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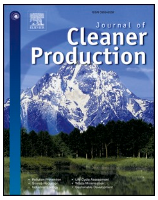

# Enteric methane mitigation strategies for ruminant livestock systems in the Latin America and Caribbean region: A meta-analysis

Guilhermo Francklin de Souza Congio [a,b,*], André Bannink [c], Olga Lucía Mayorga Mogollón [a], Latin America Methane Project Collaborators[1], Alexander Nikolov Hristov [d,**]

[a] Colombian Corporation for Agricultural Research, AGROSAVIA, Tibaitatá, Bogotá, D.C, 250047, Colombia
[b] Department of Animal Science, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba, 13418-900, SP, Brazil
[c] Wageningen Livestock Research, Wageningen University & Research, Wageningen, 6700, AH, the NetherlandsThe Netherlands
[d] Department of Animal Science, The Pennsylvania State University, 335 Agricultural Sciences and Industries Building, University Park, 16802, PA, USA

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## ABSTRACT

Latin America and Caribbean (LAC) is a developing region characterized for its importance for global food security, producing 23 and 11% of the global beef and milk production, respectively. The region's ruminant livestock sector however, is under scrutiny on environmental grounds due to its large contribution to enteric methane ($CH_4$) emissions and influence on global climate change. Thus, the identification of effective $CH_4$ mitigation strategies which do not compromise animal performance is urgently needed, especially in context of the Sustainable Development Goals (SDG) defined in the Paris Agreement of the United Nations. Therefore, the objectives of the current study were to: 1) collate a database of individual sheep, beef and dairy cattle records from enteric $CH_4$ emission studies conducted in the LAC region, and 2) perform a meta-analysis to identify feasible enteric $CH_4$ mitigation strategies, which do not compromise animal performance. After outlier's removal, 2745 animal records (65% of the original data) from 103 studies were retained (from 2011 to 2021) in the LAC database. Potential mitigation strategies were classified into three main categories (*i.e.,* animal breeding, dietary, and rumen manipulation) and up to three subcategories, totaling 34 evaluated strategies. A random effects model weighted by inverse variance was used (Comprehensive Meta-Analysis V3.3.070). Six strategies decreased at least one enteric $CH_4$ metric and simultaneously increased milk yield (MY; dairy cattle) or average daily gain (ADG; beef cattle and sheep). The breed composition F1 Holstein × Gyr decreased $CH_4$ emission per MY ($CH_4I_{Milk}$) while increasing MY by 99%. Adequate strategies of grazing management under continuous and rotational stocking decreased $CH_4$ emission per ADG ($CH_4I_{Gain}$) by 22 and 35%, while increasing ADG by 22 and 71%, respectively. Increased dietary protein concentration, and increased concentrate level through cottonseed meal inclusion, decreased $CH_4I_{Milk}$ and $CH_4I_{Gain}$ by 10 and 20% and increased MY and ADG by 12 and 31%, respectively. Lastly, increased feeding level decreased $CH_4I_{Gain}$ by 37%, while increasing ADG by 171%. The identified effective mitigation strategies can be adopted by livestock producers according to their specific needs and aid LAC countries in achieving SDG as defined in the Paris Agreement.

## 1. Introduction

The Latin America and Caribbean (LAC) region cattle population comprises 28% of the global herd (FAOSTAT, 2020), accounting for 23 and 11% of the global beef and milk production, respectively (FAO, 2020), and thereby plays a relevant role in meeting world's growing animal protein demand (Conforti, 2011; Alexandratos and Bruinsma, 2012). In addition to accounting for 46% of the region's agricultural gross domestic product (FAO, 2020), the livestock sector is especially important for livelihoods and reduction of local poverty (OECD/FAO, 2019). However, despite its social and economic importance, livestock is also a major source of greenhouse gas (GHG) emissions in LAC countries, particularly enteric methane ($CH_4$) (Arango et al., 2020), which has a

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* Corresponding author. Colombian Corporation for Agricultural Research, AGROSAVIA, Tibaitatá, Bogotá D.C. 250047, Colombia.
** Corresponding author. Department of Animal Science, The Pennsylvania State University, 335 Agricultural Sciences and Industries Building, University Park, 16802, PA, USA.
*E-mail addresses:* gcongio@gmail.com (G.F.S. Congio), anh13@psu.edu (A.N. Hristov).

[1] A comprehensive list of authors and affiliations appear at the end of the paper.

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**Abbreviations and notations**

| | |
|---|---|
| ADG | Average daily gain |
| $CH_4$ | Methane |
| $CH_4$ production | Daily methane production |
| $CH_4$ yield | $CH_4$ per unit of DMI |
| $CH_4I_{Gain}$ | $CH_4$ intensity; $CH_4$ emission per unit of ADG |
| $CH_4I_{Milk}$ | $CH_4$ intensity; $CH_4$ emission per unit of MY |
| CI | Confidence interval |
| CP | Crude protein |
| CT | Condensed tannins |
| DDGS | Dried distillers' grains with solubles |
| DMI | Dry matter intake |
| EE | Ether extract |
| GEI | Gross energy intake |
| GHG | Greenhouse gas |
| HT | Hydrolyzable tannins |
| LAC | Latin America and Caribbean |
| LAMP | Latin America Methane Project |
| MY | Milk yield |
| N | Nitrogen |
| $N_2O$ | Nitrous oxide |
| NDF | Neutral-detergent fiber |
| NPN | Non-protein nitrogen |
| OMI | Organic matter intake |
| RFI | Residual feed intake |
| SDG | Sustainable Development Goals |
| $SF_6$ | Sulfur hexafluoride |
| UNFCCC | United Nations Framework Convention on Climate Change |
| $Y_m$ | $CH_4$ energy as a percentage of GEI |

disproportionally greater impact on short-term global warming and climate change (Herrero et al., 2016; Mehrabi et al., 2020).

According to the 2020 report of The Lancet Countdown on health and climate change, ruminant livestock continue to dominate agriculture's contribution to climate change being responsible for 56% of total agricultural GHG emissions and 93% of all livestock emissions globally (Watts et al., 2021). Considering that $CH_4$ is a powerful but short-lived GHG, decreasing its emission is essentially important for limiting global warming in the short-term (European Commission, 2020; Arndt et al., 2021). This is in line with IPCC (2018) emission reduction targets and with sustainable development guidelines resulting from the Paris Agreement of the United Nations Framework Convention on Climate Change (UNFCCC) (UN General Assembly, 2015). The latter has defined 17 Sustainable Development Goals (SDG) which guide mutual efforts from the international community towards achieving emission reduction targets by 2030 (UNFCCC, 2015). The SDG include actions related to eradicating poverty and hunger, achieve food security, and urgent efforts to tackle climate change. In this context, it is important to emphasize that efforts to combat climate change, cannot jeopardize successes in eliminating poverty and hunger (Arndt et al., 2021).

In view of the above, identification of effective $CH_4$ mitigation strategies which do not compromise animal performance is urgently needed, especially in LAC countries, where livestock has a key role for livelihoods. Ideally, such mitigation strategies should increase animal productivity since about 25% of the growing global demand for beef by 2028 is expected to come from the LAC region (OECD/FAO, 2019). Previous comprehensive reviews have reported effective enteric $CH_4$ mitigation strategies (Beauchemin et al., 2008; Hristov et al., 2013b). However, these analyses did not use a quantitative approach, were applicable mostly to intensive production systems and did not evaluate animal performance variables. Therefore, the objectives of the current study [referred to as 'Latin America Methane Project' (LAMP)] were to: 1) collate a database of individual animal records from enteric $CH_4$ emission and mitigation studies conducted in the LAC region, and 2) perform a meta-analysis to identify feasible enteric $CH_4$ mitigation strategies, which do not compromise animal performance. The novelty of the current meta-analysis is that it used a quantitative approach to assess potential enteric $CH_4$ mitigation strategies along with their effect on animal performance, specifically targeting LAC livestock systems, which are typically more extensive that production systems examined in previous reviews.

## 2. Material and methods

The LAMP is an international collaborative initiative specifically designed to involve LAC animal scientists that work on enteric $CH_4$ emissions from ruminants and recommend $CH_4$ mitigation practices that are feasible for the LAC region. It is integrated with activities from the 'Global Network' project (https://globalresearchalliance.org/research/livestock/collaborative-activities/global-research-project/; accessed March 16, 2021) which is an activity of the 'Feed and Nutrition Network' of the Livestock Research Group within the Global Research Alliance for Agricultural Greenhouse Gases (https://globalresearchalliance.org/research/livestock/networks/feed-nutrition-network/; accessed March 16, 2021).

### 2.1. Database

The LAMP collated a database containing 4208 individual animal records (beef cattle $n = 2044$; dairy cattle $n = 1710$; sheep $n = 454$) from 132 published ($n = 56$) and unpublished ($n = 76$) studies carried out from 2011 to 2021 by researchers from Brazil ($n = 3000$ from 71 studies), Mexico ($n = 273$ from 17 studies), Colombia ($n = 272$ from 18 studies), Costa Rica ($n = 247$ from 4 studies), Argentina ($n = 230$ from 13 studies), Peru ($n = 89$ from 5 studies), Chile ($n = 81$ from 3 studies), and Uruguay ($n = 16$ from 1 study). The LAC database is of a comparable size to other databases developed by the 'Global Network' project team and evaluated by a meta-analysis approach (Niu et al., 2018; Van Lingen et al., 2019). For the purpose of the present analysis, the following animal output variables were evaluated: daily $CH_4$ production (g/d), $Y_m$ [$CH_4$ energy as a percentage of gross energy intake (GEI)], $CH_4$ yield [$CH_4$ production ÷ dry matter intake (DMI); g/kg], DMI (kg/d), milk yield (MY; kg/d; dairy cattle), average daily gain (ADG; kg/d; growing cattle and sheep), and $CH_4$ emission intensities [$CH_4$ production ÷ MY ($CH_4I_{Milk}$) and $CH_4$ production ÷ ADG ($CH_4I_{Gain}$); both in g/kg]. The database was comprised of different sampling methods and measurement techniques. Enteric $CH_4$ was measured using the sulfur hexafluoride ($SF_6$; 72.2% of the studies) technique, respiration chambers (17.5%), the GreenFeed system (C-Lock Inc., Rapid City, SD) (5.2%), and others (*i.e.*, laser detector, polytunnel, and head-box; 5.1%). Advantages and shortcomings of $CH_4$ measurement techniques have been discussed by the 'Global Network' project team (Hammond et al., 2016). Dry matter intake was estimated using markers (54.4% of the studies), gravimetrically (36.7%), using electronic monitoring systems (6.6%), and by sward cutting technique (2.3%) (Smit et al., 2005; Chizzotti et al., 2015; De Souza et al., 2015).

The feed management systems were grazing or pasture-based (58.3% of the studies; being 48.8% with beef cattle, 37.5% with dairy cattle, and 13.7% with sheep) and confined, including beef feedlot cattle (26.6%), dairy cattle (12.3%), and sheep (2.8%) systems. Studies in which pasture-based diets were provided to the animals in feed bunks (typically necessitated by the enteric $CH_4$ measurement method; *e.g.,* respiration chamber and polytunnel), were considered as confinement system in the database. Across animal categories, dietary forage content from pasture-based systems ranged from 20 to 100% of DMI (average ± SD; 87 ± 18.0%). For beef feedlot, dairy and sheep confinement systems, the dietary forage content ranged from 17 to 100% of DMI (62 ± 21.3%), from 44 to 94% DMI (62 ± 11.4%) and from 40 to 100% DMI (64 ± 15.4%), respectively. Among dietary forage sources from pasture-based systems *Urochloa* spp., *Megathyrsus maximus* and *Pennisetum* spp. were the main tropical species, and *Lolium* spp., *Avena* spp., *Festuca* spp., *Medicago sativa* and *Trifolium* spp. were the predominant temperate species. For beef feedlot systems, corn silage, tropical hays and fresh-cut grass were the main dietary forage sources, whilst corn silage and corn silage plus temperate hays were most representative for dairy confinement systems. For sheep confinement systems, tropical hays and fresh-cut grass were the most used dietary forage sources. Concentrate ingredients frequently used in the diets included solvent-extracted soybean meal, soybean expeller, ground corn grain, wheat meal, sorghum grain and others (Tables A.1 and A.2; Appendix A).

The studies in the LAC database were conducted with a broad variety of pure breed and crossbreed animals. The beef cattle dataset included mostly Nellore (38.9% of beef cattle observations), Angus (21.8%), Brahman (3.7%), Angus × Hereford (3.0%), Brangus (2.8%), Angus × Hereford × Nellore (2.6%), Angus × Nellore (1.8%), Charolais (1.6%), and other crosses including Zebu cattle (18.0%). The dairy dataset included mainly Holstein (36.8% of dairy cattle observations), Holstein × Jersey (21.8%), Holstein × Gyr (15.0%), Sahiwal (10%), Gyr (7.3%), and Jersey (5.2%). The database also contained some breeds that can be considered dual-purpose, such as Costeño and Brown Swiss. Sheep breeds were mostly Texel × Polwarth (22.8% of sheep observations), Santa Ines (18.9%), Texel × Suffolk (13.4%), White Dorper × Suffolk (12.0%), Ile de France (9.7%), Pelibuey (8.9%), and Texel × Ile de France (7.2%).

### 2.2. Statistical procedures

Records with missing $CH_4$ production and DMI values as well as trials without a clear assignment of treatments were removed from the database, as the objective of the study was to identify effective strategies of enteric $CH_4$ mitigation. Outliers were identified for all variables considering each trial separately, using the interquartile range method (Zwillinger and Kokoska, 2000) considering a factor of 1.5 for extremes with the boxplot procedure of SAS (SAS Institute Inc., Cary, NC; version 9.4). After outlier's removal, 2745 records (65% of the original data) from 103 studies were retained (the complete list of references used in the current analysis is given at the end of Appendix A). Each study was examined individually to assign treatments as control (*i.e.,* baseline condition) and treatment (*i.e.,* a strategy aimed at reducing enteric $CH_4$ emission). This step was performed consulting the collaborators for unpublished and individual publications for published work (Table A.1; Appendix A). Studies with a factorial arrangement with more than one factor potentially able to mitigate enteric $CH_4$ were considered separately in the database. The potential mitigating strategies were attributed to one main category and then classified in sub-category levels according to the literature (Hristov et al., 2013a; Beauchemin et al., 2020). Main and first-order categories are presented in Fig. 1 (for a complete list of potential mitigating strategies see Table A.1; Appendix A). Main categories, first-order categories and second-order sub-categories were respectively highlighted as bold, underlined and italic to facilitate the reading. Potential mitigation treatments with each strategy coming from only one study were not analyzed individually because of bias, but they were considered in the sub-category analysis they had become (*e.g.,* Nellore cattle breed was not evaluated individually but it was considered in the genetic selection and *breed composition* analyses; Table A.2; Appendix A). The effects of mitigation strategies on enteric $CH_4$ (*i.e.,* $CH_4$ production, $Y_m$, $CH_4$ yield, and $CH_4$ intensities) and animal performance (*i.e.,* DMI, MY, and ADG) were estimated as the

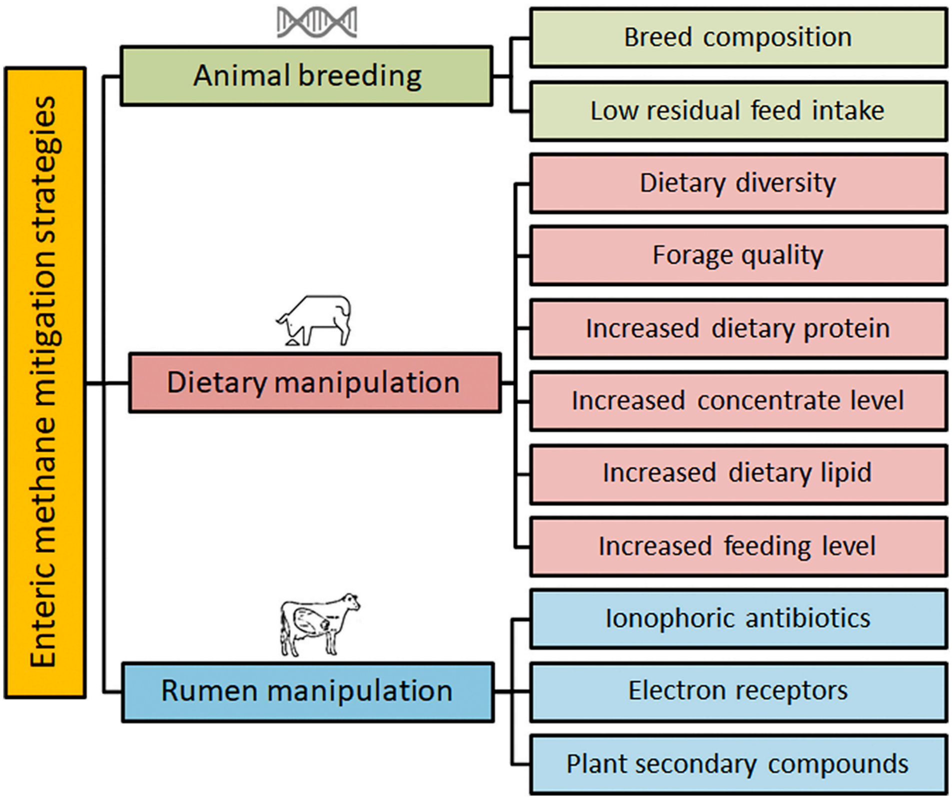


**Fig. 1.** Main and first-order mitigation strategies included in the current meta-analysis. For a complete list of potential mitigating strategies see Table A.1 (Appendix A).

difference between the mitigation treatment and its respective control, divided by control treatment, expressed as a percentage [*i.e.,* (mitigation treatment – control treatment) ÷ control treatment × 100]. All potential mitigation strategies and control treatments (to which each mitigation strategy was compared to) are described in Table A.1 (Appendix A). The meta-analysis was performed using Comprehensive Meta-Analysis software (CMA, Biostat Inc., Englewood, NJ, version 3.3.070; Borenstein et al., 2014) with a random-effects model and values weighted by inverse variance (St Pierre, 2001). Means, standard deviations and number of observations were used to compute the effect sizes. Heterogeneity between observed effect sizes was examined with the Cochran's $Q$ test and the $I^2$ statistic. Funnel plots were used to visually assess potential publication bias. The pooled effect size was reported as the relative mean ratio with its 95% confidence interval (CI), where positive values indicate an increment of the potential mitigation strategy compared with control treatments, and negative values indicate a reduction. Statistical differences were considered significant at adjusted $P \leq 0.05$.

## 3. Results

A total of 34 sub-categories were evaluated as potential enteric $CH_4$ mitigation treatments distributed in three main categories **animal breeding**, **dietary manipulation** and **rumen manipulation**, which comprised 11.4, 62.9 and 25.7%, respectively, of the total strategies assessed (Table A.1; Appendix A). Further, 32 potential mitigation strategies with each strategy coming from only one study were not evaluated individually (Table A.2; Appendix A). The complete results from the meta-analysis are presented in the Appendix A including Tables A.3 to A.6 which contain mean effect sizes, 95% CI levels, $P$-values and $n$, and forest plots with mean effect sizes and 95% CI levels (Figs. B.1 to B.8; Appendix B). Results for successful enteric $CH_4$ mitigation strategies (*i.e.,* those that decreased enteric $CH_4$ without affecting negatively animal performance) are presented in Fig. 2 and Fig. 3.

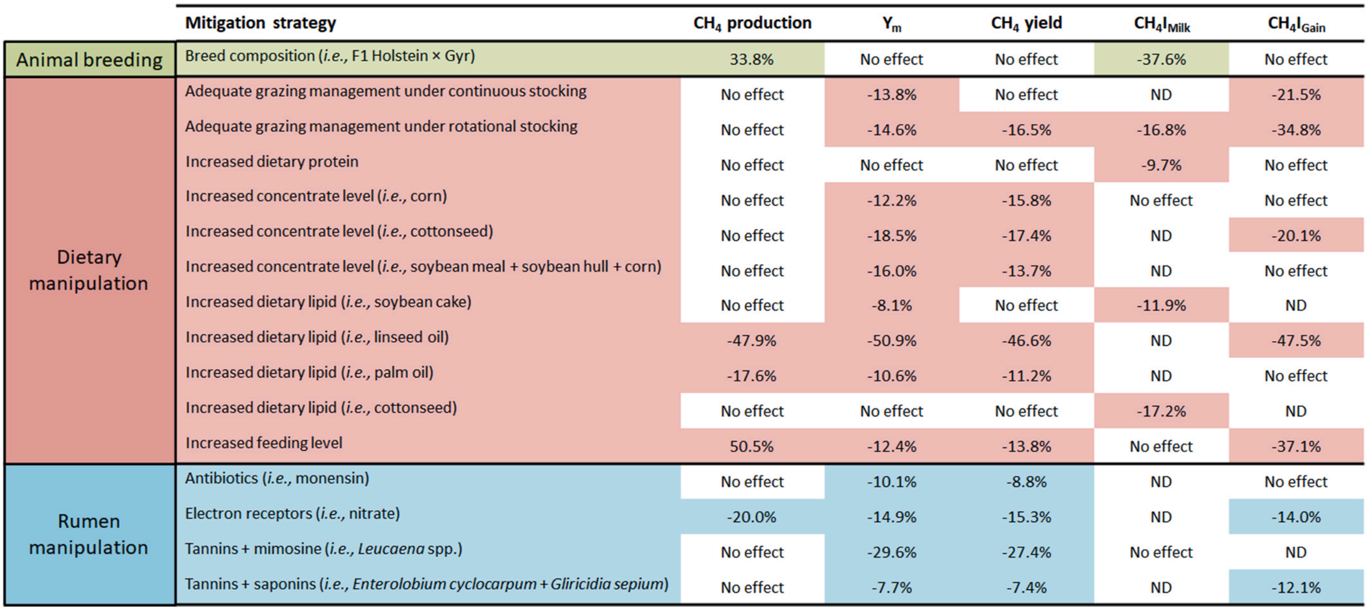
### 3.1. Animal breeding

Within the *breed composition* sub-category (included into the overall genetic selection category), Holstein increased $CH_4$ production ($P = 0.006$) by 24.1% (Table A.3 and Fig. B.1), $CH_4$ yield ($P = 0.001$) by 8.8% (Table A.4 and Fig. B.3), DMI ($P = 0.024$) by 14.0% (Table A.4 and Fig. B.4), and MY ($P = 0.012$) by 10.8% (Table A.6 and Fig. B.7), but did not reduce $Y_m$ and $CH_4I_{Milk}$. The F1 Holstein × Gyr breed increased ($P < 0.001$) $CH_4$ production, DMI and MY by 33.8, 41.0, and 98.5%, respectively, while it decreased ($P < 0.001$) $CH_4I_{Milk}$ by 37.6%, and did not influence $Y_m$ and $CH_4$ yield (Figs. 2 and 3). The ADG was 65.1 and 76.6% greater ($P < 0.001$) for F1 Holstein × Gyr and Holstein, respectively (Fig. 3; Table A.6 and Fig. B.8), but these breeds had no effect on $CH_4I_{Gain}$ (Fig. 2; Table A.5 and Fig. B.6). *Low residual feed intake (RFI)* animals increased $Y_m$ ($P = 0.022$) and $CH_4$ yield ($P = 0.019$) by 4.3% (Tables A.3-A.4 and Figs. B.2-B.3) while decreasing ($P < 0.001$) DMI by 7.2% (Table A.4 and Fig. B.4) and did not affect $CH_4$ production, $CH_4I_{Gain}$ or ADG.

### 3.2. Dietary manipulation

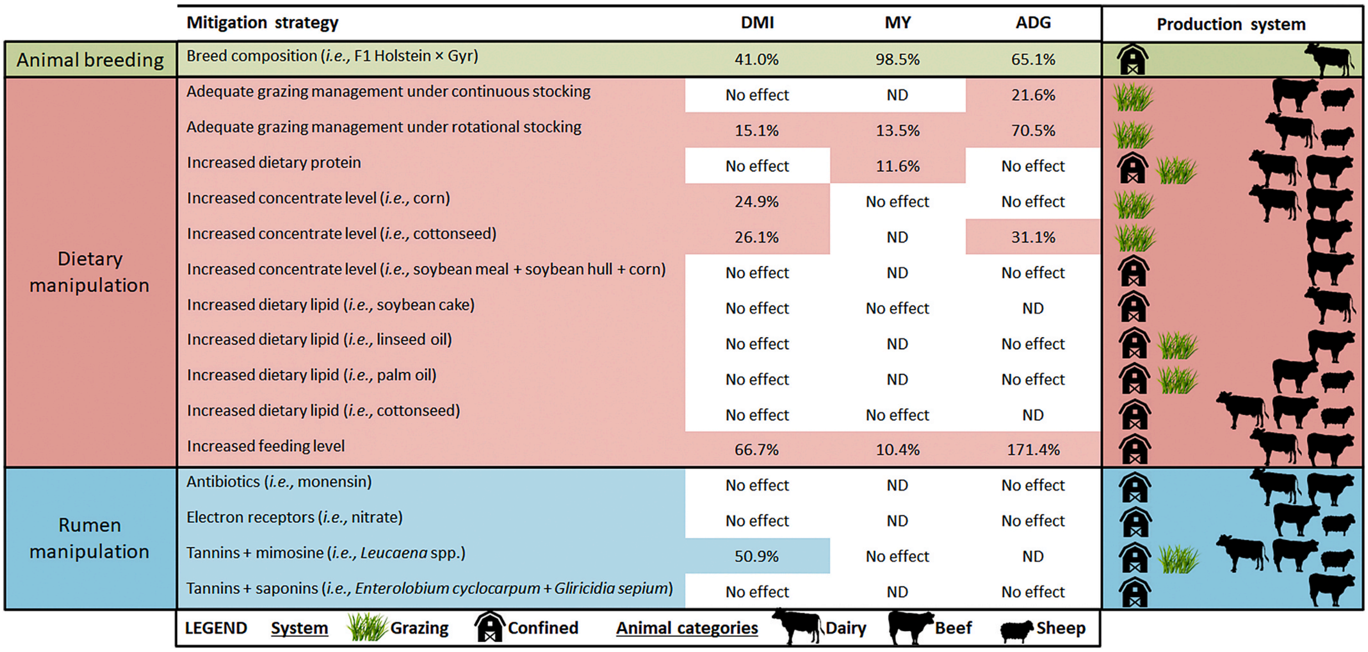
Included into the dietary diversity category, allocation of *additional forage sources* as both corn silage supplementation for grazing animals and partial pasture allocation to confined animals, did not affect enteric $CH_4$ emission or animal performance. Similarly, *partial replacement of corn grain* with alternative feeds such as crude glycerin and corn gluten feed and citrus pulp also did not affect $CH_4$ emissions or animal performance (Tables A.3-A.6 and Figs. B.1-B.8).

Improved forage quality (category) through *alternative grazing systems* like livestock-crop, livestock-crop-forest, and livestock-forest systems increased $CH_4$ production ($P = 0.033$) and DMI ($P = 0.013$) by 10.2 and 8.0% (Tables A.3-A.4 and Figs. B.1-B.4) without affecting other parameters. Continuous stocking management under moderate/low grazing intensities, within the *grazing management* sub-category, decreased ($P = 0.008$) $Y_m$ and $CH_4I_{Gain}$ by 13.8 and 21.5%, respectively, and increased ($P < 0.001$) ADG by 21.6% (Figs. 2 and 3). Additionally, rotational stocking management under lower pre-grazing herbage mass and moderate/low grazing severities decreased $Y_m$ ($P < 0.001$), $CH_4$ yield ($P < 0.001$), $CH_4I_{Milk}$ ($P = 0.022$) and $CH_4I_{Gain}$ ($P < 0.001$) by 14.6, 16.5, 16.8 and 34.8%, respectively, and increased DMI



| | Mitigation strategy | $CH_4$ production | $Y_m$ | $CH_4$ yield | $CH_4I_{Milk}$ | $CH_4I_{Gain}$ |
|---|---|---|---|---|---|---|
| Animal breeding | Breed composition (*i.e.,* F1 Holstein × Gyr) | 33.8% | No effect | No effect | -37.6% | No effect |
| Dietary manipulation | Adequate grazing management under continuous stocking | No effect | -13.8% | No effect | ND | -21.5% |
| | Adequate grazing management under rotational stocking | No effect | -14.6% | -16.5% | -16.8% | -34.8% |
| | Increased dietary protein | No effect | No effect | No effect | -9.7% | No effect |
| | Increased concentrate level (*i.e.,* corn) | No effect | -12.2% | -15.8% | No effect | No effect |
| | Increased concentrate level (*i.e.,* cottonseed) | No effect | -18.5% | -17.4% | ND | -20.1% |
| | Increased concentrate level (*i.e.,* soybean meal + soybean hull + corn) | No effect | -16.0% | -13.7% | ND | No effect |
| | Increased dietary lipid (*i.e.,* soybean cake) | No effect | -8.1% | No effect | -11.9% | ND |
| | Increased dietary lipid (*i.e.,* linseed oil) | -47.9% | -50.9% | -46.6% | ND | -47.5% |
| | Increased dietary lipid (*i.e.,* palm oil) | -17.6% | -10.6% | -11.2% | ND | No effect |
| | Increased dietary lipid (*i.e.,* cottonseed) | No effect | No effect | No effect | -17.2% | ND |
| | Increased feeding level | 50.5% | -12.4% | -13.8% | No effect | -37.1% |
| Rumen manipulation | Antibiotics (*i.e.,* monensin) | No effect | -10.1% | -8.8% | ND | No effect |
| | Electron receptors (*i.e.,* nitrate) | -20.0% | -14.9% | -15.3% | ND | -14.0% |
| | Tannins + mimosine (*i.e., Leucaena* spp.) | No effect | -29.6% | -27.4% | No effect | ND |
| | Tannins + saponins (*i.e., Enterolobium cyclocarpum* + *Gliricidia sepium*) | No effect | -7.7% | -7.4% | ND | -12.1% |

**Fig. 2.** Successful mitigation strategies and their mean effect size (%) on enteric methane emission metrics. Positive values indicate an increment by the potential mitigation strategy compared with a control and negative values indicate a reduction. All effects in the table are statistically significant at adjusted $P \leq 0.05$, whereas no effect indicates adjusted $P > 0.05$. All adjusted $P$-values as well as 95% confidence intervals were provided in tables in Appendix A. $CH_4$ production: daily $CH_4$ emission (g/d); $Y_m$: $CH_4$ energy as a percentage of gross energy intake; $CH_4$ yield: g $CH_4$/kg dry matter intake; $CH_4I_{Milk}$: $CH_4$ emission intensity for milk (g $CH_4$/kg of milk yield); $CH_4I_{Gain}$: $CH_4$ emission intensity for body weight gain (g $CH_4$/kg of average daily gain); ND: no data.



| | Mitigation strategy | DMI | MY | ADG | Production system |
|---|---|---|---|---|---|
| Animal breeding | Breed composition (*i.e.*, F1 Holstein × Gyr) | 41.0% | 98.5% | 65.1% | 🏠 🐄 |
| Dietary manipulation | Adequate grazing management under continuous stocking | No effect | ND | 21.6% | 🌿 🐂 🐑 |
| | Adequate grazing management under rotational stocking | 15.1% | 13.5% | 70.5% | 🌿 🐄 🐑 |
| | Increased dietary protein | No effect | 11.6% | No effect | 🏠 🌿 🐄 🐂 |
| | Increased concentrate level (*i.e.*, corn) | 24.9% | No effect | No effect | 🌿 🐄 🐂 |
| | Increased concentrate level (*i.e.*, cottonseed) | 26.1% | ND | 31.1% | 🌿 🐂 |
| | Increased concentrate level (*i.e.*, soybean meal + soybean hull + corn) | No effect | ND | No effect | 🏠 🐂 |
| | Increased dietary lipid (*i.e.*, soybean cake) | No effect | No effect | ND | 🏠 🐄 |
| | Increased dietary lipid (*i.e.*, linseed oil) | No effect | ND | No effect | 🏠 🌿 🐂 |
| | Increased dietary lipid (*i.e.*, palm oil) | No effect | ND | No effect | 🏠 🌿 🐂 🐑 |
| | Increased dietary lipid (*i.e.*, cottonseed) | No effect | No effect | ND | 🏠 🐄 🐂 🐑 |
| | Increased feeding level | 66.7% | 10.4% | 171.4% | 🏠 🐄 🐂 |
| Rumen manipulation | Antibiotics (*i.e.*, monensin) | No effect | ND | No effect | 🏠 🐄 🐂 |
| | Electron receptors (*i.e.*, nitrate) | No effect | ND | No effect | 🏠 🐂 🐑 |
| | Tannins + mimosine (*i.e.*, *Leucaena* spp.) | 50.9% | No effect | ND | 🏠 🌿 🐄 🐂 🐑 |
| | Tannins + saponins (*i.e.*, *Enterolobium cyclocarpum* + *Gliricidia sepium*) | No effect | ND | No effect | 🏠 🐂 |

LEGEND System 🌿 Grazing 🏠 Confined Animal categories 🐄 Dairy 🐂 Beef 🐑 Sheep

**Fig. 3.** Successful mitigation strategies and their mean effect size (%) on animal performance metrics. Positive values indicate an increment by the potential mitigation strategy compared with a control and negative values indicate a reduction. All effects in the table are statistically significant at adjusted $P \leq 0.05$, whereas no effect indicates adjusted $P > 0.05$. All adjusted $P$-values as well as 95% confidence intervals were provided in tables in Appendix A. DMI: dry matter intake (kg/d); MY: milk yield (kg/d); ADG: average daily gain (kg/d); ND: no data.

($P = 0.025$), MY ($P < 0.001$) and ADG ($P < 0.001$) by 15.1, 13.5 and 70.5%, respectively (Figs. 2 and 3). Use of corn silage as forage source (*NDF digestibility* sub-category) did not affect $CH_4$ emissions while reduced ($P = 0.026$) ADG by 15.6% (Table A.6 and Figure B.8). Mixed grass-legume swards and pasture nitrogen (N) fertilization, both included under *pasture management* sub-category, also did not affect either $CH_4$ or animal performance.

Increased dietary protein category did not reduce $CH_4$ production, $Y_m$ or $CH_4$ yield but decreased ($P = 0.003$) $CH_4I_{Milk}$ by 9.7% while increasing ($P = 0.021$) MY by 11.6% (Figs. 2 and 3). Feed sources included under the increased concentrate level category did not influence $CH_4$ production but all, except *dried distillers' grains with solubles* (*DDGS*), decreased ($P \leq 0.05$) both $Y_m$ and $CH_4$ yield by 12.2–18.5% and 13.7–17.4% (Fig. 2), respectively. *Corn ground/grain* and *cottonseed meal* increased DMI ($P = 0.038$ and $P < 0.001$) by 24.9 and 26.1%, respectively, whereas *cottonseed meal* decreased ($P < 0.001$) $CH_4I_{Gain}$ by 20.1% and increased ($P < 0.001$) ADG by 31.1% (Figs. 2 and 3).

The *by-product* soybean cake within the increased dietary lipid category did not affect $CH_4$ production, $CH_4$ yield, DMI or MY but reduced $Y_m$ ($P < 0.001$) and $CH_4I_{Milk}$ ($P = 0.005$) by 8.1 and 11.9%, respectively (Figs. 2 and 3). Within *grains* sub-category, soybean decreased ($P < 0.001$) $CH_4$ production, $Y_m$, $CH_4$ yield and $CH_4I_{Gain}$ by 19.3, 14.3, 13.5 and 22.7%, respectively, and also decreased ($P = 0.004$) DMI by 7.1% but did not affect ADG (Tables A.3-A.5 and Figs. B.1-B.6). Linseed oil (*oils* sub-category) decreased $CH_4$ production ($P < 0.001$), $Y_m$ ($P = 0.015$), $CH_4$ yield ($P = 0.012$) and $CH_4I_{Gain}$ ($P = 0.004$) by 47.9, 50.9, 46.6 and 47.5%, respectively whereas it did not compromise DMI or ADG (Figs. 2 and 3). Also, palm oil decreased $CH_4$ production ($P = 0.029$), $Y_m$ ($P < 0.001$) and $CH_4$ yield ($P < 0.001$) by 17.6, 10.6 and 11.2%, respectively, without affecting $CH_4I_{Gain}$ and animal performance (Figs. 2 and 3). *Rumen protected fat* did not affect either $CH_4$ or animal performance. Whole cottonseed (*seeds* sub-category) did not influence $CH_4$ production, $Y_m$ or $CH_4$ yield but decreased ($P = 0.033$) $CH_4I_{Milk}$ by 17.2% without compromising animal performance (Figs. 2 and 3). Increased feeding level category increased $CH_4$ production ($P < 0.001$), DMI ($P < 0.001$), MY ($P = 0.021$) and ADG ($P < 0.001$) by 50.5, 66.7, 10.4, and 171.4% while it decreased ($P < 0.001$) $Y_m$, $CH_4$ yield and $CH_4I_{Gain}$ by 12.4, 13.8 and 37.1%, and did not affect $CH_4I_{Milk}$ (Figs. 2 and 3).

### 3.3. Rumen manipulation

The ionophoric antibiotic *monensin* decreased $Y_m$ ($P = 0.008$) and $CH_4$ yield ($P = 0.005$) by 10.1 and 8.8%, respectively, without affecting $CH_4$ production, DMI, $CH_4I_{Gain}$ or ADG (Figs. 2 and 3). The electron receptor *nitrate* was effective in reducing $CH_4$ production ($P < 0.001$), $Y_m$ ($P = 0.013$), $CH_4$ yield ($P = 0.021$) and $CH_4I_{Gain}$ ($P = 0.042$) with 20.0, 14.9, 15.3 and 14.0%, respectively, and did not compromise DMI or ADG (Figs. 2 and 3).

Both *flavonoids* and *Lippia origanoides essential oil* (containing thymol and carvacrol compounds) included under plant secondary compounds category did not affect any parameter evaluated (Tables A.3-A.6 and Figs. B.1-B.8). Potential strategies based on *tannins* such as *Acacia mearnsii* also did not influence either enteric $CH_4$ emissions or animal performance, whereas *Tithonia diversifolia* increased $CH_4$ production ($P = 0.019$) and DMI ($P = 0.030$) by 20.0 and 9.2%, respectively, without affecting $Y_m$, $CH_4$ yield, $CH_4I_{Milk}$ and MY (Tables A.3-A.4 and Figs. B.1-B.4).

The combination of *tannins and mimosine* (sub-category), included both *Leucaena diversifolia* and *L. leucocephala*, decreased ($P < 0.001$) $Y_m$ and $CH_4$ yield by 29.6 and 27.4%, respectively, and increased ($P = 0.015$) DMI by 50.9% without affecting $CH_4$ production, $CH_4I_{Milk}$ and MY (Figs. 2 and 3). *Tannins and saponins* (sub-category) comprised *Enterolobium cyclocarpum* and *Enterolobium cyclocarpum* plus *Gliricidia sepium*. *Enterolobium cyclocarpum* decreased $CH_4$ production ($P = 0.004$) and $CH_4$ yield ($P = 0.032$) by 14.2 and 11.8%, respectively (Tables A.3-A.4 and Figs. B.1-B.3), but there were no data to evaluate effects on ADG and MY. *Enterolobium cyclocarpum* and *Gliricidia sepium* did not affect $CH_4$ production and DMI but decreased $Y_m$ ($P < 0.001$), $CH_4$ yield ($P < 0.001$) and $CH_4I_{Gain}$ ($P = 0.038$) with 7.7, 7.4 and 12.1%, respectively, without affecting ADG (Figs. 2 and 3).

## 4. Discussion

Only the successful mitigation strategies are addressed in this section whereas non-effective strategies are discussed in Appendix A. Fig. 3 shows animal performance effects of successful mitigation strategies by animal type and production system.

Previous comprehensive reviews analyzed strategies of GHG mitigation, including enteric $CH_4$, and overall sustainability of livestock systems (Beauchemin et al., 2008; Hristov et al., 2013b). Although these reviews are robust and exhaustively explore the literature globally, a statistical analysis is lacking. Furthermore, analysis of livestock mitigation options for the LAC region is lacking but necessary, since not all strategies mentioned by global reviews are feasible and applicable at regional or local conditions (Niu et al., 2018). Our meta-analysis covered *in vivo* individual animal data generated by researchers in the LAC region. It provides a general overview of the state of the art of enteric $CH_4$ research in the LAC region and also sheds light on the efficacy of mitigation options considered. Most of the experimental studies that delivered data for the current analysis examined one of the $CH_4$ mitigation strategies in isolation instead of considering potential interactions in the context of the whole livestock production systems and other GHG sources. Therefore, the discussion below is an attempt to consider potential synergistic and pollution swapping effects of individual mitigation practices in a sustainable development perspective.

### *4.1. Animal breeding*

Overall, **animal breeding** through genetic selection was an effective strategy to mitigate enteric $CH_4$ emissions while keeping or increasing animal performance. Most of the *breed compositions* studied have increased the $CH_4$ production which was coupled with greater DMI. For F1 Holstein × Gyr the increase in MY was accompanied with a reduction in $CH_4I_{Milk}$ whereas this was not the case for pure breed Holstein. It can be speculated that diets from LAC dairy systems, usually with lower energy content than typical dairy confinement diets from USA, for example, may have restricted the potential of Holstein cows under such conditions, and privileged crossbreeds and more locally adapted cows. It is worth mentioning that a greater ADG for F1 Holstein × Gyr and Holstein dairy cows compared to the respective baseline breeds did not decrease $CH_4I_{Gain}$, reinforcing the point that genetic breeding for those breeds has been aimed towards increasing milk production instead of beef yield. The present findings agree with the literature that increasing animal productivity through improving the genetic potential of animals using planned crossbreeding can be an effective strategy for decreasing $CH_4$ intensity under local conditions (Capper and Bauman, 2013; Hristov et al., 2013a). Capper and Bauman (2013) highlighted the importance of considering the increase of animal productivity in the discussion on GHG emissions abatement. Since the availability of cropland per person is expected to decline by 25% by 2050, there is an obvious need that effective GHG mitigation strategies are linked with enhanced yields to meet the world's growing food demand (Alexandratos and Bruinsma, 2012).

### *4.2. Dietary manipulation*

The concept of *grazing management* was derived from the general term *pasture management* according to Da Silva and Corsi (2003). Included into *grazing management* sub-category, the strategy of continuous stocking management (see Allen et al., 2011 for grazing terminologies) under moderate/low grazing intensities was effective to decrease $Y_m$ and $CH_4I_{Gain}$ while increasing ADG. Similarly, lower pre-grazing herbage mass associated with moderate/low grazing severities under rotational stocking management decreased $Y_m$, $CH_4$ yield, $CH_4I_{Milk}$ and $CH_4I_{Gain}$, and increased DMI, MY and ADG. Several papers have reported grazing strategies as an effective practice able to modulate sward and animal responses in the tropics (Carvalho, 2013; Da Silva et al., 2015). Continuous stocking managed under steady-state, with either excessively high or low grazing intensities (*i.e.,* swards constantly kept short or tall, respectively), usually jeopardizes DMI and ADG (Da Silva et al., 2013; Kunrath et al., 2020) and ultimately increasing $CH_4$ yield or $CH_4$ intensity (Barbero et al., 2015; De Souza Filho et al., 2019). For rotational stocking, an optimal combination between both grazing frequency and grazing severity is crucial for achieving high animal productivity, leading to $CH_4$ mitigation. Several studies have reported that grazing frequencies based on pre-grazing heights with canopy intercepting 95% of the incident light usually have lower pre-grazing herbage mass relative to those managed under excessively long and lower frequency of grazing periods (Da Silva et al., 2015). This lower pre-grazing herbage mass is usually coupled with a greater net herbage production built on leafy swards (Carnevalli et al., 2006; Pereira et al., 2014) with improved nutritive value (Trindade et al., 2007; Da Silva et al., 2020). Ultimately, this results in greater DMI and animal productivity (Voltolini et al., 2010; Gimenes et al., 2011) and lower enteric $CH_4$ yield and intensity (Muñoz et al., 2016; Congio et al., 2018). The severity at which a sward is grazed was also demonstrated to be effective in modulating plant and animal responses (Carvalho, 2013). As a general rule, moderate/low grazing severities (*i.e.,* not exceeding 40–50% of herbage depletion based on pre-grazing height) should be preferred instead of high severities, because this not only allows faster sward regrowth (Da Silva et al., 2009; Silveira et al., 2013), but also prioritizes greater DMI and animal productivity (Fonseca et al., 2012; Savian et al., 2020) while decreasing enteric $CH_4$ yield and intensity (Savian et al., 2014; 2018). It is worth mentioning that the optimal range of sward height (for continuous stocking) and the ideal pre-grazing height (for rotational stocking) must be considered at the individual forage species level, owing to differences in plant structure and its growth, and how the animals respond to grazing the pasture (Carvalho, 2013; Da Silva et al., 2015). Additionally, grazing strategy based on ideal pre-grazing height also mitigates nitrous oxide ($N_2O$) emission intensity from grazed pasture soils (Congio et al., 2019) while moderate/low grazing severity increases $N_2O$ and $CH_4$ emissions from feces (Savian et al., 2019). Still, from a whole farm perspective, perennial pastures in the LAC region have shown a large potential on stocking carbon into the soil (Abdalla et al., 2018; Segnini et al., 2019) offsetting most of the GHG emissions from beef cattle grazing systems (Oliveira et al., 2020). In a context where the growing demand for food must be achieved through cleaner practices, adequate *grazing management* strategies are an environmentally friendly opportunity to improve forage yields through efficiency improvements of existing resources rather than increased use of additional external resources (Congio et al., 2018).

The increased dietary protein category included the strategy of increasing dietary crude protein (CP) content without significant differences in forage to concentrate ratio, with an average of 14.6% of CP for the mitigating treatment compared to an average of 11.9% CP with the control treatments. A higher dietary CP content was achieved with (extra) inclusion of protein sources in the concentrate fraction and led to an increase on MY and a decrease on $CH_4I_{Milk}$. Based on data from 59 experiments, Sauvant et al. (2011) reported a linear negative relationship between $CH_4$ production per digested organic matter and dietary CP levels. Other studies contradicted that trend (Hynes et al., 2016; Niu et al., 2016) likely due to differences in the levels of dietary protein evaluated. When dietary protein content of the basal treatments is considerably low (*e.g.,* low-quality forage-based diets which are more common in the LAC region than in developed countries), and compromises fulfillment of metabolizable protein requirements and rumen N availability in the animal, an increase on dietary protein content is more likely to increase animal performance and decrease $CH_4$ emission intensity (Sauvant et al., 2011). For this reason, the present meta-analysis may have identified a greater animal performance and lower $CH_4$ intensity as a result of increasing dietary CP content for dairy but not for beef cattle. However, considering that increased dietary protein levels can also rise N excretion and $N_2O$ emissions from grazed soils or manure storage (Sauvant et al., 2014), precision feeding practices that allow meeting the animals' nutrient requirements (Hristov et al., 2013a, 2013b) should be adopted to avoid unnecessary N losses to the environment.

The increased concentrate level category consisted mostly of grazing studies in which the mitigation treatment was the use of supplemental concentrate feed sources. Overall, mitigation and control treatments averaged 67 and 90% forage (and 33 and 10% concentrate) in dietary DM, respectively, which resulted in an effective mitigation strategy to increase animal performance and decrease enteric $CH_4$ emissions. It is well reported in the literature that increased concentrate levels may reduce the proportion of dietary energy converted to $CH_4$ (Blaxter and Clapperton, 1965), mainly due to the shift in type of fermented substrate (*i.e.,* from fiber to non-fiber carbohydrates) and its effects on rumen fermentation such as a higher rate of fermentation, a shift in volatile fatty acids profile and a decrease in ruminal pH (Beauchemin et al., 2008). Supplementation of concentrate feeds to grazing animals does not necessarily increase the total organic matter intake (OMI) (*i.e.,* substitutive effect; Moore, 1980). The latter depends on sward characteristics (*e.g.,* sward structure and forage allowance), supplement source and level, and animal requirements (Poppi et al., 1997; Costa et al., 2020). In studies with beef cattle, as long as there is no restriction on forage allowance, it is expected that providing concentrate supplements with high-quality forage-based diets under adequate grazing management conditions has little or no effect on total OMI. On the other hand, concentrate supplementation for low-quality forage-based diets is more likely to result in an increase in total OMI (*i.e.,* additive or associative effects; Moore, 1980), leading to a mitigation of enteric $CH_4$ emissions per unit of animal product. According to Paterson et al. (1994) an adequate supplementation strategy should focus on maximizing intake and digestibility of available forage while minimizing concentrate supplementation/import to the production system. This approach minimizes costs and is in line with environmentally friendly and sustainable development principles (Beauchemin et al., 2008). However, concentrate supplementation options should be analyzed on their feasibility at the local level and diets. Furthermore, by-product ingredients that are non-edible for humans may be a good alternative.

There was substantial research regarding the inclusion of dietary lipid sources (increased dietary lipid category), due to their well-recognized enteric $CH_4$ mitigation effect (Beauchemin et al., 2008). Overall, increased inclusion of dietary lipid comprised control treatments averaging 3.2% of ether extract (EE) which were compared with 5.6% EE from mitigation treatments, without significant differences in forage-to-concentrate ratio. This mitigation practice decreases enteric $CH_4$ emissions through a replacement of fermentable feed substrate by lipid which is not fermented well in the rumen and has an inhibitory effect on fiber degradation and protozoa with their associated methanogens (Johnson and Johnson, 1995), or it affects a shift in rumen fermentation profile. Beauchemin et al. (2020) pointed out that the cost-effectiveness of this mitigation practice must be examined as well as potential negative effects on fiber digestibility and product quality (*e.g.,* milk fat and protein depression). Overall, our current meta-analysis demonstrated that increased dietary lipid is an effective mitigation strategy decreasing all enteric $CH_4$ parameters with only a small decrease in DMI which did not impact MY or ADG, suggesting an increased feed efficiency that was also reported by Rabiee et al. (2012) for dairy cattle. Considering individual dietary lipid sources, only whole soybean grain depressed DMI whereas no source decreased either MY or ADG. A comprehensive review from Hristov et al. (2013b) concluded that there is a real mitigation potential in inclusion of lipids in ruminant diets, but they highlighted that, in higher inclusion rates, it may depress DMI and digestibility, and consequently animal productivity. These authors further indicated that lipid sources such as DDGS and whole cottonseed could increase dietary N intake, which may lead to an increased potential for $N_2O$ emissions from manure during storage and land application, suggesting the potential of a pollution swapping effect.

The strategy of increased feeding level comprised mostly studies carried out in respiration chambers where the main objective was to detail the energy partition of distinct cattle breeds that were fed at different feeding levels (Carvalho et al., 2018; Ferreira et al., 2018). Understanding that a considerable fraction of LAC herd is undernourished, the dataset was used to evaluate quantitatively the effect of *ad libitum* DMI on enteric $CH_4$ mitigation compared to restricted feeding. The non-restricted group (*i.e.,* mitigation treatment) had a 66.7% greater DMI, which also increased $CH_4$ production by 50.5% relative to the control, restricted group. This strong positive relationship between DMI and ruminal $CH_4$ production is well understood (Cottle et al., 2011; Kennedy and Charmley, 2012; Hristov et al., 2018). However, increased feeding levels also increased ADG leading to a decrease in $CH_4I_{Gain}$ which agrees with reviews by Cottle et al. (2011) and Hristov et al. (2013a). Dry matter intake in ruminants can be restricted at different levels regardless of the feeding system. Under grazing conditions, excessively short sward height usually restrict DMI (Da Silva et al., 2013), whereas excessively tall (Congio et al., 2018) or stemmy swards also impair DMI (Benvenutti et al., 2009). Confining animals during part of or the whole year is a usual practice for smallholder farming systems in the LAC region. In those systems, low-quality fiber sources are fed during part of the year with no extra or a low amount of CP sources, or simply a restricted amount of feed is offered that results in limited DMI. In this context, cattle producers should adopt practices to optimize DMI through an adequate grazing management and an optimal diet formulation to achieve higher animal productivity and a reduced enteric $CH_4$ yield and intensity without other nutrient losses to the environment.

### 4.3. Rumen manipulation

Strategies in the antibiotics category included *monensin* which decreased $Y_m$ and $CH_4$ yield in growing cattle. Monensin is recognized for increasing feed efficiency through inhibiting gram-positive over gram-negative bacteria, leading towards more propionate production in the rumen (Russel, 1987; McGuffey et al., 2001). Increased propionate to acetate ratios and a reduced number of ruminal protozoa generating extra hydrogen in the rumen next to bacterial activity could be beneficial for decreasing enteric $CH_4$ emission (Beauchemin et al., 2008; Cottle et al., 2011). The literature, however, is inconsistent, in particular, regarding the effect of monensin on $CH_4$ emission under grazing systems (Grainger et al., 2008; Waghorn et al., 2008). According to Hristov et al. (2013b) this inconsistency is mostly due to the potential mitigation effect being dose-, feed intake-, and diet composition-dependent. However, a previous robust meta-analysis (Appuhamy et al., 2013) agree with our current results, indicating a moderate mitigation effect of monensin on $CH_4$ yield and $Y_m$ from growing cattle. Two further important aspects, frequently discussed in the literature, are that the mitigation effect of monensin may be temporal and that public pressure to reduce the use of antibiotics in livestock systems may limit its use in the future (Beauchemin et al., 2008).

The *nitrate* mitigation strategy, within the electron receptors category, decreased $CH_4$ production, $Y_m$, $CH_4$ yield and $CH_4I_{Gain}$. Several studies have reported nitrate being an effective additive to decrease $CH_4$ emissions from ruminants including its persistent effect for dairy and beef cattle (van Zijderveld et al., 2011; Lee et al., 2017; Feng et al., 2020). The reduction of nitrate to ammonia into the rumen is energetically a more favorable pathway than the reduction of carbon dioxide to $CH_4$. Therefore, hydrogen is partially utilized to reduce nitrate instead of being used in methanogenesis (Lee and Beauchemin, 2014). An important aspect regarding nitrate use is that it is critical to adapt the animals gradually and to acclimatize the ruminal ecosystem, avoiding poisoning through nitrite accumulation and methaemoglobin formation in blood (Leng, 2008; Lee and Beauchemin, 2014). Alaboudi and Jones (1985) showed that the rumen microbiome capacity to reduce nitrate to ammonia can return to previous levels after three weeks of nitrate withdrawal from the diet, reinforcing the needs for a re-adaptation period as soon as nitrate supplementation is interrupted. Hristov et al. (2013b) pointed out that it could be a critical issue for smallholders in developing countries where feed availability and composition may change frequently. These authors also indicated that the nitrate level in the basal diet must be taken into account when supplemental nitrate is fed, especially when forage sources come from fields under high N inputs (Lee and Beauchemin, 2014). Even with some authors reporting the possibility of increased urinary-N losses when supplemental nitrate is fed, several studies have shown that nitrate does not increase urinary-N excretion compared with urea when isonitrogenous and balanced diets are fed (Lee at al., 2015; Olijhoek et al., 2016). However, Petersen et al. (2015) suggested increased rumen $N_2O$ emissions when dairy cows were fed at high nitrate levels of intake (*i.e.,* 14 and 21 g/kg DM). Hristov et al. (2013b) noticed that the mitigation potential from nitrate should increase in low-protein diets where the rumen microbiome may benefit from nitrate as a non-protein N (NPN) source. This approach may be of a particular interest in developing countries where ruminant livestock are fed forages with low nitrate and CP contents, especially during the dry season. Cottle et al. (2011) highlighted that this mitigation strategy could be particularly useful for Australian pasture-based systems where nitrate could potentially replace urea as the main supplementary NPN source for ruminant grazing low-quality pastures. Australian pasture-based systems already manage the risk of urea intoxication when supplementing animal diets through acclimation, molasses-based licking blocks and water medication. These practices could also be used to control nitrate intake and minimize the potential risk of poisoning in the LAC region.

Plants may have a broad variety of secondary compounds that may affect the rumen microbiome and enteric $CH_4$ emission (Ku-Vera et al., 2020a). The strategy of feeding tannins (*tannins* strategy included under plant secondary compounds category) has been studied more intensively for their $CH_4$ mitigation effect (Ku-Vera et al., 2020a). Tannins belong to a subclass of plant polyphenols and are usually classified according to their chemical structures as hydrolyzable or condensed tannins (HT and CT, respectively; Vasta et al., 2019). Recent research has focused on sources of CT rather than HT due to the potential toxic effect of the latter (Beauchemin et al., 2020). The mechanism by which tannins impact ruminal methanogenesis is not fully understood, but it is accepted that the effect of HT seems to be more associated with a direct inhibition of methanogenic archaea, whereas the effect of CT is associated with an inhibitory effect on some specialized fibrolytic bacteria consequently impairing fiber digestion (Goel and Makkar, 2012; Naumann et al., 2017). Tanniferous plants are widely spread and include various shrubs, trees, both legume and non-legume plant species, mainly in warm climate regions from LAC (Hristov et al., 2013a; Ku-Vera et al., 2020a).

*Leucaena* spp. (included into *tannins and mimosine* sub-category) is a native legume shrub widely spread in the LAC tropical region and is one of the most studied and promising tanniferous plant genus (Ku-Vera et al., 2020b). The most studied species is *L. leucocephala* followed more recently by *L. diversifolia* (Gaviria-Uribe et al., 2020; Ku-Vera et al., 2020b). Although the presence of CT has been reported to be the main cause for the anti-methanogenic properties of *Leucaena*, the secondary compound mimosine was also suggested as a potential mitigation agent (Soltan et al., 2013; 2017). Mimosine is a toxic, free amino acid responsible for the inhibition of protein synthesis and the growth of several gram-positive bacteria and fungi (Soltan et al., 2017) which is found in leaves, pods and seeds of *Leucaena* (Dalzell et al., 2012). Our meta-analysis showed that 27% (DM basis) inclusion of *Leucaena* spp. on predominantly grass-based diets decreased $CH_4$ yield whereas DMI was increased, which agrees with studies conducted in tropical regions (Molina et al., 2016; Montoya-Flores et al., 2020). Increased DMI through *Leucaena* inclusion on grass-based diets is likely due to a greater supply of CP and decreased fiber content of the whole diet (Soltan et al., 2013; Piñeiro-Vázquez et al., 2018) and, in some studies, these attributes are ignored when explaining its mitigation effect (Molina et al., 2016). Studies have reported a reduced nutrient digestibility (Piñeiro-Vázquez et al., 2018; Montoya-Flores et al., 2020) at high levels of *Leucaena* inclusion, whereas others showed greater N retention and increased N partitioning from urine towards feces (Soltan et al., 2013), thus reducing the fraction of urinary-N in total N excreted. The latter has been demonstrated for other CT sources (Carulla et al., 2005) and could be an additional benefit of CT inclusion in diets because fecal-N is mainly organic, whereas urinary-N is more volatile and more rapidly nitrified in the soil, contributing to ammonia volatilization, nitrate leaching and $N_2O$ emissions.

Saponins are glycosides of a high molecular weight found in a wide variety of tropical shrubs and trees (Canul-Solis et al., 2020; Ku-Vera et al., 2020a). Their anti-methanogenic effect is often attributed to their action on cell membranes of rumen protozoa, thus affecting methanogenic archaea which are symbiotically associated with them (Patra and Saxena, 2009). Goel and Makkar (2012) reported that the concentration range for tannins to be able to reduce enteric $CH_4$ without compromising animal productivity is narrower than that for saponins. However, leguminous tree species such as *E. cyclocarpum* and *G. sepium* contain both *tannins and saponins* in their pods and leaves in substantial concentrations (Molina-Botero et al., 2019a,2019b). The current meta-analysis showed that *E. cyclocarpum* included at 32% (DM basis) in grass-based diets decreased $CH_4$ production and $CH_4$ yield. Further, the inclusion *E. cyclocarpum* plus *G. sepium* with 26% (DM basis) decreased $CH_4$ yield, $Y_m$ and $CH_4I_{Gain}$. Our results are in agreement with other studies carried out under tropical conditions (Albores-Moreno et al., 2017). Decreased nutrient digestibility has also been reported for ground pods of *E. cyclocarpum* when included at levels greater than 30% of DM (Piñeiro-Vázquez et al., 2013; Albores-Moreno et al., 2017). A similar limiting inclusion level for *E. cyclocarpum* plus *G. sepium* was reported by Molina-Botero et al. (2019a) to not impair nutrient digestibility. The use of effective enteric $CH_4$ mitigating plant sources containing tannins and/or saponins, that usually have a high CP and low fiber content such as *Leucaena* spp., *E. cyclocarpum* and *G. sepium,* is particularly important for grazing systems and small farmers in the LAC tropical region, especially during the dry season when the overall pasture quality is poor. For the temperate region of LAC, there is a lack of studies investigating alternatives of shrub species for inclusion in animal diet for mitigating enteric $CH_4$.

Most of our dataset on strategies in the category plant secondary compounds came from studies carried out using respiration chambers on the short-term, and maybe for the latter reason there were few studies where an effect on animal productivity was assessed, which weakens our analysis with regard to effects on MY, ADG, $CH_4I_{Milk}$ and $CH_4I_{Gain}$. It is critical that long-term studies, similar to that of Molina-Botero et al. (2020b), are conducted with plant secondary compounds. In addition to measurements of animal performance, N balance and nutrient digestibility should be included to address the major question of rumen ecosystem adaptation due to supplementation with tannins and saponins (Ramos-Morales et al., 2018). Further, studies conducted in respiration chambers are pointed out to be more accurate in estimating $CH_4$ emissions and DMI (Hristov et al., 2018), but they cannot simulate grazing conditions. Respiration chamber studies measure DMI more precisely compared with the use of markers under grazing conditions, and control of proportion of individual dietary ingredients (*e.g.,* grass and tanniferous legume) is more accurate. The choice of study type depends on the specific aim of the study, but results must be gathered under grazing conditions as well.

### 4.4. Practical implications

In the context of enteric $CH_4$ mitigation, it is important to consider the implications of each available metric (Eckard and Clark, 2018). Considering the Paris Agreement perspective, where several LAC countries have made commitments to reduce absolute GHG emissions (Arango et al., 2020), certainly the reduction of daily $CH_4$ production will be the more important metric to use. On the other hand, in a current market-oriented society, environmentally friendly products from certified agri-livestock systems have been increasingly required by consumers, including in LAC countries (*e.g.,* carbon neutral Brazilian beef; Alves et al., 2017). It highlights the importance of metrics based on GHG emissions per unit of animal product produced (Beauchemin et al., 2020). An additional point from an emission intensity perspective is that a considerably greater amount of food will be demanded to feed the growing population by 2050 (Conforti, 2011), requiring feed efficiency improvements from current agri-livestock systems practices. The current meta-analysis identified more options intended to decrease $CH_4$ intensity rather than $CH_4$ production. For example, strategies such as F1 Holstein × Gyr *breed composition* and increased feeding level raised daily $CH_4$ production, but decreased $CH_4$ intensity. Other strategies, such as both continuous and rotational *grazing management*, increased dietary protein, increased concentrate level, and *E. cyclocarpum* and *G. sepium*, decreased $CH_4$ intensity with no effect on $CH_4$ production, whereas increased dietary lipid and the electron receptor *nitrate* mitigated both $CH_4$ production and intensity.

The feasibility of GHG mitigation strategies is imperative for adoption by livestock producers globally (Beauchemin et al., 2020). However, in developing countries like those from the LAC region, livestock producers are usually smallholders limited economically in their capacity to make an initial investment in a high-cost strategy, even if the strategy has a positive cost-benefit ratio (Ku-Vera et al., 2020b). Taking into account that there is a widespread lack of governmental support in terms of financial allocation to allow livestock producers adopting mitigation strategies, it is critically important that the practices to be adopted are at low or non-cost to the livestock producer and that it results in productivity gains.

For pasture-based systems, adequate strategies of *grazing management* fit perfectly in this context because they are no-cost, increase animal productivity and decrease $CH_4$ and potentially also $N_2O$ emissions (Congio et al., 2018; 2019). Concentrate supplementation (*i.e.,* increased concentrate level) for grazing animals can also be an effective strategy, but its feasibility must be assessed at the local level. *Alternative grazing systems* (*e.g.,* silvopastoral) are frequently discussed as having a great potential in reducing enteric $CH_4$ emissions as well as carbon footprint through carbon stocking (Arango et al., 2020; Ku-Vera et al., 2020a,2020b). Both outcomes are possible, but there is a lack of studies under grazing conditions estimating carbon stocking *in loco* to support these hypotheses quantitatively. A potential issue against the wide adoption of silvopastoral systems is the high implementation cost in a scenario where governmental incentives are lacking.

Under confinement production systems, supplementation with tanniferous/saponiferous legume forages, electron receptors (*i.e., nitrate*), increased feeding level, and utilization of more specialized animal breeds and crossbreeds (*e.g.,* F1 Holstein × Gyr) are effective strategies. Dual-purpose cattle production systems with cows producing 7–10 kg/d milk are typical for the LAC region (Ku-Vera et al., 2020b) and highlight an opportunity that more specialized breeds or crossbreeds can improve the productivity of those farming systems whereas mitigating $CH_4$ intensity. Increased dietary lipid and increased dietary protein, when these are bellow animal requirements, can also be adopted in confined production systems.

The LAC database included the majority of available *in vivo* studies and the current analysis provides the most comprehensive quantitative approach regarding enteric $CH_4$ mitigation strategies for the LAC region current feasible. This provides sustainable mitigation solutions for livestock producers that can support LAC countries to achieve global environment targets. Our findings should be used in a holistic perspective, in conjunction with additional analysis concerning other livestock environmental issues (*e.g.,* manure emissions, N losses, water use and contamination), and thus guide policy makers to design more efficient and sustainable livestock production systems.

## 5. Conclusions and future directions

Of the 34 enteric $CH_4$ mitigation strategies evaluated in the present study, 16 decreased at least one enteric $CH_4$ metric without compromising animal productivity, and from those, only six simultaneously decreased $CH_4$ emission by on average −27% and increased animal productivity by on average + 68% [*e.g.,* F1 Holstein × Gyr (−38% and +99%; average of $CH_4$ emission decrease and animal productivity increase, respectively), adequate continuous stocking management (−22% and +22%), adequate rotational stocking management (−35% and +71%), increased dietary protein level (−10% and +12%), increased dietary concentrate level (−20% and +31%), and increased feeding level (−37% and +171%)]. The identified effective mitigation strategies can be adopted by livestock producers according to their specific needs and aid LAC countries in achieving SDG as defined in the Paris Agreement.

Even though the LAC region is very diverse in terms of production systems, management and scale, our database represents well the broad variety of diets and animal breeds found in the region. Nevertheless, research conducted in pasture-based or grazing systems (58.3% of the studies) underrepresents the relevance of this feed management system in most of the countries of the LAC region, which indicates more research should be directed towards these systems. Strategies such as supplementation with dietary lipids, increased dietary protein concentration, nitrate and monensin supplementation proved to be effective mostly under confined systems. Thus, there is an urgent need to validate these technologies for grazing ruminants under LAC conditions. Likewise, promising strategies based on feed additives such as the inhibitor 3-nitrooxypropanol and seaweeds containing bromoform (*e.g., Asparagopsis* spp.) have been shown to decrease both daily $CH_4$ emission and emission intensity, and their efficacy should be evaluated in animal studies in LAC conditions.

## Data availability

Individual animal data used in the analysis can be requested by contacting individual contributors which are co-authors on the manuscript.

## CRediT authorship contribution statement

**Guilhermo Francklin de Souza Congio:** Methodology, Validation, Formal analysis, Data curation, Writing – original draft, Visualization. **André Bannink:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Funding acquisition. **Olga Lucía Mayorga Mogollón:** Methodology, Validation, Investigation, Resources, Data curation, Project administration, Latin America Methane Project Collaborators, Methodology, Validation, Investigation, Resources, Data curation, Writing – review & editing. **Alexander Nikolov Hristov:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

## Latin America Methane Project Collaborators

Gustavo Jaurena[5], Horacio Gonda[6], José Ignacio Gere[7,8], María Esperanza Cerón-Cucchi[9], Abimael Ortiz-Chura[9], María Paz Tieri[10,11], Olegario Hernández[12], Patricia Ricci[8,13], María Paula Juliarena[8,14], Banira Lombardi[8,14], Adibe Luiz Abdalla[15], Adibe Luiz Abdalla-Filho[15], Alexandre Berndt[16], Patrícia Perondi Anchão Oliveira[16], Fábio Luis Henrique[17], Alda Lúcia Gomes Monteiro[18], Luiza Ilha Borges[18], Henrique Mendonça Nunes Ribeiro-Filho[19], Luiz Gustavo Ribeiro Pereira[20], Thierry Ribeiro Tomich[20], Mariana Magalhães Campos[20], Fernanda Samarini Machado[20], Marcos Inácio Marcondes[21], Maria Eugênia Zerlotti Mercadante[22], Leandro Sannomiya Sakamoto[22], Lucia Galvão Albuquerque[23], Paulo César de Faccio Carvalho[24], Jusiane Rossetto[24], Jean Víctor Savian[24,25], Paulo Henrique Mazza Rodrigues[26], Flávio

Perna Júnior[26], Tainá Silvestre Moreira[47,26], Rogério Martins Maurício[27], João Paulo Pacheco Rodrigues[28], Ana Luiza da Costa Cruz Borges[29], Ricardo Reis e Silva[29], Helena Ferreira Lage[30], Ricardo Andrade Reis[23], Ana Cláudia Ruggieri[23], Abmael da Silva Cardoso[23], Sila Carneiro da Silva[b], Marília Barbosa Chiavegato[31], Sebastião de Campos Valadares-Filho[21], Flávia Adriane de Sales Silva[21], Diego Zanetti[32], Telma Teresinha Berchielli[23], Juliana Duarte Messana[23], Camila Muñoz[33], Claudia Janeth Ariza-Nieto[48], Andrea Milena Sierra-Alarcón[48], Laura Bibiana Gualdrón-Duarte[34], Lorena Inés Mestra-Vargas[34], Isabel Cristina Molina-Botero[35], Rolando Barahona-Rosales[36], Jacobo Arango[37], Xiomara Gaviria-Uribe[36,37], Luis Alfonso Giraldo Valderrama[36], Jaime Ricardo Rosero-Noguera[38], Sandra Lucía Posada-Ochoa[38], Sergio Abarca-Monge[39], Roberto Soto-Blanco[39], Juan Carlos Ku-Vera[40], Rafael Jiménez-Ocampo[40,41], Ever del Jesus Flores-Santiago[40,42], Octavio Alonso Castelán-Ortega[43], María Fernanda Vázquez-Carrillo[43], Mohammed Benaouda[44,45], Carlos Alfredo Gómez-Bravo[35], Víctor Ilich Alvarado Bolovich[35], Medardo Antonio Díaz Céspedes[35] and Laura Astigarraga[46].[5] Department of Animal Production, Faculty of Agronomy, University of Buenos Aires (UBA), Buenos Aires, Argentina. [6] Department of Animal Nutrition and Management, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, Uppsala, Sweden . [7] Regional Faculty of Buenos Aires, National Technological University (UTN), Buenos Aires, Argentina. [8] National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina. [9] Institute of Pathobiology, National Institute of Agricultural Technology (INTA) (INTA–CONICET), Buenos Aires, Argentina. [10] Dairy Value Chain Research Institute (IDICAL) (INTA–CONICET), Rafaela, Argentina. [11] Regional Faculty of Rafaela, UTN, Rafaela, Argentina. [12] INTA, Santiago del Estero, Argentina. [13] Institute of Innovation for Agri-Livestock Production and Sustainable Development, INTA, Balcarce, Argentina. [14] Physics and Engineering Research Centre, National University of the Centre of the Buenos Aires Province (UNCPBA), Tandil, Argentina. [15] Laboratory of Animal Nutrition, Centre for Nuclear Energy in Agriculture (CENA), University of São Paulo (USP), Piracicaba, SP, Brazil. [16] Brazilian Agricultural Research Corporation (Embrapa) Southeast Livestock, São Carlos, SP, Brazil. [17] Associated Colleges of Uberaba (FAZU), Uberaba, MG, Brazil. [18] Department of Animal Science, Federal University of Paraná (UFPR), Curitiba, PR, Brazil. [19] Department of Animal and Food Science, Santa Catarina State University (UDESC), Lages, SC, Brazil. [20] Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil. [21] Department of Animal Science, Federal University of Viçosa (UFV), Viçosa, MG, Brazil. [22] Institute of Animal Science (IZ), São Paulo Agribusiness Technology Agency (APTA), Sertãozinho, SP, Brazil. [23] Department of Animal Science, School of Agriculture and Veterinary Science (FCAV), São Paulo State University (UNESP), Jaboticabal, SP, Brazil. [24] Grazing Ecology Research Group, Department of Forage Plants and Agrometeorology, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. [25] Departament of Pastures and Forages, National Institute of Agricultural Research (INIA) Treinta y Tres, Treinta y Tres, Uruguay. [26] Departament of Animal Nutrition and Production, Faculty of Veterinary Medicine and Animal Science (FMVZ), USP, Pirassununga, SP, Brazil. [27] Department of Bioengineering, Federal University of São João del-Rei (UFSJ), São João del-Rei, MG, Brazil. 28 Faculty of Animal Science, Federal University of Southern and Southeastern Pará (UNIFESSPA), Xinguara, PA, Brazil. [29] Department of Animal Science, Federal University of Minas Gerais (UFMG), Belo Horizonte, MG, Brazil. [30] Faculty of Veterinary Medicine, Newton Paiva University, Belo Horizonte, MG, Brazil. [31] Departments of Horticulture & Crop Science and Animal Science, The Ohio State University, Columbus, OH, USA. [32] Federal Institute of Education, Science and Technology of Southern Minas Gerais, Machado, MG, Brazil. [33] Instituto de Investigaciones Agropecuarias, INIA Remehue, Osorno, Chile. [34] AGROSAVIA, Turipaná, Cereté, Colombia. [35] Department of Animal Husbandry, Faculty of Animal Science, National Agrarian University La Molina (UNALM), Lima, Peru. [36] Department of Animal Production, Faculty of Agricultural Sciences, National University of Colombia (UNAL), Medellín, Colombia. [37] International Center for Tropical Agriculture (CIAT), Cali, Colombia. [38] Faculty of Agricultural Sciences, University of Antioquia (UdeA), Medellín, Colombia. [39] National Institute of Innovation and Agricultural Technology Transfer (INTA), Turrialba, Costa Rica. [40] Laboratory of Climate Change and Livestock Production, Department of Animal Nutrition, Faculty of Veterinary Medicine and Animal Science, University of Yucatan (UADY), Mérida, Yucatán, Mexico. [41] National Institute for Forestry, Agriculture and Livestock Research (INIFAP), Experimental Field Valle del Guadiana, Victoria de Durango, Durango, Mexico. [42] Chapingo Autonomous University, South-Southeast Regional Unit (URUSSE), Teapa, Tabasco, Mexico. [43] Laboratory of Livestock, Environment and Renewable Energies, Faculty of Veterinary Medicine and Animal Science, Autonomous University of the State of Mexico (UAEMex), Toluca, Estado de México, Mexico. [44] UMR1213 Herbivores, French National Research Institute for Agriculture, Food, and Environment (INRAE), Saint-Genès-Champanelle, France. [45] AgroSup Dijon, Dijon, France. [46] Department of Animal Science and Pastures, Faculty of Agronomy, University of the Republic of Uruguay (UdelaR), Montevideo, Uruguay.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2021.127693.

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