer PR, et al. Methane production and methanogen levels in steers that differ in residual gain. J Anim Sci. 2015; 93(5):2375–81. https://doi.org/10.2527/jas.2014-8721 PMID: 26020333.