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ABSTRACT BOOK

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Amending Dairy Manure to Reduce Ammonia Volatilisation Can Influence GHG Emissions

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As Australian dairy systems intensify with herds spending less time on pasture and more on hard surfaces, greater amounts of manure are collected and stored on farms before land application. Amending dairy manure to increase nitrogen content by reducing ammonia volatilization will increase the nutrient value as a soil amendment and decrease environmental impact. Amendments used to decrease NH₃ loss include acids, absorbents and covers. However, the impact of these amendments on greenhouse gas (GHG) emissions have not been measured for manure collected from grazing system dairy farms in Australia. We investigated the effect of addition of sulfuric acid, zeolite, and use of a cover, compared to untreated manure, on NH₃ and GHG emissions from scraped manure collected from a commercial dairy farm. The manure was stored in 120 L tubs in the laboratory and gas emitted measured continuously for 75 days, at which point the cover was removed and gas measurement continued.

Ammonia losses were reduced for the three amendments; effectively stopped for covered manure, initially slowed for acidified manure, and reduced due to zeolite addition. By contrast, CH₄ emissions were highest for the covered manure, most likely due to the anaerobic conditions formed, while the acidification had the added benefit of inhibiting CH₄ emissions. Nitrous oxide emissions from acidified manure increased strongly as NH₃ emission rate declined. Greenhouse gas emissions from zeolite amended manure did not *significantly* differ to that from the untreated control. Carbon dioxide equivalent emissions were greatest for covered manure and least for acidified manure, suggesting that acidification could have a double benefit for Australian dairy systems of mitigating both methane and ammonia loss.

GHG Emissions Affected by Collection and Management of Manure from an Australian Grazed Dairy Farm

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Methane emissions from manure are approximately one third of that produced enterically and therefore a significant contribution to greenhouse gas (GHG) losses by the Australian grazing based dairy industry. As dairy systems intensify, lactating cows spend more time on concrete where greater volumes of manure need to be managed. Technologies like solid-liquid separation are recommended to improve manure management, although their impact on GHG emissions from manure needs to be quantified in these systems. In this study, we measured continuous CH₄, CO₂, and N₂O emissions from manure sources collected on a commercial dairy farm and stored for 85 days. The sources investigated were scraped manure, effluent (washed manure), and the liquid and solid fractions after separation of effluent using a screen and a screw-press. Approximately 100 kg of each manure source was placed in each of three replicate tubs in the laboratory, and gas emissions from each tub were measured hourly using a Gasmeter DX4015 portable FTIR gas analyser.

Each gas emission profile differed for the four manure sources. Carbon dioxide emitted by the separated solid fraction was highest compared to the other sources at all times, while CH₄ emission from scraped manure, although initially delayed for the first 4 days, was the greatest, particularly by 30 days of storage. At the end of the study cumulative CO₂e emissions from the separated solids (8161 g) were three times the emissions from slurry and 13 times that from effluent and the separated liquid manures. By contrast slurry generated the greatest emissions of CH₄ (94 g; 2632 g CO₂e) and N₂O (5.8 g; 1537 g CO₂e). High CO₂e emissions were most likely due to composting of solids, which consistently had the largest C:N ratio. Solid-liquid separation may contribute to increasing CO₂e emissions from dairy systems through pollution-swapping.

Carbon Footprint of Milk Production under Smallholder Dairy Systems in the Salale Milk Belt of the Central Highlands of Ethiopia

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Smallholder dairy farming is facing a tremendous challenge of simultaneously increasing milk production while mitigating accompanied GHG emissions, thereby enhancing resilience to climate change. The GHG emissions of smallholder milk production is expected to increase significantly with increasing demand for dairy products under business as usual scenario. In this study using data from households we estimate the carbon footprint (CF) of smallholder milk production and examine factors influencing CF under different farming systems to identify viable options for mitigation of GHG emissions. For this purpose we applied a cradle to farm-gate life cycle assessment (LCA) approach to estimate GHG emission of 456 smallholder farmers (SHF) from four dairy districts in the Salale milk belt, central highlands of Ethiopia, Oromia regional state. According to our findings enteric fermentation was the primary source of GHG emissions and CH₄ emission from enteric fermentation and manure management comprised the bulk of total emissions across all farming systems. The estimated average CF varied depending on farming system, and global warming potential (GWP) and allocation methods used. The overall average CF reported was 3.57 and 4.43 kg CO₂e/kg fat and protein corrected milk (FPCM) using GWPs of IPCC (2007, 2014) and when all GHG emissions were allocated to a single product (milk) respectively. When GHG emissions were allocated to multiple products using economic allocation to milk, beef, finance and insurances, the CF of milk production was 1.91 and 1.98 CO₂e/kg FPCM and on average 72% (urban = 88%, peri-urban = 82%, rural = 45%) of total GHG emissions were allocated to milk. With regard to farm typology, rural SHF tended to have significantly higher CF per kg of milk than urban and peri-urban SHF systems. Regression output indicated that feed digestibility had a negative and significant (P<0.01) association with CF of SHF. Improved feeding by increasing the proportion of concentrate and improved forage digestibility in the daily ration at the expense of straw and crop residue could be an important area of intervention for all farming systems. For rural SHFs increasing the proportion of crossbred cows in the herd will have a paramount importance in improving milk yield and reproductive performance and maximizing the overall herd productivity, which in turn, would be expected to reduce GHG emission intensity.

Key words: Dairy cattle, Greenhouse gas, Carbon footprints, Life Cycle Assessment, Smallholder

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Environmental Footprints of Conventional and Alternative Natural Productivity-Enhancing Technologies in Beef Production

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The objective of this study was to evaluate the effects of using conventional (growth implants, beta-agonists [BAA], and ionophores) with or without other natural (fibrolytic enzymes, essential oils, and probiotics) productivity enhancing technologies (PETs) on the growth performance, carcass traits and environmental impacts of feedlot cattle. Cross-bred yearling steers (499±28.6 kg; n=384) and heifers (390±34.9 kg; n=384) were offered a barley grain-based basal diet and divided into implanted or non-implanted groups. Steers were allocated to diets that contained (i) No additive (Control); (ii) Enzyme (Enz); (iii) Oleobiotec Ruminant (Oleo); (iv) Direct-fed-microbial (DFM); (v) DFM+Enz+Oleo (DFMEnzOleo); (vi) Monensin+Tylosin+Ractopamine hydrochloride=Conventional (Conv); (vii) Conv+DFM+Enz (ConvDFMEnz); (viii) Conv+DFM+Enz+Oleo (ConvDFMEnzOleo). Heifers received the following diets: (i) Control; (ii) Enz; (iii) Oleo; (iv) CitriStim® (Citr); (v) Oleo+Citr (OleoCitr); (vi) Melengesterol acetate (MGA)+Oleo+Zilpaterol hydrochloride (ZC; MGAOleoZC); (vii) Monensin+Tylosin+MGA+ZC=Conv; (viii) Conv+Oleo (ConvOleo). Data from these trials were used to estimate greenhouse gas (GHG) emissions using Holos, a whole-farm GHG emission model (www.agr.gc.ca/holos-ghg), while ammonia (NH₃) emissions and resource (land and water) use were generated using spreadsheet calculations. Conventionally treated and implanted cattle exhibited improvements in growth and carcass traits as compared to the other treatments ($P<0.05$). Consequently, replacing the conventional feed additives with or without the natural feed additives increased resource use by 7.9% and 10.5% for steers and heifers, respectively. Further, GHG emission intensity for steers and heifers increased by 5.8% and 6.7%, and NH₃ emission intensity by 4.3% and 6.7%, respectively. Ceasing implant usage also increased resource use by 14.6% and 19.5%, GHG emission intensity by 10.5% and 15.8% and NH₃ emission intensity by 3.4% and 11.0% for heifers and steers, respectively. These results demonstrate that use of conventional PETs increased animal performance while reducing the environmental impacts of feedlot cattle production; hence, removal would increase the environmental footprint of beef produced for domestic and international markets.

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Emission Intensities in Sheep Production Located in Various Geographical Regions of Norway

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Sheep currently account for approx. 4% of GHG emissions from livestock. Human population growth will likely elevate consumption of sheep products. To limit GHG emissions, it is essential to reduce emission intensities, i.e., GHG emissions from one unit of product. Thus, on-farm GHG emissions must be accurately estimated, and mitigation options need to be evaluated using whole-farm models that account for trade-offs between emission sources. HolosNorSheep is a whole-farm model describing GHG emissions from Norwegian dual-purpose meat and wool production, using a cradle to farm gate approach. The model is based on IPCC methodology and considers direct CH₄ from enteric fermentation and manure management, direct and indirect N₂O from manure management and soils, direct CO₂ emissions from energy use (fuel) and indirect CO₂ emissions from the production of input factors (electricity, fertilizers, and pesticides). Soil carbon balance is estimated using the ICBM-model. Emission intensities for sheep and mutton carcass and wool were calculated for five geographical regions: East, South-West, East, Mid and North Norway. These regions differ in resource availability, pasture periods, animal performances, manure management, etc. Inputs were based on farm operational data and data from the Sheep Recording System. The resulting emission intensities for wool and sheep and mutton carcass varied from 45.2 and 17.1 kg CO₂-eq. in Mid Norway to 52.7 and 20.0 kg CO₂-eq. in North Norway, respectively. The difference was mainly due to higher CO₂ and N₂O emissions from greater fuel and N-fertilizer use in North Norway, related to a greater ley area needed to compensate for a shorter growth season and normally one cut to produce grass silage for indoor winter feeding. Based on the distribution of sheep in the different regions, weighted average emission intensities for wool and sheep and mutton carcass of 45.6 and 17.3 kg CO₂-eq were estimated.

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On Average, Modelled Methane Matches Measured Methane

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The Dutch government has committed to 50% reduction of greenhouse gas emissions by 2030 and 90% by 2050. To help achieve this goal, each dairy farm in the Netherlands has methane emission predicted annually, for policy, research, and management decisions. Recently methane was measured within barns on several farms. Our objective, with support from the Ministry of Agriculture, Nature and Food Quality, was to determine the agreement between modelled and measured methane. For modelling, the KringloopWijzer was used, which was developed using recent research. This model uses farm specific ration of the herd to estimate total emissions of the farm. Typically, this model uses a complete year; however, weekly feed and production data were available for one farm, so weekly emissions were estimated instead. For measuring, three methods were used; a barn sensor (air samples collected near the roof were measured with a laser), a GreenFeed, and a near-infrared sensor (sniffer). The model, barn sensor, and GreenFeed results were converted to methane emission (kg/animal/year). The sniffer measured concentration and could not be converted. The measured methods captured more weekly variation than the model. The Pearson's correlation was low between the model and barn sensor (0.10), and higher with GreenFeed (0.49). The R-squared value from linear regressions between the model and measurements, were low (zero to 0.24). On a weekly basis there was poor agreement; however, the model long-term average was a reasonable predictor of methane measured with the barn sensor (141.3 ± 7.7 and 159.7 ± 27.7 kg/animal/year, respectively), and a good predictor with GreenFeed (146.4 ± 14.9 and 145.8 ± 8.0 kg/animal/year, respectively). In conclusion, measuring methane captures more variation across weeks than modelling, thus it may not be appropriate to make weekly comparisons with a model designed for annual emissions. However, on average, the model and measured methods are in agreement.

Re-Establishing Native Mixed Grassland Species into Annual Cropping Land in the Canadian Prairies

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The loss of native grassland due to conversion to cropland is a critical problem across the United States and Canadian Great Plains. The objective of this study was to investigate the impacts of re-establishing native mixed grassland species into annual cropping land on pasture productivity and quality, animal response and soil organic carbon (C). The study was conducted at the Agriculture and Agri-Food Canada, Swift Current Research and Development Centre (semi-arid, Brown soil zone). Three different pastures were established in 2006 on 9 paddocks of 0.81 ha (2 acre) that had been primarily in wheat and summer fallow: i) native grass mix (7 native grass species) + native legume (NG+NL), ii) native grass mix + alfalfa (NG+TL), and iii) meadow brome grass + alfalfa (TG+TL). All pastures were continuously grazed with commercial yearling steers to a utilization rate of 50-60%. Soil samples were collected in 2008, 2012, 2015, and 2018 at different sampling depths. Average daily gain (kg/day) was independent of pasture type; however, total live weight production (kg/ha) was lower ($P < 0.001$) for NG+NL (100) relative to NG+TL (228) and TG+TL (222). This could be linked to the observed differences in available pasture yield and stocking rate among treatments. Organic matter digestibility was affected ($P < 0.001$) by pasture mixture; 52, 58 and 59% for NG+NG, NG+TL and TG+TL, respectively. Annual soil C sequestration for the top 15 cm sampling depth was not affected by treatment and ranged between 0.06 and 0.69 Mg/ha. However, for the 30 cm sampling depth, annual C sequestration was lower ($P = 0.002$) for NG+NL (0.62 Mg/ha) relative to NG+TL (2.10 Mg/ha) and TG+TL (2.35 Mg/ha). Overall, native species can be established into annual cropland to support beef production and improve soil C sequestration.

Enhanced Foliar Nitrogen Formulations Persistence as Affected by Rate, Rainfall Intensity and Timing

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Low recoveries of nitrogen (N) applied as foliar fertilizer are probably related to the lack of foliar interception and the superficial runoff of the leaves. The objective of this work was to determine the persistence of the application of different enhanced foliar formulations applied to *Lolium perenne* L tested in intact soil core lysimeters kept in a climate-controlled room under controlled conditions (16/8 hours light/dark, LED lamps T8 Glass tube 18w, 6500k daylight, FSLT812 18W; 20° C). We tested dissolved Urea (Urea-d), Nanoformulation-urea (Nano-urea) and a Nanoformulation-NO₃ (Nano-NO₃), all applied in a 1:2 dilution with nano pure water, at rates equivalent to 25, 50, 75 and 100 kg N ha⁻¹ applied at 0 h, 6 h and 24 h post application, under different rainfall intensities (5, 10, 20, 30 mm d⁻¹), all organized in a randomized design (n=3). Additionally, a control treatment without N application was included (water only). There were interactions between the formulation used, the N rate, the rainfall intensity and the time elapsed after application (P<0.05). Runoff concentrations of available N (N-NH₄ + N-NO₃) increased with the shortest time elapsed after fertilizer foliar application, and increasing rainfall intensities, associated to the dragging effect of rain drops on the fertilizer present on the leaves. The time elapsed between the N foliar fertilization and the occurrence of rainfall was more relevant when high doses of N were applied. Regardless of the dose of N used, the application of Urea-d or Nano-urea resulted in lower total N losses. This suggests that the size of the enhanced formulation and its N release dynamic at 24 h significantly affected the rate of absorption by the leaf, allowing a greater permanence on the surface.

Multi-Study Quantification on the Abatement Potential of the 3-Nitrooxypropanol in Feedlot Diets

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Lately, global forums have been discussing the role of enteric methane contribution in greenhouse gas emissions. The Australian livestock industry has been working on innovative ways to reduce its methane emission outputs to aid in the climate crises. Aiming to quantify the potential methane abatement of 3-nitrooxypropanol, a meta-analysis with all previously published papers using feedlot cattle was performed. A database was built with five studies using 3-nitrooxypropanol in backgrounding and finisher feedlot diets and was comprised of 26 treatments. The statistical analysis was performed using the MIXED procedure of SAS (version 9.4, SAS/STAT, SAS Institute Inc., Cary, NC), considering study as a random effect. Furthermore, to account for variations in precision across studies, the inverse of the squared standard error of the mean of methane yield was used as a factor in the WEIGHT statement of the model. All analysis were performed using methane yield (g/kg of dry matter intake) to account for any differences in intake across studies. The standardized mean effect of methane yield (estimated as the ratio of methane yield of 3-nitrooxypropanol fed beef cattle divided by control cattle emissions) revealed that 3-nitrooxypropanol in feedlot backgrounding and finisher diets can suppress methane emissions in cattle by 29 and 27%, respectively. Moreover, the linear regression of the methane yield (g/kg of dry matter intake) on the 3-nitrooxypropanol dose (mg/kg of dry matter) showed a reduction of 416000 mg of methane emitted for every mg of 3-nitrooxypropanol added in one kilo of backgrounding or finisher diet (CH_4 yield = $23.2 \pm 1.72 - 0.0416 \pm 0.00997 \times 3\text{-nitrooxypropanol dose}$; $P < 0.01$; RMSE = 5.15). In conclusion, 3-nitrooxypropanol is one of the most promising of the dietary methane inhibitors known and reduces methane emissions by approximately 30% in ruminants.

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The Red Seaweed *Asparagopsis taxiformis* Inhibits Methane Emissions in Feedlot Beef Cattle

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It has been confirmed in confined studies that the red seaweed *Asparagopsis taxiformis* when included at low level in cattle feed will induce significant reductions in enteric CH₄ emissions. However, efficacy has not been demonstrated in commercial beef feedlots. The objective of this study was to confirm and quantify the antimethanogenic efficacy of *Asparagopsis* in a commercial feedlot for the first time.

Sixty-four *Bos taurus* heifers with initial live weight of 399±7.69 kg and approximately 18 months of age were allocated by weight into 4 blocks of 16. Heifers were randomly allocated to a control group on a feedlot finisher diet, or a treatment group on the finisher diet containing 0.4% (DW) of freeze dried *Asparagopsis*. The 77-day feedlot finishing period was carried out at the “Tullimba” commercial feedlot operated by University of New England in Kingstown, NSW, Australia, (30°20’S, 151°10’E; altitude 560 m). The study was approved by the UNE Animal Ethics Committee (Authority No. AEC-21-03). Heifers were housed in 8 pens of 8 animals and individual feed intake was recorded through an automated feed intake system (Autofeeder [AF] pen; Ruddweigh International Scale Company, NSW, Australia). The barley-based feedlot diet contained 14.9% crude protein and 13.7 MJ/kg of dry matter of metabolizable energy. The heifers were fed daily between 9.30 and 10.00 am and live weight measured on days 0, 28, 56 and 76. Individual methane emission was measured for 77-days using 8 Greenfeed Methane Emission monitors (C-Lock Inc., Rapid City, SD). Dry matter intake (9.58±0.576 kg/d), final weight (518±10.5 kg) and gain-to-feed ratio (0.167±0.061) did not differ between treatments (P>0.05). *Asparagopsis*-fed heifers produced 67% less methane than the control heifers during the feeding period (112 vs 36.9 g/d). The study demonstrates that the significant methane mitigating potential of *Asparagopsis* at low feed inclusion is maintained in a commercial feedlot environment.

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Insertion Depth of Oral Stomach Tubes in Cows Affects *In Vitro* Methane Inhibition of Seaweed

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This study aimed to quantify the effect of rumen fluid (RF) collection site (central or ventral) on *in vitro* fermentation parameters and methane inhibition by *Asparagopsis taxiformis*. We expected a greater methane inhibiting effect with batch incubations with RF from the central rumen layer, the site of more intense fermentation activity. Rumen fluid was collected first centrally and then ventrally via an oesophageal tube from 6 dry Norwegian Red cows. Rumen-cannulated cows were selected to manually control sampling location. Equal volumes of RF from cows 1-3 and cows 4-6 were mixed, diluted with buffer, and incubated for 24 h with ANKOM Gas Production System. Approximately 1 g DM of substrate (50:50 grass silage and concentrates, with or without 9.0 mg seaweed) was incubated in 100 mL buffered RF in triplicates using a 2 x 2 x 2 factorial experiment [2 rumen locations (central, ventral), 2 groups of cows (1-3, 4-6), 2 substrate treatments (feed ± seaweed)]. Total gas production was quantified. After 24 h concentrations of methane in headspace and of short-chain fatty acids (SCFA) in incubation fluid were analyzed by gas chromatography. Linear mixed-effect model in R was used with location and treatment as fixed effects and cow group as random effect. Total gas production was affected by seaweed, causing a 10% reduction ($P < 0.01$), but not by RF sampling site ($P = 0.48$), whereas methane production was affected both by seaweed ($P < 0.01$) and RF sampling site ($P = 0.03$). Seaweed led to a 99% reduction of methane with central RF incubations but only to an 87% reduction with ventral RF incubations. Only iso-SCFA was affected by both seaweed and RF sampling site. In conclusion, *in vitro* batch technique may underestimate the methane mitigation potential of test substrates when using RF from ventral rumen.

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Methane Related Phenotypes and Their Correlations with Production Phenotypes in Holstein Dairy Cows

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Genetic selection of low methane producing animals has been proposed as a strategy to decrease enteric methane emissions of cattle. There is debate as to the methane related phenotypes that should be selected to breed dairy cows for low greenhouse gas emissions. The most common phenotypes considered have been methane production, methane yield and methane intensity. Recently, residual methane production, the difference between actual and predicted methane output has been proposed as a suitable trait that could be used. A key attribute of any trait used to select low methane producing cattle is that it should not be antagonistic to production traits. The aim of this research was to evaluate the relationship between methane production, methane yield, methane intensity and residual methane production and production phenotypes of lactating Holstein cows. The experiment included 480 lactating Australian Holstein cows. Methane production was measured with the sulfur hexafluoride tracer technique over a five-day period, with dry matter intake recorded through an electronic feeding system. Methane production was positively correlated ($P < 0.01$) with body condition score ($r = 0.31$), bodyweight ($r = 0.19$), bodyweight change ($r = 0.22$) and dry matter intake ($r = 0.43$). Methane yield was positively correlated ($P < 0.01$) with body condition score ($r = 0.23$), body weight change ($r = 0.13$) and concentration of milk fat ($r = 0.29$). Methane intensity was positively correlated ($P < 0.01$) with days in milk ($r = 0.25$), body condition score ($r = 0.32$), body weight change ($r = 0.16$) and concentrations of milk fat ($r = 0.54$) and milk protein ($r = 0.32$). Residual methane production was positively correlated ($P < 0.01$) with milk fat concentration ($r = 0.22$), but not correlated with any other phenotypic variables. Residual methane production was identified as the most appropriate phenotype for breeding purposes as it had the smallest correlations with major production phenotypes.

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Effect of Arbuscular Mycorrhizal Fungi in the Reduction of N₂O in Soybean Cultivated Soils

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Agricultural soil is an important source of nitrous oxide (N₂O), a potent greenhouse gas involved in the destruction of the protective ozone layer that contributes to global warming. A few recent studies have revealed the potential effects of Arbuscular Mycorrhizal Fungi (AMF), a widely distributed soil fungi on controlling N₂O emissions. However, how AMF regulates N₂O production from soil remains poorly understood. To address the identified knowledge gap, we manipulated the abundance of AMF in two independent greenhouse experiments using two different approaches (sterilized and re-inoculated soil and non-mycorrhizal soybean mutant) and two different soils. The results showed that N₂O emissions were increased by 56% and 45% in microcosm with reduced AMF abundance compared to microcosms with a well-established AMF community thus suggesting that AMF regulates N₂O emission. The abundance of key genes (*nirK* and *nosZ*) responsible for denitrification significantly decreased in AMF treatments indicating that the regulation of N₂O emission is transmitted by AMF-induced changes in the denitrification process. Therefore, we propose a possible mechanism that enhances N₂O-consuming denitrifiers in legumes (soybean) because it holds promise for mitigating N₂O emission.

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Climate Impact of Improved Genetic Resources from a Novel Beef Production System in the Colombian Orinoquia

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The climate impact of livestock activities is key in the achievement of climate targets of developing economies, which usually rely on the agricultural sector. Farm, and particularly life cycle models are commonly used to assess the climate impact of cattle. However, there are major methodological challenges to overcome due to their multifunctional nature. This paper presents for the first time an assessment of the greenhouse gas emissions from the production of genetic resources in cattle farms combining a model at the farm level with the life cycle of the animals. The object of the study was a cattle farm in the Colombian Orinoquia oriented towards a low carbon intensity production by means of a high-productivity cattle breed, and a rotational grazing system with improved pastures. The functional unit was the annual production of the determining co-products “1 unit” of embryo, and semen, and “1 kg genetics” of female and male breeding stock, and weaned heifer. In an attributional approach the allocation was based on the live weight, the energy requirement for metabolic processes, and the price. Following a consequential approach, the substitution by system expansion of the dependent co-product “1 kg beef” of cull females and males was estimated. In the life cycles of embryo-female and semen-male the contribution of the embryo and semen to the emissions ranged from zero in the allocation based on live weight to almost 100% in the economic allocation. In the life cycle of weaned heifer-cull female the emissions can be halved by substituting beef from farms with a lower efficiency. The study evidences the climate benefits of the introduction of animals and pastures of high genetic quality in cattle farms. The developed framework can be used to enhance monitoring, reporting, and verifying systems in the assessment of climate smart solutions.

Nitrogen Cycling and N₂O Emissions in *Brachiaria*-Based Grass-Alone Pastures and Silvopastoral Systems in a Grazing Trial in Colombia

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Silvopastoral systems provide a number of environmental and productive benefits compared to grass-alone pastures in terms of i) increased forage biomass offer and quality, ii) nutrient cycling, iii) biodiversity, iv) cash flow, among other ecosystem services. With the aim of evaluating the nitrogen (N) cycling differences in grass-alone pastures and silvopastoral systems we established a field trial in the CIAT campus in Palmira, Colombia consisting of two grass-only, and two silvopastoral treatments: i) *Brachiaria* hybrid cv. Cayman, ii) *Brachiaria* hybrid cv. Cayman + *Leucaena diversifolia*, iii) *B. brizantha* cv. Toledo, and iv) *B. brizantha* cv. Toledo + *L. diversifolia*. Over a six months period we evaluated every 50-55 days the aboveground biomass production after standardization of the *Brachiaria* grass, the *L. diversifolia* foliage, forbs, and plant litter. The dry matter production per treatment for each botanical fraction, and nutrition quality parameters of the grasses and the legume were determined. The isotopic ¹³C and ¹⁵N natural abundance of all samples were measured to estimate N fixation by the legume and transfer to the associated grass. The soil-borne nitrous oxide (N₂O) emissions and inorganic N transformations in the soil were monitored over a period of 50 days after simulation of urine deposition during the rainy season. Soil chemical and physical characterization were performed at the beginning and at the end of the experiment. Our hypothesis is that plant biomass and N concentration in forage is higher in the silvopastoral systems compared to the *Brachiaria* alone pastures via N fixation by the legumes, and that N₂O emissions intensity is lower in the silvopastoral treatments. Currently diverse technologies have been proposed pointing for sustainable livestock production, however, silvopastoral systems stand out among them for the potential of dual benefit increasing livestock productivity while providing ecosystem services, including reduction of the carbon balance of the system.

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Nitrogen Use Efficiency and N₂O Emissions of Three Tropical Forage Grasses Fertilized with Different Nitrogen Rates

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Cattle raising is an important agricultural activity because of its role in food security, the generation of jobs throughout the value chain and its contribution to the global economy. However, it is necessary to optimize its management to increase productivity and mitigate adverse effects on the environment. The objective of this study was to evaluate the influence of different doses of nitrogen (N) on the productivity and N use efficiency (NUE) of three common pastures of low-land conditions of the tropics, *Urochloa* hybrid cv. Cayman, *Megathyrsus maximus* cv Mombasa and *Cynodon nlemfuensis* - Stargrass. In a cattle system located in the municipality of Santander de Quilichao, Cauca - Colombia, plant biomass production, nutritional quality parameters, NUE and N₂O emissions were evaluated in response to four fertilization rates (0, 10, 20 and 30 kg N ha⁻¹). Results showed that the highest NUE was achieved with 20 kg N ha⁻¹, with 93% efficiency. Cayman pasture with a rate of 20 kg N ha⁻¹ had a higher dry matter (DM) production than with 30 kg N ha⁻¹ (i.e., 22% more) and 47% less N₂O total emissions that translated into 1.65 µg N-N₂O kg DM⁻¹ of emissions intensity. In Stargrass, the 20 kg N ha⁻¹ rate allowed an increase in productivity and a decrease in N₂O emissions with respect to the other rates; the emissions intensity was 4.6 µg N-N₂O kg DM⁻¹. Mombasa grass with 30 kg N ha⁻¹ showed an increase of approximately 60% in dry material productivity, at a very low emission cost (1.43 µg N-N₂O kg DM⁻¹). Among tropical pastures there is substantial genetic variability to improve NUE and reduce N₂O emissions. This observation can be complemented with agronomic management such as the use of optimal N doses.

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How Predictive Methane Emissions Models, Developed under Experimental Conditions, Can Help Assess Methane Emissions from Ruminants in the Sahel

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In the Sahel region of West Africa, pastoral ruminant grazing systems are characterized by very low levels of forage intake and low-quality forage of around 40% digestibility during most of the year, especially the dry season. Consequently, the latest estimates of enteric CH₄ emissions from Sahelian cattle herds considering the specificity of their diet (intake and digestibility parameters) have shown that CH₄ levels are around half of the values of the initial IPCC Tier 1 predictions. The IPCC Tier 1 hypothesized that intake was at its full value. A database including 602 digestibility experiments on small ruminants and 87 experiments on cattle, performed in Senegal from the early 60s to the end of the 90s, was used to predict potential CH₄ enteric emissions. These experiments accurately measured parameters which are indispensable for emissions estimations but quite difficult to assess in the field (intake, chemical composition of feed and feces) and which vary a lot through seasons. The experiments covered a wide diversity of livestock feed from natural Sahelian pastures, agricultural by-products (groundnuts haulms and husks, rice and millet straw) to available supplements (groundnuts or cotton cakes, local pellets, molasses). This diversity explains the great range of crude protein (16 to 283 g CP/kg DMI), fibre (226 to 965 g NDF/g DMI), fat (3 to 137 g EE/kg DMI) and lignin (25 to 302 g ADL/kg DMI) contents of the diets considered. In the literature, models that fit with these feeding conditions are scarce. In addition, they do not always consider as predicting indicators some chemical components (EE, CP, tannins, lignin) which vary significantly among diets according to the seasons and regions. Recently available CH₄ prediction models established in experimental conditions considered to be closer to this study's digestibility trials were selected, discussed, and compared to the IPCC Tier 1 and 2 methods as a default reference. This work aimed to find models more suited to Sahelian livestock conditions. However, *in vivo* measurements are still lacking to assess and improve their accuracy. Preliminary results of a new research project (CaSSECS) measuring *in vivo* enteric CH₄ emissions from Sahelian cattle provide ways forward for a more holistic understanding of their enteric CH₄ production.

Keywords: Sahelian ruminants; enteric CH₄ emissions; predictive models

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Effects of High-Oil Rapeseed Cake or Natural Additives on Methane Emissions and Performance of Dairy Cows

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Eight Red Nordic dairy cows (81 ± 21 d in milk and 41.0 ± 1.9 kg of milk yield) were randomly assigned to a replicated 4×4 Latin square design with 21-d periods. Treatments comprised grass silage-based diets (45:55 forage to concentrate ratio on DM basis) including 1) control containing 19.3% rapeseed meal, 2) control with full replacement of rapeseed meal with rapeseed cake, 3) control plus 50 g/d of yeast hydrolysate product and coniferous resin acid-based compound, and 4) control plus 20 g/d of combination of garlic-citrus extract and essential oils in a pellet. Total-tract digestibility was measured using total collection of feces, and CH₄ emissions were measured in respiratory chambers on 4 consecutive days. Data collected during d 17-21 in each period were statistically analyzed using a mixed model. Treatments did not affect feed intake while feeding rapeseed cake increased crude protein and ether extract digestibility compared with the other diets. Emissions of CH₄ per day, per kilogram of DMI, and per kilogram of energy-corrected milk, and gross energy intake were lower for rapeseed cake compared with other diets. There was no effect of yeast-resin on daily CH₄ emissions, whereas CH₄ yield (g CH₄/kg DMI or as percentage of gross energy intake) decreased with garlic-citrus compared with control. Treatments did not influence energy balance. Further, rapeseed cake reduced the proportion of nitrogen intake excreted in feces, and yeast-resin improved nitrogen balance compared with control diet. Feeding rapeseed cake resulted in greatest yields of milk and energy-corrected milk, and feed efficiency. Relative to control diet, rapeseed cake decreased saturated fatty acids by 10% in milk fat by increasing *cis*-monounsaturated fatty acids but also increased the proportion of *trans* fatty acids. Proportion of odd- and branched-chain fatty acids increased with garlic-citrus and yeast-resin compared with control.

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Understanding Variability of Enteric Methane Production in Beef Cattle to Identify Low-Methane Animals

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Breeding ruminants for low methane (CH₄) production can make permanent reductions in emissions. Thus, there is a need to better understand variability of CH₄ production among cattle to accurately assess low-CH₄ phenotypes. Our objectives were to: 1) investigate the between-animal and within-animal variation in CH₄ production of finishing beef cattle, 2) identify consistently low-CH₄ emitters, and 3) examine factors that contributed to the variability. Crossbred beef heifers (n=77; BW=450 kg) were allocated to 3 pens and offered a diet of 90% concentrate and 10% silage (DM basis). The study was conducted over 3 consecutive 6-week periods (total of 126 days). GrowSafe bunks measured daily DM intake (DMI) and rumen fluid was sampled orally each period. A GreenFeed system measured individual animal emissions for 2 weeks/period. Methane production was calculated by animal within period using fluxes that were ≥3 min, compiled into six 4-h blocks corresponding to time of day, and averaged over blocks. Animals with <5 blocks were omitted for the period and animals with ≥2 periods of good CH₄ data were used in the final analysis (n=52). Animals were ranked based on CH₄ yield (g/kg DMI) from low to high and grouped as Very low (≤10% of animals), Low (11-25%), Intermediate (26-74%), High (75-89%), and Very high (≥90%) emitters (mean±SD, 12.63±2.184). CH₄ yield was 16% less (*P*<0.05) for Very low compared with Intermediate animals, and there was a 36.6% difference between the top and bottom groups. Lower CH₄ yield was due to lower CH₄ production (g/d, *P*<0.05) rather than decreased DMI (*P*>0.05). Total VFA concentration decreased as CH₄ yield decreased, although molar proportions of VFA remained unchanged, suggesting lower extent of ruminal digestion rather than a shift in fermentation. There were no differences in feeding behavior or average daily gain among groups (*P*>0.05). A period×group interaction (*P*<0.001) for CH₄ yield indicated that the rankings for animals changed over time; however, there were no extreme changes in rankings. We conclude that when using a GreenFeed system not all animals will be characterized for CH₄, and measurements need to be repeated over time to accurately identify low CH₄-emitting animals.

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Methane and Carbon Dioxide Emissions from Crossbred Beef Cattle Grazing Native Pastures

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Enteric methane (CH₄) from ruminants accounts for about 6% of global anthropogenic greenhouse gas emissions, and individual animal contribution varies with feed efficiency. Past studies measuring CH₄ emissions in beef cattle have often used drylot conditions with uniform diets. To better understand CH₄ production from cattle grazing diverse diets on open-range, we quantified CH₄ and carbon dioxide (CO₂) production from beef cattle previously measured for residual feed intake, adjusted for off-test backfat thickness (RFI_{fat}), in drylot. In addition, cattle were measured while grazing two different pasture conditions (SUM: higher quantity and quality in summer; FAL: higher quantity but lower quality in fall). Crossbred beef cows (n=34) and replacement heifers (n=19) were monitored using the GreenFeed emissions monitoring (GEM) system for 26 ± 12 days in each pasture to collect their CH₄ and CO₂ emissions (g/day). Cows had higher average daily CH₄ (SUM: 260.1 ± 3.1 vs. 193.3 ± 4.3 g/day; FAL: 290.4 ± 3.0 vs. 230.0 ± 4.0 g/day) and CO₂ emission (SUM: 9297.6 ± 92.3 vs. 7010.7 ± 124.9 g/day; FAL: 9250.5 ± 90.8 vs. 7327.5 ± 121.9 g/day) than heifers ($P < 0.01$). Cattle emitted less daily CH₄ (16% for heifers; 10.4% for cows) and CO₂ (4.3% for heifers) when grazed on summer pasture compared with fall pasture ($P < 0.01$). A positive relationship was evident between cow CH₄ emission and RFI_{fat} ($R^2 = 0.008$; $P < 0.05$) during fall grazing. Similarly, CO₂ emission was positively related to cow RFI_{fat} in both pastures ($R^2 = 0.015$; $P < 0.01$). In conclusion, beef cattle CH₄ emissions increased from summer to fall with advancing vegetation senescence. Although other factors might also influence enteric CH₄ emissions of cows grazed on high quantity-low quality pasture, RFI_{fat}, measured in a feedlot setting, can explain a small but significant proportion of this variance.

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Developing Prediction Models of Enteric Methane Production in Sheep Using an Intercontinental Database

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Measurement of enteric methane is expensive and not practical in most farms. Reliable prediction models have been developed recently for dairy and beef cattle based on intercontinental databases; however, equivalent sheep models are not yet available. This study aimed to: 1) collate an intercontinental database from individual sheep; 2) identify the key covariates for predicting enteric methane production (g/d); and 3) develop and cross-validate global equations as well as the potential need for age, diet, or climate region-specific equations. The refined intercontinental database included 2,135 individual observations across 13 countries and linear models were developed by incrementally adding covariates. A universal methane production equation using only DMI led to a root mean square prediction error (RMSPE) of 25.4% and a RMSPE-standard deviation ratio (RSR) of 0.69. Increasing the complexity of universal models by including DMI+BW, DMI+BW+OMD or DMI+BW+rumen VFA molar proportions tended to increase the prediction performance (RSR=0.62, 0.60 and 0.64, respectively), whereas the diet composition had a negligible effect. These universal equations had higher precision and less prediction error with similar accuracy than the IPCC extant equations. Similar prediction performance was observed when universal equations were used for warm or temperate climate regions indicating that within this database there is no need for developing region-specific models. Development of diet-specific models (forage vs mixed-diets) led to minor changes in the prediction performances. On the contrary, prediction can be improved through the development of age-specific models for adult sheep (>1-year-old), including DMI and propionate (RMSPE=18.6%, RSR=0.57). For young sheep (<1-year-old), the universal models can be applied if DMI and BW are included (RMSPE=23.0%, RSR=0.68). In conclusion, DMI is the key factor in predicting sheep methane production and the utilization of age-specific equations adding information on the BW and rumen fermentation pattern can substantially increase prediction accuracy.

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Effect of Dietary Metabolizable Protein Level on Energy Partitioning of Ayrshire and Holstein Dairy Cows

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This study aimed to determine the effect of dietary metabolizable protein (MP) level on energy partitioning of Ayrshire and Holstein dairy cows. Six Holstein and 6 Ayrshire lactating cows were used in a replicated 3 × 3 Latin square (35-d periods; 14-d adaptation) with a 2 × 3 factorial arrangement of treatments. Cows were fed (ad libitum) a total mixed ration formulated (NRC, 2001) to provide 85%, 100% or 115% of MP requirements. Energy expenditure in methane (respiration chambers; 5 d) and in milk, feces, and urine (7 days) were measured. Main effects of breed, MP level and interaction (breed × MP) were determined using the MIXED Procedure of SAS. Linear and quadratic contrasts were used to determine effects of MP level on variable responses. Significance was declared at $P \leq 0.05$. The breed × MP level interaction was not significant for measured variables. Gross energy (GE) intake was not affected by increasing MP level but was less for Ayrshire versus Holstein cows (93 vs. 117 Mcal/d). Loss of energy (Mcal/d) in methane and urine increased linearly with increasing dietary MP level, while fecal energy losses were not affected by MP level. When expressed as proportion of GE intake, only urinary energy losses increased with increasing level of MP, while proportional methane and fecal energy losses were not affected. Fecal energy losses (% GE intake) were less for Ayrshire than Holstein cows (30.0% vs. 32.2%), but urinary and methane energy losses were not affected by breed (2.5%, 5.4%, respectively). Milk energy expenditure (Mcal/d) or efficiency (% GE intake) increased linearly with increasing dietary MP level. Compared with Holstein cows, daily milk energy expenditure of Ayrshire cows was 20% less (24.0 vs 30.3 Mcal/d), but milk energy efficiency averaged 25.8% and was not affected by breed. Results from this study show that energy expenditure in Holstein and Ayrshire dairy cows responded similarly to increasing dietary MP level, even though energy intake and milk energy expenditure were very different between the two breeds.

***Cajanus cajan* Consumption on Performance and Mitigation of Enteric Methane Emissions from Nellore Steers**

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Solutions to more productive grazing systems capable of mitigating enteric methane (CH₄) emissions are needed. This study aimed to evaluate the performance and the enteric CH₄ emission of *Nellore* steers in three different continuous grazing systems, including *Cajanus cajan* consortium. The experiment occurred from July 2020 to July 2021 at Embrapa Pecuária Sudeste, in Sao Carlos, SP, Brazil. Twenty-seven animals (15-16 months) were weighed monthly and their enteric CH₄ production measured by the Sulfur hexafluoride (SF₆) tracer gas technique for five days. The treatments were: 1) recovered pasture with a mixture of *Urochloa decumbens* cv. Basilisk and *Urochloa brizantha* cv. Marandu with 200 kg N-urea/ha per year in the rainy season, with moderate stocking rate (REC); 2) degraded pasture of *U. decumbens* cv. Basilisk with low stocking rate (DEG); 3) pasture with a mixture of *U. decumbens* cv. Basilisk, *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* cv. BRS Mandarin with moderate stocking rate (CON). Each treatment were in three replicated areas with 1.5 ha, submitted to stocking rate adjustments using “Put and take” technique. The performance and CH₄ data were subjected to analysis of variance and comparison of means by the Fisher test (5%), using the SAS PROC MIXED. Individual average daily gain (ADG) was statistically different ($P \leq 0.05$) between treatments: CON (478 g/day), followed by REC and DEG (387 and 302 g/day, respectively). The daily emission per animal and daily emission per kg of gain were, respectively: 207 g/head per day and 1054 g/kg in REC; 211 g/head per day and 2023 g/kg in DEG; and 214 g/head per day and 614 g/kg in CON, having statistical difference only in emission per kg of gain. The efficient use of intercropping increased the weight gain of the animals and emitted less methane per kg gain.

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Methane and Short-Chain Fatty Acid Production from Nellore Steers in Pasture Intercropped with *Cajanus cajan*

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The study evaluated the enteric methane (CH₄) emission and short-chain fatty acid (SCFA) production of *Nellore* steers in three grazing systems under continuous stocking. The experiment was conducted at Embrapa Pecuária Sudeste, in Sao Carlos, SP, Brazil. Nine rumen-cannulated animals (15-16 months), weighing 280 kg, were evaluated in two seasons: rainy (January) and dry (July) seasons of 2021. The emission of CH₄ and production of SCFA were assessed by the Ex-situ ruminal fermentation technique at 0, 4, 8, and 12 hours after the onset of the evaluation and quantified by gas chromatography. The treatments were: 1) pasture with mixture of *Urochloa decumbens* cv. Basilisk and *Urochloa brizantha* cv. Marandu recovered with 200 kg N-urea/ha per year in the rainy season (REC); 2) degraded pasture of *U. decumbens* cv. Basilisk (DEG) and 3) pasture with a mixture of *U. decumbens* cv. Basilisk, *U. brizantha* Stapf cv. Marandu intercropped with *Cajanus cajan* cv. BRS Mandarin (CON). Each treatment was allocated in three replicated areas with 1.5 ha. The means were subjected to analysis of variance and compared by the Fisher test ($p < 0.05$), using the PROC MIXED of SAS. The CH₄ production differed between seasons: 23.1 and 13.2 g CH₄/kg DM per day during the rainy and dry seasons, respectively. The CH₄ emission in the DEG was greater than the other treatments with 19.7 g CH₄/kg DM per day, compared to 17.7 and 17.2 g CH₄/kg DM per day of the CON and REC, respectively. The total production of SCFA did not differ among the treatments but was different between the seasons with averages of 191 and 130 g CH₄/kg DM per day in the rainy and dry seasons, respectively. Cattle grazing degraded pasture emit more CH₄ than fertilized pasture or grass-legume consortium.

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The Productivity-Profitability-Carbon Nexus of Livestock Systems under Increasingly Variable Climates

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Future climate change will likely result in increased frequencies of extreme climatic events. Here, we modelled the impacts of historical and future climates on pasture and livestock productivity, considering greenhouse gas emissions (GHG) and carbon sequestration for farms in two diverse agro-ecological regions of Tasmania (a beef farm in the cool wet north-west Tasmania (NWT), and a sheep farm in the relatively low rainfall Midlands (MT)). Future climate data were developed by introducing additional extreme events into downscaled projections from global climate models. Climate data were used in the GrassGro™ model to quantify pasture and animal production; GHG were computed using the Australian National Greenhouse Gas Inventory, and soil carbon fluxes and stocks were computed using RothC model. Co-developed with a regional farmer group, a package of whole-farm adaptations for each case study farm was modelled under future climates: “Low-hanging fruit” (animal management/genetics, feedbase management, plant breeding and improved soil fertility), hereafter LHF, and “Towards Carbon Neutral” (“Low-hanging fruit” in addition to deep-rooted species, injection of methane vaccine to livestock and planting trees), hereafter TCN. Despite increasingly variable climates, pasture and livestock production by 2050 were either similar to or greater than historical values. The LHF and TCN adaptation increased livestock production and reduced historical net emission intensities (EI). For NWT, EI ranged from 12 kg CO₂e/kg LW (historical) to 8-10 kg CO₂e/kg LW (TCN). For MT, EI decreased from 6 kg CO₂e/kg LW and 34 kg CO₂e/kg CFW (historical) to 5 kg CO₂e/kg LW and 26 kg CO₂e/kg CFW (TCN). The TCN adaptation reduced net farm emissions relative to LHF and even further compared to historical values (up to 31% and 20% for NWT and MT in 2050, respectively). Ongoing research will investigate the profitability of these adaptations, as well as adaptations allowing income diversification and farming systems transformations.

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Enteric Fermentation Flagship Project: Profiling Rumen Microbiomes for Methane Emission Prediction in Bovine Species

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The Enteric Fermentation Flagship project, funded by the Global Research Alliance, was initiated in 2018 to investigate rumen microbiomes to predict methane emissions in bovine species. A major objective of this project was to implement a low-cost, high-throughput method for profiling the rumen microbiome in bovine species across different production stages and systems used around the globe. The aim was to utilize this method for identifying and selecting low methane emitting animals while retaining or improving productivity. In this project, we implemented a protocol successfully tested and applied in sheep that utilizes freeze-dried rumen samples and restriction enzyme reduced representation sequencing for microbial sequencing to generate rumen microbiome profiles. To date, approximately 1015 rumen samples from Brazil (Buffalo), Ireland (Beef), New Zealand (Dairy/Beef) and Uruguay (Beef) have been collected and sequenced using this protocol. These samples were from animals across a diverse range of environments and production systems. Methane emission and feed intake were measured on 62% and 90% of the animals, respectively. We explored the variation in the rumen microbiome profiles across all samples and found large differences not only between countries but also between different systems within a country. Phenotype prediction was performed using a linear mixed model with a cohort-adjusted microbial relationship matrix as a random effect. We obtained prediction accuracies around 40%-60% for methane and 20%-30% for residual feed intake when predicting across cohorts. This work suggests that there is potential for using rumen microbiomes to predict important livestock traits in diverse production systems used around the world.

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Estimation of Methane Emission from Cattle of Bangladesh

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In Bangladesh, cattle numbers are increasing day by day as not only unemployed people but also employed people are now involved in farming. The increasing cattle population is a matter of great concern for Bangladesh because greenhouse gases are also increasing gradually due to the greenhouse gases (methane, carbon dioxide) in the expiratory gases of cattle respiration. In our study, we determined the methane emission from cattle in Bangladesh. We followed the Intergovernmental Panel on Climate Change (IPCC) guidelines using Tier-1 and Tier-2 methods for the calculation of total methane emission from cattle. The calculation showed the emission of methane from cattle using Tier-1 method based on emission factors provided by both IPCC-2006 and IPCC-2019. We also showed the emission value by using the Tier-2 system based on dry matter intake. Finally, we compared our different calculated values for different IPCC provided emission factors and for different methods. After calculation, in the year of 2016, 2017, 2018 and 2019 total emission of methane from cattle using Tier-1 method based on the emission factors provide by IPCC-2006 are 932.43, 938.08, 942.48 and 947.15 gigagrams respectively and based on emission factors provided by IPCC-2019 total emission are 1348.93, 1357.10, 1364.08 and 1371.52 gigagrams, respectively. Using the Tier-2 method, total emission of methane in those years are 970.95, 976.51, 981.20 and 986.19 gigagrams, respectively.

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A Simple Spot- sampling Method for Estimation of Methane Flux from Ruminants

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The rearing of ruminants for domestic consumption and export invariably leads to the emissions of methane, a potent greenhouse gas. However, limited data are available on methane emissions from ruminants in countries like Bangladesh because most methane flux estimation systems are complex and costly. The objective of this study was to establish and test a simple and cost-effective *in vivo* methane estimation system. The system consisted of a face mask with piping to a 500 L tank and manual gas sampling from the tank, into a vacutainer, before and after animal measurements. Methane in the air samples were analyzed using gas chromatography. A study was conducted to estimate methane output of a sheep with a body weight of 25.5 kg. The sheep grazed approximately 3 h, from 9.00 to 11.00 am, and then moved back indoors in a shed with access to approximately 250 g concentrate and water. Eight spot-samples were collected from the tank using the face mask system. The first sampling started at 11.00 am and then every six hours on measurement day one. Then there was a break of thirteen hours followed by collecting samples every six hours on day two. The methane estimate of each spot-sample was extrapolated to L/day and the average of the 8 spot-samples calculated. The average methane production in the current study was 2.58 L/day and diurnal variation was from 0.04 before morning grazing to 3.87 L/day at the evening. This suggests that the simple system developed can be used to estimate methane emissions from ruminants.

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Greenhouse Gases Emissions and ^{13}C Signature from Two Different Tropical Pasture Systems

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Intercropped systems have been suggested to replace monoculture grazing system as a strategic management for achieving a more sustainable ruminant production. An experiment was conducted to evaluate the effects of two different tropical pastures systems on carbon dioxide, methane, and nitrous oxide emissions, as well as the carbon isotopic signature of methane ($\delta^{13}\text{CH}_4$) and carbon dioxide ($\delta^{13}\text{CO}_2$). Treatments were eight pastures of *Urochloa brizantha* and eight pastures of *Urochloa brizantha* intercropped with *Tithonia diversifolia*. Sixteen polyvinyl-chloride static chambers were randomly distributed within paddocks ($n=8$). Greenhouse gases flux measurements were performed in dry and cold environment conditions in August 2021, for five consecutive days, during five minutes, between 0900 to 1100 AM using a real-time cavity ring-down spectrometer (CRDS) coupled to a sample inlet system. A subsample of gas was collected using plastic syringes and glass vials before opening the lid of the chamber for determination of the carbon isotopic signature. The greenhouse gases fluxes were calculated considering the linear increase of gas concentration during the incubation period, air temperature, pressure, and chamber volume. Data were analyzed as a randomized complete design and the means were compared using Tukey test at 5% of significance. No differences were found for the carbon dioxide or nitrous oxide fluxes between monoculture system and intercropped system ($P = 0.67$ and $P = 0.61$, respectively). However, for methane, the monoculture system showed greater uptake of methane ($P = 0.01$) than the intercropped system, ($-0.031 \text{ mg m}^{-2} \text{ h}^{-1}$ vs. $-0.016 \text{ mg m}^{-2} \text{ h}^{-1}$, respectively). The isotopic ^{13}C signature of methane and carbon dioxide were not different between treatments. In conclusion, in such environmental conditions and soil type, *Tithonia diversifolia* intercropped with *Urochloa* was not effective in mitigating greenhouse gases from soil with no effect on the carbon isotopic signature of the gases after eight months of the pasture system establishment.

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Use of Microbiota Composition Data to Improve Predictive Models of Methane Emissions from Dairy Cows

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The use of predictive models for evaluating enteric methane emissions from ruminants could be used to assess mitigation strategies on farms. Predictive models of these emissions often use animal and diet characteristics as predictors. As microbes are key players in methanogenesis, we hypothesized that inclusion of microbial data improves existing models' performances. In this work, we developed linear mixed-effect models with milk composition, animal and diet characteristics data for predicting methane production (g/day), methane yield (g/kg dry matter intake) and methane intensity (g/kg milk) from dairy cattle. We used data from 4 lactating dairy cows fed diets supplemented with concentrates rich in fiber or starch and without or with bicarbonate in a 4x4 Latin-square design. Enteric methane emissions were measured. Microbial taxa from rumen and faeces samples were grouped on the family level to have more consistent variables; row counts were transformed with the centered Log ratio transformation. Root-mean-square prediction error expressed as a percentage of the observation mean (RMSPE%) and concordance correlation coefficient (CCC) were used to compare the models. Including the bacterial (*Campylobacteraceae* and *Prevotellaceae*) or archaeal (*Group9_sp*, *Methanocorpusculum_sp*, *ucl_Euryarchaeota* and *ucl_Methanomicrobia*) families from faeces to methane production model and including the archaeal (*Group11_sp*, *Methanobrevibacter_ruminantium_clade* and *ucl_Euryarchaeota*) families from rumen to methane intensity model strongly improved their respective performances with a considerable decrease in RMSPE% (-56%, -52% and -33%, respectively) and a considerable increase in CCC (+32%, +41% and +54%, respectively). However, microbial taxa had no effect on methane yield. This study highlighted the interest in considering faecal microbial composition in the model most commonly used under field conditions. This unexpected result could be due to the dietary treatments tested. Moreover, rumen archaeal improved methane intensity prediction. These results suggest that microbial data might significantly improve the predictive ability of current models.

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***Desmodium heterocarpon*: A Stoloniferous Forage Legume for Low GHG Emission Livestock Production**

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A two-year study was conducted to investigate the impact on weight gain of Nellore heifers and greenhouse gas emissions of the introduction of the forage legume *Desmodium heterocarpon* into a *Urochloa brizantha* (cv. Marandu) pasture compared to grass-alone pastures with or without the addition of 3 x 50 kg N ha⁻¹ year⁻¹ urea fertilizer. The experiment was conducted at the CEPLAC pastures station in Bahia (16° 39' S, 39° 30' W) with two replicates of each treatment. Nitrous oxide emissions and ammonia losses from dung, urine, forage residues and N fertilizer were evaluated on five occasions using fixed chambers. Excreta production and forage intake by the heifers were monitored. Proportion of legume in the consumed forage was determined from ¹³C abundance of the dung. Introduction of the legume increased weight gain of the heifers per ha with the same impact (+60 %) as 150 kg N fertilizer. Nitrous oxide emissions averaged 0.40% of urine N and 0.07% faecal N. Ammonia volatilization losses from urine averaged 4.5% and 1.2% from dung. There were large variations in these emissions between sampling times attributable to variations in rainfall, but minor differences between pasture treatments. Of the fertilizer N, 0.2% was emitted as nitrous oxide and 4.9% volatilized as ammonia. The tannin content of this legume was high (2 to 6% dry matter) and there was a mean of 17.3% legume in the diet, which suggests that enteric methane emissions could be mitigated. The direct and indirect nitrous oxide emissions amounted to 77.7, 334.5 and 161.2 g N₂O-N, for the unfertilized, N fertilized and mixed grass legumes pastures, respectively. Considering an emission of 4.5 kg fossil CO₂/kg N for the manufacture and transport of the N fertilizer, the emissions (excluding methane) totalled 32, 814 and 67 kg CO₂eq ha⁻¹ year⁻¹, respectively.

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The Impact of Sward Type on Animal Performance and the Carbon Footprint of Beef Production

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Ruminant livestock production systems globally are faced with numerous challenges including but not limited to the need to reduce greenhouse gas (GHG) emissions, reduce the reliance on fertilizer nitrogen (N), and reduce the reliance on therapeutic veterinary medicines while maintaining productivity to meet rising global demand for animal proteins. The objective of this study was to determine the impact of sward type on animal performance, economic implications and GHG emissions associated with a traditional dairy calf to beef production system. A total of 120 early maturing dairy beef steers were enrolled in a 23-month-old dairy calf to beef production system, over two full production cycles. Three sward types (one per farmlet) were investigated in a farmlet system experiment; perennial ryegrass (*Lolium perenne*) (PRG; 205 kg N/ha/yr); PRG and white clover (*Trifolium repens*) (PRGWC; 90 kg N/ha/yr); PRG, Timothy (*Phleum pratense*), white and red (*T. pratense*) clover, chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*) (MSS; 90kg N/ha/yr). Compared to PRG the MSS sward had a higher herbage DM production ($P<0.05$). Animal average daily gain (ADG; average 0.85 kg/day) during the first season at pasture did not differ with sward type ($P>0.05$). During the first winter indoors, when animals were fed forage conserved from their respective farmlet, ADG of animals offered PRGWC or MSS did not differ but were higher than animals offered PRG ($P<0.05$). During the second grazing season animals offered PRGWC or MULTI had an ADG of 1.02 and 1.09 kg/day respectively, significantly higher than the performance achieved by animals offered PRG (0.88 kg/day; $P<0.05$). Days to slaughter were reduced by 21 days because of the accelerated growth rate on the PRGWC and MULTI swards. Altering sward types presents an opportunity to reduce the carbon footprint of beef production through reduced fertilizer inputs and improved animal performance.

Carbon Footprint from Enteric Methane and Feed Production When Feeding Extreme Amounts of Concentrate

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The aim was to study the effect of feeding diets with increasing levels of concentrate on enteric methane and carbon footprint (CF) of feed production, since the mitigating effect of concentrate on enteric methane may be counteracted by the CF of feed production. Three diets were fed to Holstein and Jersey cows in both a production trial and a trial where the effect of the three diets on methane was studied. The diets contained either 49% (C49), 70% (C70) or 91% (C91) of DM from concentrate. The C91 diet also contained 9% of chopped barley straw. The C49 diet contained 1% straw, 26% grass silage, and 24% maize silage, whereas the C70 diet was made by mixing the two other diets in a 1:1 ratio on DM basis. The concentrate feedstuffs replacing the forage were (% on DM basis) 25 of dried beet pulp, 20 of NaOH treated wheat, 17 of dried distillers grain, 17 of rape seed cake, 16 of ground barley, and 5 of other ingredients. The CF of feed production was based on a LCA analysis. Holsteins had a lowered methane production per kg DMI of 44%, whereas it was only lowered by 4% for Jerseys for the C91 diet compared to C49. For the C49 diet about 40% of the daily total CF came from enteric methane, and the rest from feed production and net soil carbon storage. For the C91 diet the proportion of CF from methane was reduced to 26% for Holsteins, and to 36% for Jerseys. Total CF per kg DMI was decreased by 9% for Holsteins, but was even increased by 8% for Jerseys. In conclusion, the mitigating effect due to extreme concentrate amounts on enteric methane production was larger in Holsteins than Jerseys; however, this mitigating effect was counterbalanced by including the CF of feed production.

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A Static Balance Model Quantifying Nutrients in Feces, Urine and Gasses of Cattle and Pigs

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National inventories of methane emissions from livestock and stored manure are commonly based on fixed emission factors. However, there is a great variation among farms in the animal performance level, feed intake, and the ingredient composition of feed as well the management of manure. Such variations have a great impact on the enteric methane emission and methane emissions associated with manure storage and management. Undigested nutrients are potentially substrates for methanogenesis during the storage and management of manure. The objective of this study is to develop a model for dairy cattle and pigs quantifying enteric methane emission and the excretion of nutrients in feces and urine based on the nutrient input and performance level. Table values of the chemical composition of feed, the apparent total tract digestibility of nutrients in feed ingredients for cattle and pigs are used to quantify the amount of organic matter, crude protein, sugar and starch, crude fat, and fiber in feces. The nutrients excreted in feces are further divided into fast and slowly degradable fractions with regards to methanogenesis in stored manure, based on their degradation potential. Nutrient intake will be used to quantify enteric methane emissions from the animals. Excretions in urine will be quantified as the difference between digested and retained nitrogen, i.e., milk, body deposition, and fetuses. The partitioning of nutrients in the feed for these purposes will be compared with data from cattle and pig studies allowing us to model nutrient balance on an animal level. The comparison between model predicted values and measured values in series of experiments with pigs and cattle will reveal the accuracy and the underlying assumptions of the model. The developed models will be useful to quantify the nutritional impact on enteric methane emission and the potential methanogenesis in the stored manure.

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Carbon Balance and Global Warming Potential of Typical Florida Cow-Calf Production Systems

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Grazing lands dominate land use in the US, with 130 million acres in the southeastern states. In Florida, about 4.4 million acres are used for cattle production, 1.4 million as native rangelands and 3 million as planted pastures. Rangelands are used during the winter as a source of forage for non-lactating, non-pregnant multiparous cows, and pastures as the major source of food during the growing season. The objectives of this study are to evaluate the carbon balance, greenhouse gas balance and warming potential of cow-calf production systems in Florida. The study is conducted in two areas, a native rangeland and a planted Bahia grass pasture located in Ona, FL (Range Cattle Research & Education Center), as part of the USDA-LTAR network. Carbon dioxide (CO₂) and methane (CH₄) exchanges are being measured at an ecosystem level (eddy covariance), and soil (static chambers), including nitrous oxide. Preliminary results at an ecosystem level indicate that daily CH₄ fluxes in the rangeland fluctuate from -2.7 mg CH₄ m⁻² day⁻¹ during the dry season to 60 mg CH₄ m⁻² day⁻¹ at surface soil water saturation, while for the pasture site, CH₄ fluxes fluctuated from 49 to 290 mg CH₄ m⁻² day⁻¹. Instantaneous CO₂ uptake during the peak of the growing season reaches -0.5 mg CO₂ m⁻² s⁻¹ at the rangeland, and were maintained through the year, while for the pasture site it reached -1.32 mg CO₂ m⁻² s⁻¹ during the growing season and decreased to values > -0.4 mg CO₂ m⁻² s⁻¹ by the end of the year. Daily, monthly, and seasonal CO₂, CH₄ and N₂O balance will be determined by the end of the year 2021.

Potential of ZELP to Improve the Cargill Holistic Approach to Mitigate Enteric Methane Emissions

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To support farmers to reduce methane emissions, Cargill implements a holistic approach including best farm management practices, feed nutrition and formulation, additives, and digital solutions. Prototypes of ZELP, a wearable digital device that can oxidize methane to carbon dioxide and water were evaluated in two preliminary trials to understand its effects on methane emissions, performance, and animal behavior. In Trial 1, four beef steers (462 kg of body weight) were adapted to respiration chambers, diet and ZELP. Chamber measurements of methane concentrations were obtained over four consecutive days on two separate weeks, where ZELP devices were fitted for two days or removed for two days each week in a crossover design. Methane production ranged from 24.3 to 30.4 g/kg dry matter intake without ZELP and from 13.0 to 22.5 g/kg dry matter intake with ZELP, showing a potential for ZELP to mitigate methane emissions from 25.8 to 53.3%. In Trial 2, 10 Holstein cows (37.4 ± 8.8 kg/d milk yield; 271 ± 53 days in milk) were used in a crossover design with 8-d periods. Cows were randomly assigned to treatments, which were absence or presence of ZELP. Data and samples were collected during the last 3 days of each experimental period. ZELP did not affect dry matter intake (26.1 kg/d), yields of milk (36.4 kg/d), fat (1.55 kg/d) and protein (1.27 kg/d), rumination time (569 min/d) and eating time (183 min/d; all $P > 0.25$). It was concluded that ZELP is a promising technology that can reduce methane emissions from ruminants without impairing animal welfare.

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Evaluation of Empirical Models for Predicting Methane Emissions of Dairy Cattle from Latin American Studies

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Dairy cattle in Latin America may have constraints to express their genetic potential and this may reflect in lower feed efficiency and likely increased methane (CH₄) emissions when compared with production records of animals raised in other latitudes. The objective of this study was to evaluate the performance of ten empirical models of varying complexity (including both animal and diet-related factors) for predicting total CH₄ emissions (g/day), with this being measured using the SF₆-tracer technique. Data were collected (means by treatment) from peer-reviewed literature studies (n=13), mostly conducted under grazing conditions (n= 10). Both lactating and non-lactating females (n=202) were included in the dataset with 69.3% of these being *Bos Taurus* (mainly Holstein), 24.9% crossbred *B. taurus* x *B. indicus*, and 5.9% *B. indicus* (Gyr). Average body weight (BW) was 474±100 kg, dry matter intake (DMI)=14.5±4.49 kg/d, total CH₄ emissions=337±146 g/day, and milk yield=20.2±4.68 kg (10 studies). Mean concentrate proportion offered to the animals was on average 12% of the diet on an as fed basis. Model evaluation was conducted using mixed model regression. Observed values were adjusted for the random effect of study. Mean biases were evaluated by the deviation of regression intercepts from zero while the deviation of the slopes of the regression equations from unity was used to determine the presence of linear biases. Model accuracy was assessed by the calculation of the root mean square prediction error (RMSPE). Among evaluated models, the non-linear equation proposed by Mills et al. (2013), which includes DMI as single prediction factor, displayed the best performance in the model ranking (lower RMSPE = 35.8 g of CH₄/day) and slightly under predicted total CH₄ emissions by 3.39 g/day. The results of this study confirmed DMI as a key factor driving enteric CH₄ emissions in dairy cattle.

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Impacts of Native Grassland Grazing Management in Uruguay on National Enteric Methane Emissions of Beef Cattle Systems

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Improving management of native grasslands in Uruguay increases the weaning rate and reduces the age at first calving for beef cattle. These factors significantly impact livestock production because 80% of the area devoted to livestock in Uruguay is native grassland. We hypothesize that improved management reduces enteric methane emissions from livestock production. To assess this hypothesis, total DM intake of non-pregnant cows (420 kg LW) and growing heifers from weaning to mating age (150 to 350 kg LW) was modeled and enteric methane emissions estimated for two levels of diet digestibility (DOM; 50 and 61%). We followed the IPCC models (methane conversion factor = 0.065) and used the 2020 national inventory of beef cattle. We simulated two scenarios: first, the reduction in cow number (4.2 to 3.5 million) required to produce 3 million calves resulting from an increase in weaning rate from 65 to 85%. Second, we accounted for the reduction in total DMI of heifers associated with a mating age of 24 instead of 36 months for each level of diet DOM. We assumed all non-mating heifers of 24-36 months of age (482,000 head) are mated by 36 months. Decreasing cow numbers (700,000) reduces methane emission by 44.94 and 35.14 Gg year⁻¹ (64.2 and 50.2 kg CH₄ animal⁻¹ year⁻¹), for 50 and 61% DOM, respectively. Additionally, reducing mating age reduces total DMI, and methane emissions decline by 19.68 and 15.91 Gg year⁻¹ (41 and 33 kg CH₄ animal⁻¹ year⁻¹), for 50 and 61% DOM, respectively. Improvement in diet DOM from 50 to 61% for heifers of 6 to 24 months of age reduces required DMI by 25% and consequently, methane emissions. We conclude that improving management of native grasslands in Uruguay increases energy use efficiency and simultaneously reduces the 'luxury' methane emissions (almost 10% of national enteric methane emissions) of livestock systems.

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CO₂ Fluxes from Three Different Temperate Grazed Pastures using Eddy Covariance Measurements

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Grasslands cover around 25% of the global ice-free land surface. They are used predominantly for forage and livestock production and are considered to contribute significantly to soil carbon (C) sequestration. Recent investigations into using 'nature-based solutions' to limit warming to <2 °C suggest up to 25% of GHG mitigation might be achieved through changes to grassland management. In this study we evaluate pasture management interventions at the Rothamsted Research North Wyke Farm Platform, under commercial farming conditions, over two years and consider their impacts on net CO₂ exchange. We investigate if our permanent pasture system (PP) is, in the short-term, a net sink for CO₂ and whether reseeding this with deep-rooting, high-sugar grass (HS) or a mix of high-sugar grass and clover (HSC) might increase the net removal of atmospheric CO₂. In general CO₂ fluxes were less variable in 2018 than in 2017 while overall we found that net CO₂ fluxes for the PP treatment changed from a sink in 2017 (-5.40 t CO₂ ha⁻¹ y⁻¹) to a source in 2018 (6.17 t CO₂ ha⁻¹ y⁻¹), resulting in an overall small source of 0.76 t CO₂ ha⁻¹ over the two years for this treatment. HS showed a similar trend, changing from a net sink in 2017 (-4.82 t CO₂ ha⁻¹ y⁻¹) to a net source in 2018 (3.91 t CO₂ ha⁻¹ y⁻¹) whilst the HSC field was a net source in both years (3.92 and 4.10 t CO₂ ha⁻¹ y⁻¹, respectively). These results suggested that pasture type has an influence in the atmospheric CO₂ balance and our regression modelling supported this conclusion, with pasture type and time of the year (and their interaction) being significant factors in predicting fluxes.

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Diurnal Variability of Soil Nitrous Oxide Fluxes from Different Soil Land Use

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Mean daily flux estimation is a key factor to quantify correctly nitrous oxide production from soils. However, mean daily fluxes may occur at different day moments according to the soil use. We aimed to quantify the diurnal variation of nitrous oxide fluxes from crop, forest, and grassland soils. The treatments were soil usage (corn, forest, and palisade grass grassland). Nitrous oxide fluxes were measured every 3 hours for 5 days. In each soil use, five replications were used. Gas quantification was run using the static closed chamber technique and gas chromatography. Regressions of mean data against the data obtained for each sampling hour were made to find the most probable time for gas sampling that would represent the daily mean N₂O flux. Regression analysis showed that the times that the flux best represented the daily mean varied according to the soil land use. For crop, the time 9:00-12:00 in the morning best represented the mean daily flux, for forest the times early morning 6:00-12:00 and late afternoon 15:00-18:00 best fit the daily average, while for grassland, we confirmed the previous knowledge that the evening (21:00-24:00 h) and morning (09:00-12:00 h) is the most suitable time to capture the daily mean N₂O flux.

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Impact of Supplementation in Greenhouse Gas Emissions from Beef Cattle Production

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Animal supplementation can be a strategy to mitigate the greenhouse gas emissions from beef cattle production. In this study we used a life cycle analysis approach to evaluate the impact of supplementation strategies of beef cattle production on greenhouse gas emissions. The study boundary included the whole cycle of beef cattle production from calf birth until slaughter. GHG emission was expressed in CO_{2eq} (global warming potential of 100 years) as a function of body weight gain (BWG). We used the IPCC methodologies with national emission factors when it was available to calculate emission of enteric methane and from dung, direct and indirect nitrous oxide from dung, urine and fertilizer applied in pasture, carbon dioxide from energy, including supplement and fertilizer manufacture. We also included emission from corn silage, soybean and corn used in the feedlot. We accounted for 7 possible scenarios in Brazilian beef cattle production: 1) Mineral mix for all phases, 2) Mineral mix for cow-calf and rearing and supplementation for finishing, 3) Mineral mix for cow-calf and rearing and feedlot for finishing, 4) Mineral mix for cow-calf, supplementation for rearing and finishing, 5) Mineral mix for cow-calf, supplementation for rearing and feedlot for finishing, 6) Supplementation for all phases, and 7) Supplementation for cow-calf and rearing and feedlot for finishing. The carbon footprints for each scenario were 1) 18.6, 2) 18.5, 3) 17.3, 4) 17.6, 5) 16.0, 6) 14.0, and 7) 12.1 kg CO_{2eq}/kg BWG. GHG emissions were not mitigated by supplementation for finishing (Scenario 2). Two strategies presented the greatest potential to mitigate greenhouse gas emissions; supplementation in the cow-calf phase (Scenarios 6 and 7), which increased weaning rate and total body weight produced per herd per year, and finishing animals in a feedlot (Scenarios 3, 5 and 7), which reduced the slaughter age and consequently total GHG emissions per herd.

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Impact of a Tannin and Saponin Blend on Methane and Ammonia Emissions of Lactating Dairy Cows.

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Cattle are considered a major source of greenhouse gas emissions. In the United States, animal agriculture accounts for 38% of methane emissions, 10% arising from manure management, and 28% from enteric fermentation. With California being the leading dairy state in the United States, it is imperative that various strategies be assessed for reducing methane emissions in California dairy cows. The objective of this research was to evaluate the effects of a blend of quebracho and chestnut tannin extracts with saponins (TAN) on enteric emissions, manure emissions, and production performance in dairy cows. Twenty early- to mid-lactation dairy cows were blocked by days in milk and parity and were assigned one of two treatments: TAN or control (n=2 per block). Control and TAN were administered as a top dress with TAN being supplemented at a rate of 0.07% of dry matter. Cows were individually fed their assigned treatments, group-housed in a free-stall pen, and were milked twice daily. Each cow block underwent enteric methane emission sampling in head chambers for a 12-hour period on their respective treatment days 0, 16, 32, and 48. All urine and manure produced by each cow during enteric emission sampling were collected and stored. After the conclusion of enteric emission sampling, urine and manure were homogenized separately and were then combined into a slurry at 1:1.7 (urine wt: feces wt) per cow. Slurry methane and ammonia emissions were measured for 24 hours. Supplemental TAN tended to decrease enteric methane emissions though it had no impact on slurry methane emissions. Slurry ammonia emissions were significantly increased with supplemented TAN. Cow performance variables including energy-corrected milk, milk fat yield, milk protein yield, and dry matter intake were not affected by TAN supplementation. Future research should assess the impacts of TAN at varying dosage levels.

Examining the Impact of *Ascophyllum nodosum* to Reduce Emissions Tested Using *In Vitro* RUSITEC Technique

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There is an urgent need to identify viable means to reduce the environmental impact of ruminant animal production. One potential strategy is the inclusion of dietary additives such as seaweed extracts. The objective of this experiment, therefore, is to test various *Ascophyllum nodosum* (*A. nodosum*) inclusion rates using the *in vitro* rumen simulation technique (RUSITEC). The study assessed the impact of three inclusion levels of *A. nodosum* on methane and total gas production using the RUSITEC. The extracts were included at 0.75, 1 and 1.25% of the total feed volume provided to the system. The diets were based on grass silage and concentrate. Both the control and the three inclusion levels of *A. nodosum* were allocated at random to three RUSITEC vessels with three replicates per treatment. Each vessel was inoculated with 500 ml of rumen fluid and 350 ml of artificial saliva. Each seaweed extract was added to the vessels in combination with a nylon bag containing grass silage 16 g and a bag containing 4 g of concentrate. Following the 10-day acclimatization period dry matter degradation, gas production and outflow of fermentation products was measured on days 11-18. A sample of outflow liquor was collected on those days for VFA and ammonia analysis. On the final day of experimentation (d 14) a 15-ml sample of rumen fluid was collected, immediately snap frozen and used for microbial DNA extraction and subsequent 16s rRNA gene sequencing. The inclusion of 0.75% of the *A. nodosum* extract reduced methane over the course of the experimental period ($P < 0.05$). Both the 0.75 and 1% inclusion of *A. Nodosum* significantly reduced daily gas production compared to the control diet ($P < 0.05$). These data provide promising findings in relation to the potential of the native Irish seaweed *A. nodosum* in reducing methane emissions. Further work will establish the impact on the rumen microbiome and fermentation and ammonia.

Exploratory Area Integrated Measurements of Nitrous Oxide Emissions from a Texas Beef Cattle Feedyard

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Nitrous oxide, a potent greenhouse gas is produced at animal production facility manure management systems. At beef cattle feedlots, the primary manure management system source is the pen surface. Measurements of these emissions from the pen surface using non-flow-through – non-steady-state chambers have shown that emissions are spatially heterogeneous, vary with time, temperature and in response to environmental inputs such as rainfall. Measurement techniques with small chambers are unable to obtain good estimates of whole feedyard emissions in part because of the spatial variability. Micrometeorological techniques have been used to obtain area integrated emissions estimates from feedyards of gaseous emissions such as ammonia. Unfortunately, equivalent open path measurement systems are not available for nitrous oxide. The objective of this research was to investigate whether useful estimates of N₂O emission rate could be obtained from downwind point measurements of N₂O concentration using BLS dispersion modelling techniques. A climate controlled, instrument trailer was deployed on the predominantly downwind edge of a 50,000 head feedyard on the Texas High Plains for 2 weeks during summer 2020 and a further 2 weeks in the following spring. A Los Gatos enhanced performance N₂O analyzer was installed in the instrument trailer continuously drawing ambient air at 3 m height. The analyzer recorded the N₂O concentration every two seconds. A co-located meteorological station recorded meteorological data for use in dispersion modelling. Periodic measurements were made of upwind N₂O concentrations by repositioning the instrument trailer to the upwind side when suitable conditions were expected. Using the Windtrax BLS atmospheric dispersion model, N₂O emission fluxes were determined based on 15-minute average downwind concentrations, the meteorological data and the source configuration. This paper describes the experimental configuration and discusses the results. Analysis indicates that this monitoring approach has the potential to produce useful N₂O emissions estimates.

Evaluation of the Anti-Methanogenic Effects of Three Herbs on Beef Cattle Fed a Feedlot Finishing Diet

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Cattle are a significant source of greenhouse gases (GHG). In 2010, cattle emitted 5.0 gigatons of CO₂ equivalent globally, which represents about 62% of the livestock sector emissions. Therefore, mitigating GHGs such as methane (CH₄) originating from cattle offers an opportunity to reduce GHG emissions and climate change. In the present study, two experiments were conducted to evaluate the anti-methanogenic effect of *Cymbopogon citratus* (CC), *Matricaria chamomilla* (MC) and *Cosmos bipinnatus* (CB) on enteric CH₄ production by beef cattle. The inclusion of increasing supplementation levels of CC (0%, 2%, 3%, and 4% of the daily dry matter intake (DMI)) on enteric methane production from beef cattle was also evaluated. In experiment 1, eight Charolais x Brown Swiss steers were used distributed in a Latin square 4 × 4 design repeated twice. They were fed a 19.4% forage and 80.6% concentrate diet; and 4 treatments were evaluated: 1) control diet (CO), 2), CO + 365 g dry matter (DM) / d CB, 3) CO + 365 g DM / d MC, 4) CO + 100 g DM / d CC. In experiment 2, four Charolais x Brown Swiss steers were distributed in a 4 × 4 Latin square design and were fed a 49.3% forage and 50.7% concentrate diet. In experiment 1, it was observed that 100 g DM / d of CC and 365 g DM/d of CB reduced the CH₄ yield with respect to CO by 32.5% and 27.6% respectively (P ≤ 0.05). In experiment 2, CC had a quadratic effect, showing the lowest CH₄ production at the 2% DM inclusion level, with the digestibility of DM, fibre and energy following the same trend (P ≤ 0.05). It is concluded that the effects of CC depend on the diet, level of inclusion and content of secondary metabolites.

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Effects of Corn and Soybean Supplementation on Performance and Methane Emission in Young Llamas

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The South American camelid production is one of the most important activities for the socio-economic development of the high-Andean farmers. Llamas are versatile and rustic animals that have adapted to the harsh climatic conditions of the Andes, and they are bred primarily for their meat and fiber. This study was aimed at determining the effects of dietary supplementation on animal performance and enteric methane (CH₄) emission in young llamas (*Lama glama*) grazing in the Argentina highlands. The experiment was conducted at Agricultural Experimental Station of INTA Abra Pampa, Jujuy at 3500 m, using 18 animals equally divided into two groups, presenting averages of initial body weight of 68.8 kg and body condition score of 3.1 (scale 1 to 5). Animals were randomly allocated to dietary treatment: P, pasture (mainly *Festuca scirpifolia* "Chillagua") and PS: P + supplementation (2% body weight, containing 80% ground corn and 20% expeller soybean) for 45 days. Drinking water was freely available. Enteric CH₄ emissions were estimated using the sulfur hexafluoride (SF₆) tracer technique (Johnson et al. 1994). The average daily gain (ADG) did not differ between dietary treatments (P>0.05). The PS diet tended (P<0.1) to decrease enteric CH₄ emission (g/d; 13.5± 2.7 vs. 19.0 ± 6.8) and CH₄ intensity (g CH₄/kg ADG; 71.9 ± 27.9 vs. 105.2 ± 44.2) compared to the P diet. The trend to reduce CH₄ emission without affecting ADG with the PS diet could be associated with the high inter-animal variability or SF₆ technique variability. Results should be examined with further studies as it could be a potential alternative to reduce enteric CH₄ emission.

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***In Vitro* Assessment of *Lippia turbinata* and *Tagetes minuta* on Fermentation and Ruminal Microbial Populations**

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Argentina has a wide diversity of botanical species that contain bioactive compounds traditionally used for medicinal purposes. To date, there is little information about their antimicrobial properties as ruminal fermentation modifiers. The objective of this study was to determine methane (CH₄) production, digestibility and ruminal microbiota parameters in response to the inclusion of *Lippia turbinata* (poleo) and *Tagetes minuta* (suico), using the *in vitro* batch fermentation technique (at 0, 12, 24 and 48 h of incubation) using a substrate similar to a dairy diet. Fifty mg of ground plant material was added to 450 mg of substrate (excepting control). A positive control with monensin (1.87 mg /L) was also included. Three cannulated Hereford steers (518 ± 51 kg) fed alfalfa hay and ground corn grain (80:20 on a dry basis) were used as donors of ruminal fluid. *In vitro* CH₄ production with respect to neutral detergent fiber incubated was lower than that of the control (P=0.001) at 12 h, 24 h, and 48 h incubation with poleo (33, 35 and 31%) and with suico (30, 29 and 30%). While poleo reduced CH₄ production and methanogenic *archaea* populations (1, 3 and 7%) as compared to control (P=0.001), suico decreased CH₄ without affecting these microorganisms (P=0.001). It was not possible to establish a direct relationship between the reduction in the number of methanogenic *archaea* and the number of protozoa. Total bacteria increased, but changes were not of biological relevance as they did not affect any of the fermentation parameters evaluated. Regarding protozoa, only monensin significantly reduced their population (39%), while both poleo and suico had no effect. In addition, poleo and suico decreased the digestibility of the substrate used, and reduced ruminal CH₄ production, likely by different modes of action.

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Mitigating Ammonia Emissions from Litter of Cage-free Laying Hens

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Animal feeding operations are important sources of aerial pollutant emissions such as ammonia (NH_3), greenhouse gases (GHG), and particulate matter (PM). For instance, animal production contributed over 60% of atmospheric NH_3 in the United States (US). Ammonia emissions and deposition are indirect sources of Nitrous Oxide (N_2O), a GHG that has a 265–298 times of global warming potential that of CO_2 for a 100-year timescale. Atmospheric NH_3 is the precursor for fine particulate matter ($\text{PM}_{2.5}$). In addition, NH_3 emissions and deposition may cause surface water eutrophication and acidification of terrestrial ecosystems. Therefore, mitigating NH_3 emissions from animal production will benefit our environment and ecosystems. Egg production is a key sector of US animal food systems. Concerns from the general public on animal welfare have led to pledges of sourcing only cage-free eggs by many U.S. food retailers and restaurants such as Walmart and McDonald's by 2025 or 2030. The objective of this study was mitigating NH_3 generations and emissions from the cage-free hen houses. Different methods were tested and discussed for controlling NH_3 generations from the litter of cage-free hens. According to lab experiments based on litter collected from commercial cage-free houses, the litter moisture content (LMC) and pH level were two key factors affecting NH_3 generations. Ammonia emissions at 20-23% LMC were 5-6 times higher than the emissions at 10-13% LMC. However, lower LMC resulted in higher dust levels. Top application of dry shavings at different amount is an efficient way to adjust LMC. For the litter pH adjustment, a commercial acidifier (i.e., Sodium bisulfate, NaHSO_4) was tested and that had to 30-80% reduction efficiency at the application rate of 0.3-0.9 kg/m^2 floor area. Aforementioned findings are currently under verification in four cage-free laying hen facilities (200 hens/facility) on the poultry research farm at the University of Georgia.

Keywords: laying hen production; air emissions; mitigation strategy; litter management.

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Effect of *Leucaena leucocephala* intake on *In vivo* CH₄ Emission and Milk Production in Dual Purpose Cows in the Dry Caribbean Region of Colombia

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Leucaena leucocephala has demonstrated its ability to improve fermentation efficiency, reduce CH₄ emissions and increase bovine productivity. The objective of this research was to determine the effect of *L. leucocephala* on enteric emissions and on milk production in dual-purpose cows in the north of Colombia. Two diets (Diet 1: *Megathyrsus maximus* 100%; Diet 2: *L. leucocephala* 20% + *M. maximus* 80%) were evaluated with the polytunnel technique under a randomized complete block design with four cows. Animals had two 12-day adaptation periods followed by two-day measurement periods. During the two measurement events, gas samples were taken every 60 minutes for 48 hours and CH₄ concentration determined using a gas chromatograph. Total methane produced was calculated based on CH₄ concentration and total volume of the polytunnel. Voluntary dry matter intake (DMI) differed between diets ($p=0.0025$), with a consumption equivalent to 2.23 and 2.42% of animal weight for Diet 1 and 2 respectively. On average, *L. leucocephala* represented 23.9% of the total DMI for Diet 2. Dairy parameters differed significantly for Diet 1 and 2 for milk production (4.07 vs. 4.87 kg/animal/day; $p=0.033$), protein (132.5 vs. 158.9 g/animal/day; $p=0.0102$), fat (168.1 vs. 211.6 g/animal/day; $p=0.0002$) and total solids (524.6 vs. 636.5g/animal/day; $p=0.006$) respectively. Methane emissions per animal/day were 219.7 g/day for Diet 1 and 207.3 g/day for Diet 2 and did not differ between diets ($p=0.38$) despite the higher DMI for Diet 2. However, the inclusion of *Leucaena* significantly reduced CH₄ emissions per kg of DMI (22.9 vs 20.1 g/kg; $p=0.0469$), per kg of degraded DM (40.6 vs 33.5 g/kg; $p=0.0129$), and per kg of fat and protein corrected milk (56.4 vs 43.21 g/kg; $p=0.0118$). The introduction of *L. leucocephala* in grass-based diets is an effective strategy to reduce GHG emissions from cattle and improve productivity in tropical dry conditions.

Key words: enteric fermentation, forage shrub, GHG mitigation, grazing, silvopastoral systems.

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Using a UAV as a Platform for Micrometeorological Measurements of Gas Emissions from a Feedlot

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Micrometeorological techniques are well-suited to measuring gas fluxes from landscape surfaces, but large area sources often pose challenges to practical implementation of the required procedures. Unmanned aerial vehicles (UAVs) may provide a practical and inexpensive solution to alleviating some of these challenges. Our objective in this study was to assess the potential of an inexpensive UAV-based measurement approach for large area sources, in terms of accuracy and practicality. A 25,000 head capacity cattle feedlot represented the source area for this study and methane emissions were calculated based on measurements obtained from a small UAV modified to collect air samples. Two micrometeorological techniques were used: inverse dispersion modelling (IDM) and air mass balance (AMB). With IDM, the emission rate was estimated from a set of gas samples collected during 26 flights over the feedlot. By spacing the IDM measurements over the 24-hour day we were able to observe a strong diurnal cycle in emissions. We concluded that the daily emission rate was accurately measured with IDM, as it was within 10% of that calculated from feed information (based on well-known relationships between the enteric emission rate of cattle and diet). With AMB, emissions were estimated from gas concentration and wind measurements along four flight transects at different heights downwind of the feedlot. When we compare the AMB measured rates with afternoon rates derived from the IDM measured diurnal emission curve, the two techniques agreed within 10% of each other. Our study demonstrates that a UAV-based measurement platform can provide a relatively simple and inexpensive way to apply micrometeorological techniques for emission measurements from large area sources that may otherwise be difficult to monitor.

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Prediction of Enteric Methane Production and Yield of Beef Cattle Using a Latin America Database

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Methane production from rumen fermentation contributes to anthropogenic greenhouse gas emissions. Several prediction equations have been developed using empirical modeling approaches as an alternative to costly *in vivo* methane measurement techniques. However, these recent meta-analyses which used minimal data from the Latin America region, suggested that region-specific models are more accurate in predicting enteric methane than general models. Therefore, the objectives of this study were to: (1) collate a database of individual beef cattle enteric methane emission data from studies conducted in the Latin America region; (2) determine the key dietary and animal variables for predicting daily enteric methane production (g/day per animal) and yield (g/kg dry matter intake) and their relationships; (3) develop and cross-validate these newly developed models; and (4) compare their predictive ability with extant models, which are currently used to support national greenhouse gas inventories in the region. After outlier were removed, 48.7% of the original data (1100 individual dairy cattle records) from 55 studies (from 2011 to 2021) carried out in 6 countries were retained in the database. Linear mixed models were developed by incrementally adding covariates. Simple regression equations containing either dry matter intake or average daily gain as covariates performed better than extant equations from the Intergovernmental Panel on Climate Change, with smaller root mean square prediction error (32.2 vs. 35.3%), and smaller mean (1.72 vs. 2.70%) and slope (8.79 vs. 28.81%) biases. Prediction of methane production was not improved by increasing model complexity. Additionally, methane yield was satisfactorily predicted by a simple regression equation containing only feeding level as predictor variable. The newly developed equations can support improvements in national greenhouse gas inventories in the Latin America region.

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Prediction of Enteric Methane Production and Yield of Dairy Cattle Using a Latin America Database

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Methane produced from enteric fermentation in ruminants is a significant contributor to anthropogenic greenhouse gas emissions. Empirical prediction models can be useful to estimate enteric methane emissions without undertaking extensive and costly experiments. Recent meta-analyses, based primarily on data from the U.S. and the E.U. with minimal data from the Latin America region, concluded that region-specific models are more accurate in predicting enteric methane than global models. Therefore, the objectives of this study were to: (1) collate a database of individual dairy cattle enteric methane emission data from studies conducted in the Latin America region; (2) determine the key dietary and animal variables for predicting daily enteric methane production (g/day per animal) and yield (g/kg dry matter intake) and their relationships; (3) develop and cross-validate these newly developed models; and (4) compare their predictive ability with extant models, which are currently used to support national greenhouse gas inventories in the region. After outliers were removed, 610 individual dairy cattle records (46% of the original data) from 34 studies (from 2012 to 2021) carried out in 8 countries were retained in the database. Linear mixed models were developed by incrementally adding covariates. The developed methane production equation using only dry matter intake as covariate performed better than extant equations from the Intergovernmental Panel on Climate Change, with smaller root mean square prediction error (20.5 vs. 21.1%) and smaller mean (0.56 vs. 7.75%) and slope (0.64 vs. 5.43%) biases. The prediction of methane production was further improved by a combination of dry matter intake and energy corrected milk yield. In addition, methane yield was satisfactorily predicted by either simple or multiple regression equations containing feeding level or/and animal's body weight as predictor variables. The newly developed models can be used to improve national greenhouse gas inventories in the Latin America region.

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Use of a Multi-Omic Approach for Identifying Rumen Microbiome Mechanisms in Cows Modulated by an Anti-Methanogenic Additive

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The rumen microbiome allows ruminants to feed on forages not adapted for monogastrics' consumption. Nevertheless, ruminant production contributes to the greenhouse effect through the production of enteric methane by rumen archaea. The aim of this work was to get insight into how rumen microbes adapt and function when an anti-methanogenic compound inhibits methane production in cows.

The experimental setup consisted of 25 lactating Holstein cows fed a total mixed ration of corn silage, grass hay and concentrate with (n= 12) or without (n= 13) a specific methane inhibitor. In week 5, rumen fluid samples were collected before the morning feeding from each cow via stomach tubing and subjected to RNASeq (Illumina HiSeq) and metabolomics (RPLC-QToF/MS, HILIC-Orbitrap, LC-MS/MS and GC-FID) analysis. In week 6, cows were transferred into respiration chambers for measuring methane emissions for 4 days. The MetaTrans pipeline was used for metatranscriptomic analysis and metabolomic data were processed using the web-based Galaxy Workflow4Metabolomics. KEGGs (mapped mRNA), OTUs (based on rRNA), and metabolomic data were integrated via causality relationships using Bayesian Networks.

In the treated group, enteric methane emissions were reduced by 23%. Our initial analysis uncovered novel relationships between OTUs, KEGGs, and metabolites. The treated group of cows had two OTUs and 57 KEGGs differentially expressed, together with 39 discriminant metabolites, in comparison with control. After integration, the anaerobic carbon-monoxide dehydrogenase catalytic subunit, upregulated in the treated group, was related with the genera *Methanosphaera*, *Butyrivibrio*, *Ruminococcus*, and *Methanobrevibacter*, whose abundance did not differ significantly between the groups. This enzyme is involved in the initial step of carbon fixation in methanogens and acetogens. Other associations will be performed using this multi-omic approach and microbes associated with the decrease in methane emissions may be identified.

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Decreased Methane Production in Angus Cattle Fed 3-Nitrooxypropanol (Bovaer 10®)

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We aimed to quantify the effect of increasing diet concentration of 3-NOP (Bovaer®) on rumen fermentation and methane emissions of Angus steers fed typical Australian feedlot diets. Twenty Angus steers of initial liveweight of 356 ± 14.4 kg were allocated in a completely randomized block design. The experimental period was 112 days in which steers were housed in individual indoor pens, including the first 21 days of adaptation. Five different 3-NOP regimens in dry matter basis were compared: Control=no added 3-NOP; Low=50 mg/kg 3-NOP from d 0 to 112; Medium A=50 mg/kg from d 0 to 7, 75 mg/kg from d 8 to 112 ; Medium B=50 mg/kg from d 0 to 7, 75 mg/kg from d 8 to 14, 100 mg/kg from d 15 to 112; and High=50 mg/kg from d 0 to 7, 75 mg/kg from d 8 to 14, 100 mg/kg d 15 to 21, 125 mg/kg from d 22 to 112. For the 3-NOP titration regimens, the adaptation period followed a step-wise approach in terms of both increasing 3-NOP diet concentration and increasing tempered barley content in the diet. The CH₄ emissions were measured with respiratory chambers, and rumen function and performance traits also recorded. This project demonstrated that all 3-NOP regimens significantly decreased methane of feedlot cattle from day 21 to 112 of the feeding period. The maximum CH₄ production abatement from 3-NOP was observed in Medium B regimen on day 28, averaging 99% reduction compared to control steers. For the overall feeding period, methane emissions (g/d) were up to 78% lower in the animals fed 3-NOP compared to control animals. Although this experiment was not designed to make conclusions on an effect of 3-NOP on feedlot performance no negative effects of any 3-NOP regimen were detected on production or rumen function parameters.

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Effect of Microalgae Oil Supplementation on Feed Intake, Growth and Methane Emissions of Finishing Lambs

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The study assessed the effects of supplementing microalgae oil at different levels on feed intake, growth and methane emissions of finishing lambs. Sixty-four male lambs, Texel crossed, were blocked into 16 groups according to age and body weight (BW). Within each group, lambs were assigned to one of four dietary treatments. All treatments were fed TMR diets with 50:50 grass silage and concentrate (DM basis). Diets included: 0.00% (Control; TMR-C), 0.54% (Low; TMR-L), 1.08% (Medium; TMR-M) and 1.62% (High; TMR-H) microalgae oil. Daily feed intakes and weekly BW were recorded from individually penned lambs during 76 days. Average daily gain (ADG), dry matter intake (DMI) and residual feed intake (RFI) were individually calculated. At day 55 of treatment, methane emissions were measured from 24 lambs (6 groups) in 6 individual respiration chambers during 3 days. Response variables were analyzed using a linear mixed model via the REML estimation method; where treatment was used as the fixed effect, whereas random effects were as follows: lamb and block for animal performance variables and run (1 to 4) and chamber for methane emission variables. Pairwise differences between treatments were examined using Fisher's LSD test ($P \leq 0.050$). Microalgae oil supplementation decreased DMI ($P=0.002$), ADG ($P=0.010$), and methane production ($P=0.009$) and yield ($P=0.041$), without affecting RFI ($P=0.514$). Feed intake decreased 17.5% in TMR-M ($P=0.004$) and 18.9% in TMR-H ($P=0.002$) compared to TMR-C. Daily weight gain decreased 23.2% ($P=0.010$) in TMR-M and 21.8% ($P=0.016$) in TMR-H compared to TMR-C. Lambs fed TMR-H had 21.5% lower methane production ($P=0.003$) and 20.3% lower methane yield ($P=0.014$) than TMR-C lambs. We concluded that microalgae oil supplementation at high levels in a TMR diet decreases methane emissions, however, microalgae oil at medium and high levels reduced DMI and ADG without affecting feed efficiency (RFI) in finishing lambs.

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Estimating Year-Round Greenhouse Gases and Ammonia Emissions in a Naturally Ventilated Dairy Cow Building

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The understanding of greenhouse gases (GHG) and ammonia (NH₃) emissions in cattle housing systems is essential for the development of management practices to reduce their emissions in the agri-food sector. The study aimed to estimate and analyze GHG (CO₂, CH₄ and N₂O) and NH₃ emitted within the year in a naturally-ventilated dairy house using a multichannel photoacoustic and CO₂ balance method. Gases concentrations, environmental factors, and cow's number and production parameters were monitored from August 2020 to June 2021, in a naturally-ventilated dairy house in Hillsborough, Northern Ireland. The barn (70x21 m) had a loose housing system with cubicles and solid-slurry-floor. Gases were measured using a photoacoustic monitor equipped with a multipoint sampler. Sampling points, 19 indoor and 5 outdoor, were located according to the VERA's protocol. Samplings were run at an interval of 72 seconds/point. Indoor temperature, relative humidity and wind speed were measured across the house. Nitrogen (N) excretion in feces and urine were estimated from measured N intake. Air flow rates through the house were calculated using the CO₂ balance with CO₂ emissions from cows predicted from measured DM intake. During the measurement period, 70±33 cows were housed, with live weight of 643±13 kg and milk yield of 33.7±5.6 kg. Temperature (°C), relative humidity (%) and air speed (m/s) were 9.7±3.7, 81±7 and 0.12±0.12, respectively. Production of CH₄, N₂O and NH₃ per cow were 481, 7.3 and 46.8 g/d, respectively. Methane yield and per energy corrected milk yield were 21.4 g/kg DM and 13.4 g/kg respectively. The NO₂-N/manure-N ratio was 0.02 and ammonia-N/urine-N ratio was 0.30. The results showed a high accuracy in CH₄ and CO₂ estimations using the photoacoustic gas monitor and CO₂ balance method in a naturally-ventilated dairy cow building, while small variations were observed for NH₃ and N₂O estimations with respect to reports elsewhere.

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Trypan Blue Method for Ruminal Protozoa Counting, While Assessing Yeast Supplementation on Fermentation and Methane Emissions

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Yeast supplementation of ruminant diets has been shown to increase ruminal bacteria population, potentially increasing livestock productivity. Culturing and counting ruminal microbes are essential to determine the effects of yeast on microbial growth dynamics. This study aimed to quantify growth dynamics of protozoa using the trypan blue exclusion method (TBEM) for ruminal protozoa counting under different culture conditions. Ruminal fluid was extracted from 4 ruminally-cannulated steers (783 ± 26 kg) receiving a high-grain diet with (YEAST) or without (CON) 28 g/d of a yeast fermentation substrate. Ruminal fluid was filtered, clarified, and incubated at 39°C under CO₂ in 125-mL bottles without substrate (NOSUB), with 0.7 g of diet (SUB), in 500-mL Erlenmeyer flasks (ERL), or in 16×125 mm Hungate tubes with anaerobic-antioxidant medium (TUB). Additionally, pH, volatile fatty acids (VFA), and CH₄ production were determined at 12, 24, and 48 h of incubation. Protozoa viability (PV) and concentration (PC) were determined through TBEM. Data was analyzed as a factorial split plot design. Protozoa growth dynamics showed a lag phase from 0-3 h, exponential phase from 4-17 h, and stationary after 17 h. A time × treatment interaction was observed ($P < 0.01$), and differences within times were determined after adjusting by Tukey. PC and PV were greater in CON-SUB vs YEAS-SUB ($P < 0.01$). Bottles and ERL resulted in lower PV and PC when compared with TUB at all timepoints ($P < 0.001$). Total VFA concentration at 12 h was greater ($P < 0.001$) in CON-NOSUB, then at 24 and 48 h YEAST-SUB was higher ($P < 0.001$). Total gas production and CH₄ concentration in NOSUB were lesser than CON-SUB and YEAST-SUB ($P < 0.001$). In agreement with the lesser PV, YEAST-SUB resulted in decreased CH₄ concentration and increased pH than CON-SUB at 24 ($P < 0.05$). In conclusion, the TBEM was a viable methodology to characterize protozoa growth dynamics, particularly when cultured in Hungate tubes.

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Lactational Performance, Rumen Fermentation and Enteric Methane Emission of Dairy Cows Fed Amylase-Enabled Corn Silage

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This study investigated the effect of an amylase-enabled corn silage on lactational performance, enteric CH₄ emission, and rumen fermentation of lactating cows. Following a 2-wk covariate period, 48 Holstein cows were blocked based on parity, days-in-milk, milk yield (MY), and CH₄ emission and randomly assigned to 1 of 2 treatments in an 8-wk randomized complete block design experiment: (1) corn silage from an isogenic corn (CON) without α -amylase trait and (2) Enogen[®] hybrid corn silage (ECS) containing a bacterial transgene expressing α -amylase in the endosperm of the grain. The ECS and CON silages were included at 40% of the dietary dry matter (DM) and contained 43.3 and 41.8% DM and 36.1 vs. 33.1% starch, respectively. Rumen samples were collected from 10 cows on experimental wk 3. Enteric CH₄ emission was measured using the GreenFeed system. Dry matter intake was similar between treatments. Compared with CON, MY (38.8 vs. 40.8 kg/d), feed efficiency (1.47 vs. 1.55 kg/kg), and milk true protein (1.20 vs. 1.25 kg/d), and lactose yields (1.89 vs. 2.00 kg/d) were increased, whereas milk urea nitrogen (14.0 vs. 12.7 mg/dL) was decreased, by ECS ($P \leq 0.05$). There was a trend for increased ECM feed efficiency (1.45 vs. 1.50 kg/kg) for cows fed ECS ($P = 0.09$). Daily CH₄ emission was not affected by treatment but emission intensity (per unit of milk, but not of ECM) was decreased by ECS-diet ($P = 0.007$; 11.1 vs. 10.3 g/kg milk, CON and ECS, respectively). Rumen fermentation, apart from a reduced ($P = 0.04$) molar proportion of butyrate in ECS-fed cows, was not affected by treatment. Overall, ECS inclusion increased milk feed efficiency and tended to increase ECM feed efficiency. The increased MY with ECS led to a decrease in CH₄ emission intensity, compared with the control silage.

Keywords: dairy cow, corn silage, enteric methane

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Nexus Project: Pathways for Greenhouse Gas Mitigation and Climate Change Adaptation of Australian Livestock Industries

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Australian sheep and beef production systems face the dual challenges of reducing net greenhouse gas emissions and adapting to a warmer climate with changing rainfall patterns. The 'Nexus project' is exploring pathways for livestock production systems to meet these challenges using farm systems modelling approaches. The objective of this component of the project was to estimate the GHG emissions intensity (GHG EI) from sheep and beef farms. Seven case study farms in eastern Australia were investigated across a diverse range of agroecological zones from the high rainfall, cool-temperate climate in Tasmania through to the arid, tropical region of northern Queensland. The production systems were one sheep-only farm (Midlands, Tasmania), three beef-only farms (north-west Tasmania, central Queensland and northern Queensland) and three mixed sheep and beef farms (Gippsland Victoria, northern Victoria and central New South Wales). Data on current farm management and production was collected through interviews with farm managers and used to estimate GHG EI using the Australian National Greenhouse Gas Inventory Report methodology. For sheep production, emissions were partitioned between meat and wool using the protein allocation method. Results were expressed as tonnes carbon dioxide equivalents (CO₂e) per tonne of production sold (liveweight for beef and sheep meat, and greasy wool). GHG EI of beef production ranged from 9.8-15 t CO₂e/t liveweight sold, with the lowest value on the farm in north-west Tasmania and the highest value in the central Queensland. For sheep production, the GHG EI of meat production ranged from 5.3-8.7 t CO₂e/t liveweight sold and wool production from 19.7-31.0 t CO₂e/t greasy wool sold. In general sheep and beef production GHG EI's were lower where the reproduction rates were higher. Further research will investigate options to reduce both total GHG and GHG EI using combinations of mitigation and sequestration opportunities on-farm.

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Increasing Grassland Sward Composition Can Lower the N₂O Emissions Intensity of Intensive Production Systems

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Nitrous oxide (N₂O) is a powerful greenhouse gas (GHG) with 265 times the global warming potential of carbon dioxide. Inorganic nitrogen (N) fertiliser is a major source of N₂O and of substantial monetary cost at farm-level. Conventional monoculture-based grassland systems receive high inorganic N fertiliser inputs in order to maintain yields. Reducing N fertiliser inputs will be crucial in decoupling GHG emissions from agricultural production systems. More recently, there has been increased interest in the use of multi-species swards (MSS) (mixtures of grasses, legumes and herbs) in intensive livestock production systems as research has shown MSS to have higher N use efficiency, increased yield production and improved livestock performance relative to monocultures. During this yearlong field experiment, we measured N₂O emissions (static gas chamber methodology) and forage yield from plots of systematically varying proportions of productive species from three functional groups: grasses (*Lolium perenne*, *Phleum pratense*), legumes (*Trifolium pratense*, *Trifolium repens*) and herbs (*Cichorium intybus*, *Plantago lanceolata*). All plots received 150 kg N ha⁻¹ year⁻¹ inorganic N fertiliser with an additional *L. perenne* comparison receiving 300 kg N ha⁻¹ year⁻¹. A simplex experimental design was used to quantify species identity and diversity effects on N₂O emissions and emissions intensity (N-yield and DM-yield scaled N₂O emissions). Increasing inorganic N fertiliser application from 150 to 300 kg N ha⁻¹ year⁻¹ doubled annual N₂O emissions from 1.39 to 3.18 kg N₂O-N ha⁻¹ year⁻¹. Species identity rather than diversity was the main driver of N₂O emissions with higher emissions associated with the legume functional group. Regarding emissions intensity, the 6-species mixture had a significantly reduced N-yield scaled emissions than the *L. perenne* monoculture at both 150 and 300 kg N ha⁻¹ year⁻¹. This study highlights the role of MSS in improving the sustainability of intensive production systems.

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Multi-Species Swards: A Climate Smart Solution for Grass-Based Milk Production Systems

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Dairy cows in grass-based milk production systems typically graze monocultures or a simple grass-legume mixture. Switching from a monoculture or grass-legume mix to a multi-species sward diversifies the diet of a dairy cow and reduces reliance on inputs such as synthetic fertilizers and herbicides. The goal of this research was to evaluate the potential for multi-species swards (MSS) to mitigate the climate impact of milk production at a farm scale. Two milk production systems, located on well-drained soils in the southeast of Ireland, were assessed. The control system, grass-white clover (GWC), spread 194 kg fertilizer N/ha and offered 870 kg concentrate DM/cow. The MSS system applied less fertilizer (82 kg N/ha) and offered more concentrate feed to the herd (962 kg/cow) than the control. Spring-calving Holstein-Friesian cows were rotationally grazed in the GWC and MSS systems. The structure and genetic merit of the herd did not differ between production systems. The climate impact of both systems was quantified in terms of greenhouse gas (GHG) emissions using a life cycle assessment (LCA) approach. The methodology computed pre-farm GHG emissions from background processes e.g., electricity and on-farm emissions from farming activities e.g., milking of cows. The total amount of GHG released by the GWC and MSS systems was expressed in terms of CO₂ and scaled to milk production. Preliminary results from the first year of the study indicate the GWC emitted 0.82 kg CO₂ equivalent/kg milk and 15 t CO₂ equivalent/ha. The MSS system had a similar GHG intensity (0.83 kg CO₂ equivalent/kg milk) in 2020. Greater feed imports in this system partly explained the lack of difference in GHG emissions, along with the coating of urea fertilizer with a urease inhibitor. Spreading fertilizer N as CAN would have led to MSS mitigating GHG intensity by ≈5%, which will be verified as the trial progresses.

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Acidification with Inorganic and Organic Acids to Reduce Greenhouse Gas Emission from Pig Slurry

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Methane emission from livestock manure contribute significantly to the total climate footprint of agriculture. Acidification of manure is known to reduce methane emission. However, the mode of inhibition by acidification is not completely understood and may depend on the acid or acids used.

In this work, we examined the effect of organic, inorganic, and mixed acids on greenhouse gas emission from pig slurry. Initially, pig slurry was incubated in glass vials and acidified to pH 5.5. Methane production was measured with a GC-TCD. After initial screening, a continuous gas monitoring setup was used to simulate conditions occurring in a pig house. Greenhouse gas emission was continuously measured with a cavity-ring-down spectrometer, while different acid combinations were used to treat the pig slurry to pH 5.50 - 6.25. The effect of daily-added pig slurry, as well as weekly slurry removal, and acidification of the residual slurry was examined using this laboratory setup as well.

In the initial screening, nitric acid reduced methane emission > 99% ($p < 0.001$) when acidified to pH 5.5. Combinations of acetic acid with sulfuric acid or nitric acid showed no further inhibition of methane production compared to nitric or sulfuric acid alone. In the continuous gas monitoring setup nitric acid reduced methane emission, but increased nitrous oxide emission, reducing the treatment effect on total CO₂-e considerably. Treatment with HNO₃ to pH 6.25 increased nitrous oxide emission by > 800% ($p = 0.042$). Treatment with different ratios of nitric acid and sulfuric acid to pH 5.5, was not better than pure sulfuric acid treatment, which resulted in 92% CO₂-e reduction ($p = 0.002$). When daily addition of slurry was applied, the efficiency of acidification decreased significantly after one week and CO₂-e was not significantly different from the control ($p = 0.085$ for H₂SO₄ treatment).

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The Anaerobic Biodegradation Model (ABM): A Flexible Tool for Predicting Methane Emission from Livestock Manure

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The current IPCC approach for estimating methane emissions from stored livestock manure employs emission factors related to annual or monthly temperature and volatile solids in excreta, which offers little flexibility for most effects of storage management alternatives and manure characteristics or treatments. To accurately predict methane emissions at a farm level a more comprehensive model is needed.

We present a new microbial model that tracks organic matter flow and conversion through combined hydrolysis and fermentation, followed by methanogenesis via multiple methanogen populations with different temperature optima. These features allow the model to predict methane emission dynamics in response to short- and long-term changes in temperature and other variables. Inhibition of methanogens by pH, ammonia, and hydrogen sulfide is included based on previously described responses. A population of sulfate reducers competes with methanogens for substrate.

The model is available as an R package from <https://github.com/sashahafner/ABM>. The package provides a tool that can reflect management (e.g., manure removal frequency, washing of manure pits, manure production rate, surface area, and acidification) and environment in ways that have been shown to be important in experimental studies of CH₄ emission during manure storage. This can be used to understand processes leading to gas emission and guide the selection of mitigation technologies.

As a demonstration, important model parameters were fitted to a comprehensive dataset from a pig facility with continuous methane emission measurements over 350 days. The ABM was then used to predict methane mitigation potential of management practices, including frequent manure removal from the barn to the storage tank, and storage tank acidification during the summer period where higher methane emissions are expected. Results show that frequent flushing reduce methane emissions 50-60% in the barn, but the reduction including emissions from outside storages were only 15–35% depending on other input variables.

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Nitrogen Fertilizers Sources Did Not Increase Soil Methane Emissions in Tropical Pasture under Continuous Grazing

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Nitrous oxide and methane together with carbon dioxide are the main greenhouse gases that provide an increase in global warming. Nitrogen fertilization may change methane emissions in grassland soils. The aim of the study was to evaluate the effects of different sources of nitrogen fertilization in marandu-grass (*Urochloa brizantha*) pastures on soil methane emissions in a continuous grazed area. The treatments were: 1 – No nitrogen fertilization; 2: Urea fertilization; 3: Ammonium sulfate fertilization; 4: Ammonium nitrate fertilization. The design used were completely randomized, consisting of 4 treatments and 5 replications per paddock (chambers), totaling 20 experimental units. Nitrogen fertilization was split in 3 times, totaling 150 kg nitrogen ha⁻¹. The beginning of each period coincided with the application of fertilizers, where after each application were three consecutive gas collection days, then every two days for a week and then every 7 days until a new application. The methane production was quantified using the static chamber methodology, with four repetitions and concentration determined by gas chromatography. During the entire period analyzed, the treatment ammonium nitrate was sink of methane, however, no differences were identified between treatments (P=0.103). The average emissions were 25.07 mg methane m⁻², 16.35 mg methane m⁻², 17.046 mg methane m⁻², and -7.082 mg methane m⁻² for treatments no nitrogen, urea, ammonium sulfate and ammonium nitrate. The results of this study suggest that soil methane emissions are not influenced by use of nitrogen fertilizers in tropical pasture.

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What Are the Opportunities of Biological Nitrification Inhibition for Reducing N₂O Emissions from Livestock Systems?

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Nitrous oxide (N₂O) is a powerful greenhouse gas, with agriculture contributing around 52% of global anthropogenic emissions. Animal urine is the largest N₂O source in grazed livestock systems. The inhibition of soil nitrification has been shown to reduce N₂O emissions from urine patches, with much of the existing understanding based on studies using synthetic nitrification inhibitors. However, there is increasing evidence that plant-induced biological nitrification inhibition (BNI), mediated predominantly by root exudates, also reduces N₂O emissions. We conducted a review of the current understanding of BNI to identify knowledge gaps and research questions to accelerate the development of BNI as a N₂O mitigation strategy for livestock systems. Key barriers that need to be overcome include: identifying agronomically viable species with the genetic capacity to synthesize BNI compounds (discovery), maximizing the BNI capacity of these compounds in soils (proof of concept), managing species within systems to maintain BNI activity and productivity (proof of function) and having systems in place to reward farmers for adopting BNI as a N₂O mitigation strategy (recognized mitigation option). We propose that the initial research focus should be on the discovery and proof of concept stages. Firstly, the systematic screening of agronomically desirable plants to identify their BNI capacity requires the development of rapid *in-situ* screening methods that can be combined with reliable N₂O emission measurements. To ensure that any N₂O reduction can be assigned to BNI, these measurements should be accompanied by microbial and metabolomic analyses to confirm the selective inhibition of nitrification by BNI compounds. Whilst understanding the genetic regulation of BNI is a key first step, an equally important challenge will be to discern the apparent influence of soil N fertility or other soil/climatic factors on the expression of the BNI trait, particularly for more intensively managed livestock systems.

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Assessing Feed Intake, Enteric Methane Emissions and Performance of Beef Steers on Different Pasture-Based Systems

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The objective of this trial was to evaluate the dry matter intake, animal performance, and enteric methane emissions of beef steers in different pasture-based production systems. Sixty Canchim beef steers (5/8 Charolais x 3/8 Zebu) with initial liveweight 255 ± 7 kg were evaluated, distributed in five treatments (pasture-based system); Intensive, Integrated Crop-Livestock, Integrated Crop-Livestock-Forest, Integrated Livestock-Forest and Extensive. The experiment was carried out at Embrapa Southeast Livestock, Sao Carlos, SP, Brazil, evaluating four seasons (Summer, Autumn, Winter, Spring) during two consecutive years (2013-2015). The data were analyzed using mixed models. Means were separated by the Tukey test and the effects were considered significant when $p < 0.05$. Final liveweight (484a, 466a, 416b, 414b, 429b kg), dry matter intake (8.2ab, 7.5ab, 8.9a, 8.3ab, 7.4b kg), CH₄ emissions intensity in g/kg of dry matter intake (30.4a, 33.0a, 22.8b, 26.7ab, 29.2a) and in g per kg of carcass/ha/year (0.68b, 0.81b, 0.94b, 0.78b, 1.36a) differed ($p < 0.05$) between Intensive, Integrated Crop-Livestock, Integrated Crop-Livestock-Forest, Integrated Livestock-Forest and Extensive, respectively. System x season interaction was observed for the average daily gain (Summer: 0.71a, 0.97a, 0.52b, 0.69a, 0.64ab and Winter: 0.39a, 0.40a, 0.09b, -0.09b, 0.31a kg/day), and the CH₄ emission per kg of average daily gain (Summer: 0.36a, 0.17b, 0.34ab, 0.22ab, 0.37a Autumn: 0.36b, 0.46b, 0.62ab, 0.57ab, 0.69a g/kg), respectively for Intensive, Integrated Crop-Livestock, Integrated Crop-Livestock-Forest, Integrated Livestock-Forest and Extensive. Pasture systems that receive technologies such as soil correction and fertilization, are managed with rotational stocking or are integrated with corn, can produce animals with higher average daily gain and consequently higher final liveweight, which dilutes the CH₄ emissions per kg of liveweight gain. Animals produced in intensive and integrated systems emit less methane per kg of carcass produced per area per year than those raised in systems managed extensively.

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Short-Term Effects of Stockpiling Pastures on Soil Physical Properties in Acre State, Southwestern Brazilian Amazonia

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Grass-legume mixed pastures are claimed as an option for intensification of pasture-based livestock production in Brazilian Amazonia. In Acre State, the legume *Arachis pinto*i has been planted in over 80,000 ha during the last 20 years, supporting a double stocking rate when compared with traditional pastures. There is a concern if these higher stocking rates could affect soil physical properties, especially in poorly-drained soils which predominate in the region. In this study we tested if stockpiling forages in the end of wet season as a strategy to supply feeds to cattle in the dry season could also improve soil physical properties. The commercial pastures (M1, 2C and 2D) evaluated were established in 1981 and kept without fertilization and under rotational stocking until nowadays. The 2D was not stockpiled, 2C was stockpiled from April to June 2019 (77 days) and M1 was stockpiled from April to May 2020 (62 days). The soil resistance to penetration, volumetric water content and bulk density were assessed in June 2019 and in July 2021 at 0.0-0.20 m depth with 40 field repetitions. Resistance to penetration readings were carried out using a penetrometer equipped with pin penetration of 0.6 m height and conical tip with angle of 30°, penetration in constant rate of 0,035 m s⁻¹, recording the values at intervals of 0.01 m down to 0.2 m depth and resistance to penetration at each 0,025 m. The resistance to penetration in 2C was reduced in 2019 (depths of 0.025 m, 0.05 m, 0.075 m and 0.10 m) in comparison to M1 and 2D and was equal among treatments for the other depths. There were no differences among treatments for all depths in 2021. Our results show that the impact of stockpiling pastures in soil physical properties are of short duration.

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Energy Efficiency Improvement and Carbon Dioxide Emission Reduction in Piglet Rearing Using Air-to-Air Heat Exchangers

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Pig farming requires high amounts of electrical energy to operate the technical equipment inside the barns (e. g. ventilation, exhaust air treatment). Simultaneously, piglet rearing requires much thermal energy to meet the animal demands concerning air temperatures. The provision of energy is associated with carbon dioxide (CO₂) emissions. As heat exchangers can recover thermal energy, the study's objective was thus to investigate the energy efficiency and CO₂ emissions of piglet rearing using a heat exchanger. The study's long-term measurements were conducted in a barn (4140 piglets and 238 gilts) under practical conditions. The exhaust air was collected in the central exhaust air duct and could be led into two air-to-air heat exchangers. Fresh and exhaust air temperatures were recorded every 15 minutes. The air flows were calculated using the fan speed and air differential pressure (based on pre-tests and manufacturer's data). The energy consumption of the barn facility was recorded by electricity meters and the manual liquid gas documentation. The CO₂ emissions were calculated with 0.427 kg CO₂ kWh⁻¹ for electricity and 0.237 kg CO₂ kWh⁻¹ for liquid gas combustion. In the one-year trial, a total of 561,261 kWh energy was provided to operate the barn, including 291,518 kWh of thermal energy that was recovered. The operation of a heat exchanger is associated with additional consumption of electricity by the fans (42,034 kWh for compensation of increased pressure losses); however, 6.9 times more thermal energy could be recovered on an annual average. Overall, the use of heat exchangers reduced the energy consumption of the barn by 48%, the CO₂ emissions by 36.5% and the energy operating costs by 10.4%. This corresponds to savings of 12.36 kg CO₂ piglet place⁻¹ year⁻¹. Consequently, the use of heat recovery systems can improve the energy efficiency and sustainability of pig farming.

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Formation of Greenhouse Gases by Corn Silage during the Ensiling Process

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Silage is an essential global feedstuff. The microbiological ensiling process forms carbon dioxide, methane and nitrous oxide, and silage additives can affect this process. The study's objective was to measure the climate-relevant emissions during the ensiling process depending on silage additive application. Corn was chopped under practical conditions. Twelve barrels were filled with 10.2 kg fresh mass each (44.9% dry matter concentration). Three variants (n=4) were used: control variant (CON), biological additive variant (BIO; 10^6 colony-forming units *Lactobacillus Buchneri* (kg FM)⁻¹ and 10^5 colony-forming units *Lactobacillus Plantarum* (kg FM)⁻¹) and chemical additive variant (CHE; 0.5 g sodium benzoate (kg FM)⁻¹ and 0.3 g potassium sorbate (kg FM)⁻¹). The quantity and quality of gas emissions were measured by gas bags connected to the barrels and gas samples taken from the barrels' headspaces and subsequently analyzed by gas chromatography. CON silage showed a gas formation of 7.93 L (kg DM)⁻¹ in the first 25 ensiling days, BIO 8.18 L (kg DM)⁻¹ and CHE 6.16 L (kg DM)⁻¹, respectively. Most gases were produced within the first 72 ensiling hours; CHE showed a significantly slower gas formation and CO₂ concentration increase (p<0.05). All variants peak up to 7.00–7.65 ppm methane between hours 120–148; in detail, CHE indicated lower concentrations between ensiling hours 36–64 (p<0.05). In contrast, this variant showed the highest nitrous oxide concentrations between ensiling days 2–11 (p<0.05) with values up to 36.15 ppm at the typical peak between ensiling hours 36–44. The total emissions after 25 days were 6.48 g CO₂-eq. (kg DM)⁻¹ for CON, 6.55 g CO₂-eq. (kg DM)⁻¹ for BIO and 4.36 g CO₂-eq. (kg DM)⁻¹ for CHE, respectively. Compared to chemical ensiling additives, biological additives could not influence gas formation or reduce emissions; however, both promise higher aerobic stability.

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Methane to Carbon Dioxide Ratio to Predict Methane Yield for Cows Fed Fresh Forages

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The methane to carbon dioxide ratio (CH₄:CO₂) has been suggested as a proxy of methane production (CH₄P), but its relationship with methane yield (CH₄Y: g/kg dry matter intake) has not been tested. This study evaluated the relationship between CH₄:CO₂ and CH₄P and CH₄Y for two forages. Methane and carbon dioxide emissions were measured using respiration chambers from 16 non-lactating dairy cows fed either ryegrass (RG) or plantain (PLT) in two periods (P1, P2). Feed offer was restricted to ~9 kg dry matter delivered in two equal meals at ~0830 and ~1530 h. The 24h-CH₄:CO₂ was averaged within cow and period, and used as an explanatory variable for CH₄P and CH₄Y using linear regressions. A predicted CH₄Y was also calculated from the predicted CH₄ production (from the linear regression equation) and the observed dry matter intake. The 24h-CH₄:CO₂ (g/g) was greater in RG than in PLT (0.023 and 0.026 vs 0.021 and 0.022, P1 and P2, respectively). The CH₄:CO₂ explained 80% of variability in CH₄ production for the whole dataset. The CH₄:CO₂ explained most of the variation in CH₄P for PLT-P1 and PLT-P2 ($R^2 = 0.75$ and 0.79 ; respectively) and RG- P1 ($R^2 = 0.90$), but not for RG- P2 ($R^2 = 0.18$). When the CH₄:CO₂ was regressed against the observed CH₄y, the ratio explained 54 %, 24% and 30% of its variability in PLT-P1, RG-P1 and in the overall data, respectively. For both forages in P2, CH₄:CO₂ explained little of the variability of observed CH₄y ($R^2 \leq 0.11$). The correlation between CH₄y predicted and observed was weak ($r = 0.18$). In conclusion, the CH₄P can be estimated precisely using the CH₄:CO₂, but the predictive ability of this ratio was different between feeding periods for RG. The CH₄:CO₂ cannot estimate precisely the CH₄y directly or indirectly through estimation of CH₄P.

Variation in Methane Emission Patterns from Cows Fed Forages: Potential for Precision Mitigation?

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The methane (CH₄) daily emission patterns could differ depending on forages fed to ruminants. Understanding this variation could provide opportunities for targeted application of feeding management in grazing systems to mitigate emissions, e.g., timing of supplementation. The objective of this evaluation was to determine the variation in daily emission patterns of CH₄ resulting from feeding two fresh forages. Sixteen non-lactating dairy cows were fed either ryegrass (RG) or plantain (PLT) in two periods (P1, P2) and their methane emissions were measured in respiration chambers. Feed offered was restricted to ~9 kg dry matter delivered in two equal meals at ~0830 and ~1530 h. The CH₄ production (expressed as g/d) was 12% (P1) and 24% (P2) less after the morning meal and 28% (P1) and 33 % less (P2) after afternoon meal for cows fed PLT than for those fed RG. The CH₄ production rate increase from pre-meal to peak emissions was greater (except in P1 after the morning-meal) for cows fed PLT (1.8- to 3.3-fold increase) compared with those fed RG (1.5 to 2.3-fold increase) across both periods, mainly due to a 33 -47% lower pre-meal CH₄ emission rate for cows fed PLT. The CH₄ peak emission rate (g/h) was similar between forages in P1 and 19% lower in PLT than in RG in P2. The CH₄ decay rate (/h) after the emission peak was faster for PLT than for RG after morning and afternoon meals in P1 and after the afternoon meal in P2. These differences suggest there is scope for harnessing within-day and between forage variation in emission patterns to optimise interventions to reduce methane emissions from grazing ruminants.

Impact of Carbon Sequestration on the Carbon Footprint of Beef Produced on a Florida Ranch

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Buck Island Ranch is a cow-calf operation in central Florida. The ranch manages over 10,000 acres of semi-native and improved pastures, and over 3,000 head of cattle. The operation has the unique distinction of being both a working ranch and a conservation site with extensive monitoring of variables from biodiversity to nutrient dynamics in pastures and wetlands for the past 30 years. As a result of managing for both profitable beef production and conservation, the ranch provides key ecosystem services to their community through conservation-oriented management practices. There were two objectives for this project: (1) perform a cradle-to-ranch life cycle assessment of environmental impacts and resource consumption for 1 kg of live-weight sold from the ranch from 2014-2017; (2) develop reproducible methods for multi-functional allocation of environmental impacts between beef and ecosystem services. Four economic allocation approaches were piloted: (1) allocate all emissions to beef; (2) multi-functional allocation using the “highest and best use” price based on real estate evaluation of Buck Island Ranch land; (3) multi-functional allocation using USDA Agricultural Conservation Easement prices; and (4) multi-functional allocation using USDA Conservation Stewardship Program payments for conservation management practices. Production of 1 kg live-weight leaving the ranch resulted in a carbon footprint of 12 and 24 kg CO₂-eq/kg live-weight with and without carbon sequestration, respectively. Carbon footprints were highly sensitive to assumptions for land N₂O emissions, which contributed 32-47% of the footprint. Eutrophication potential was 37 g N-eq/kg live-weight. Resource consumption for 1 kg live-weight was 322 L water (without precipitation), 44 m² annual crop-eq, and 2 MJ energy surplus. When emissions were allocated between beef and ecosystem services, beef impacts were reduced 2% using the Conservation Stewardship Program approach, 39% using the highest and best use approach, and 42% using the USDA Agricultural Conservation Easement Program approach.

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Urine Patch Nitrogen Level Affects Nitrous Oxide and Dinitrogen Fluxes from a Grazed Pasture Soil

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Ruminant cattle grazing pasture result in urine deposition at high nitrogen (N) rates that can impact the environment including via gaseous emissions. Among those lost pathways, nitrogen gas emission, especially nitrous oxide (N₂O) and dinitrogen (N₂), is the primary way where N can be lost to the atmosphere. There is limited information on the magnitude or fluxes of N₂ losses from grazed-pasture systems after urine deposition due to the method limitation. We used the ¹⁵N flux method and high sampling frequency to explore N₂ and N₂O fluxes over time after urine application at two rates (400 and 800 kg N ha⁻¹) on a New Zealand grazed pasture soil. The higher N rate significantly increased daily N₂O fluxes but has no significant effect on daily N₂ fluxes in our study compared with the lower rate. N₂ is the predominant gaseous N form lost from the applied urinary-N which contributed 32.1 ± 4.1% and 14.4 ± 1.7% of the total deposited-N from 400 kg N ha⁻¹ and 800 kg N ha⁻¹ respectively, over 95 measurement days. Denitrification and codenitrification co-occurred in the pasture system, with denitrification being the predominant N₂ production pathway, contributing 97.9 – 98.5 % of total N₂ production. The N₂O/(N₂+N₂O) product ratio was generally higher during periods of nitrification (the first month after urine application) but with no clear relationship to other measured variables. Contrary to our hypothesis, an elevated urine-N rate did not enhance N₂ loss. This is speculated to be due to enhanced ammonia volatilisation and transfer of N as nitrate, to deeper soil layers. Soil relative gas diffusivity indicated that high N₂ fluxes resulted from entrapped N₂ diffusing from the draining soil.

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CARBON AGRI, a Result-Based Carbon Farming Scheme for Boosting Carbon Initiatives in Mixed Crops-Livestock Sector

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In France, the agriculture sector is developing low carbon initiatives at national scale (Low Carbon Dairy Farm and Beef Carbon) to reduce carbon intensity by 20% in 2025 compared to 2015. Regarding the 15 000 farmers involved, there is a strong interest in improving methods for measuring, reporting and verifying (MRV) mitigation actions and a need in providing payments for the delivery of climate mitigation benefits, quantified as CO₂ emissions avoided or sequestered. In 2018, the French Government adopted the *Label Bas Carbone* (Carbon Standard), a framework for voluntary carbon reduction project. Complying with this standard, CARBON AGRI, a MRV process for mixed crops-livestock systems, has been developed and certified by the French government. To capture emissions reductions and soil carbon sequestration, CARBON AGRI measures progress from a range of 40 mitigation practices. Based on the national life cycle assessment tool farm CAP'2ER[®], the reductions are assessed against a baseline level of GHG emissions per unit of product (milk, beef and crop) before the project starts. Each participating farm uses its own figures to calculate its baseline and its mitigation action plan. For the five years crediting period, carbon reduction represents the carbon intensity differences between the baseline and the project. An average French farm which reduces carbon footprint from 15 to 20%, generates a reduction of 300 to 350 tons of CO₂. These result-based emissions reductions are traded for payment from an external party voluntarily offsetting their emissions. Thanks to environmental co-benefits (water preservation, contribution to biodiversity), farmers are selling CO₂ avoided for prices of 30-40 €/t CO₂ which represent 6 000 to 12 000 € per farm. This carbon offset scheme is following the European carbon farming initiative. It plays a strategic role in catalysing carbon transition, providing financial resources and giving a rigorous framework to monitor mitigation effort in agriculture.

Keywords: MRV, agriculture, mitigation, carbon

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Effect of Grass Herbage Quality and Sheep Breed on Enteric Methane Emission from Ewes

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Globally, sheep account for approximately 3.5% of the GHG emissions from the livestock sector, mainly through enteric methane (CH₄) emissions. Improved feed digestibility is one of several potential mitigation options. The aim of this study was to compare enteric CH₄ emissions for two breeds offered grass herbage (zero-grazing) with decreasing quality. The two breeds were the modern Norwegian White Sheep (NWS), selected for production and maternal traits and the Old Norwegian Spæl (ONS), a lighter, rural breed. The grass herbage was from a grass-clover based sward. When starting the experiment, the grass herbage was of 3 weeks of regrowth, and was harvested in the morning each day, and offered twice daily. Grass herbage with decreasing quality, due to increased maturity stage of the sward, was fed *ad libitum* to individually housed mature ewes, 20 of each breed, during a 6-week period in August/September. Feed intake was continuously logged throughout the experiment. Individual 50 min accumulated CH₄ emissions were captured repeatedly 7 times per week in Portable Accumulation Chambers (PAC) in week 2, 4 and 6 of the experiment. Ewes were weighed just prior entering the PAC. NWS and ONS had an average body weight increase from 83.8 kg to 90.9 kg and from 53.8 kg to 61.7 kg, respectively. Likewise, CH₄ emission in gram per hour increased from 2.05 to 2.19 for NWS and from 1.27 to 1.47 for ONS indicating a larger average increase in emission for ONS compared to NWS, and lower emission when providing grass herbage of early maturity than late maturity. The mean daily dry matter intake (DMI) was 3.07 kg and 2.01 kg for NWS and ONS and decreased slightly for both breeds throughout the experiment. When accounting for grass DMI, ONS had lower mean enteric CH₄ emission than NWS (1.42 vs 2.13 grams CH₄/hour).

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Mitigation of Methane Emission Using Direct Seeding in Rice Cultivation in Chile

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Rice cultivation is one of the main sources of greenhouse gas (GHG) emissions in the agricultural sector. In Chile, research in agronomic practices that allow reducing GHG emission associated with this crop, is limited and preliminary. Therefore, the objective of this study was to evaluate a technology that allows to keep standard production, reducing water consumption and thereby mitigating GHG emissions. The field trial was developed in the location of Parral (Chile), using two sowing systems (direct dry sowing and broadcast sowing with pre-germinated paddy seeding) and alternate wetting and drying as a water management technique. To evaluate the emission of methane (CH₄, GHG emitted in rice crop), the static chambers technique was used; the static chamber was closed at the time of measurement to quantify the accumulation of CH₄ at three times (0, 20 and 40 min). The amount of water used during the growing season, the grain yields, and the accumulated CH₄ fluxes were determined. The results of two seasons shown a reduction of CH₄ emission and water consumption, by 67 % and 28 %, respectively, with dry direct seeding system, compared with the broadcast sowing with pre-germinated rice. The alternate wetting and drying showed a reduction of CH₄ emission and water consumption of 24 % and 16 %, respectively, compared with traditional irrigation method. There were no significant differences in the grain yield (8.4 t ha⁻¹) between irrigation systems. In conclusion, the dry direct seeding could be a better alternative to mitigate GHG emissions in Chile, reducing the water use and maintaining the grain yield.

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Exploring the Potential of Nature-Based Solutions to Generate Social, Environmental, and Economic Co-Benefits in Livestock Systems

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Nature-based Solutions can provide a range of environmental and economic co-benefits on farms, including reduced GHG emissions, greater carbon sequestration, reduced input costs, improved profitability, and a more resilient, sustainable farming system. Nature-based Solutions focus on restoring landscapes by improving soil health and biodiversity, while reducing soil disturbance and chemical inputs. The challenge that exists for farmers is to reduce the GHG emissions from their farms while simultaneously adapting to climate change, especially in managing different weather patterns and seasonal rainfall trends. It is therefore increasingly important to consider how mitigation and adaptation options can complement each other on livestock farms. Farmers are more likely to implement greenhouse gas mitigation strategies that have environmental, economic, social, or cultural co-benefits. Yet it can be difficult for farmers to know which mitigation options to prioritize on their farms. Information is needed on the non-financial barriers and benefits of mitigation options, as well as their economic impact. The objective of this study was to use a multidisciplinary approach to document the benefits of Nature-based Solutions on livestock farms, such as the potential for more consistent animal production, increased revenue through additional income streams, higher price premiums for products from farms that practiced Nature-based Solutions, and possible social impacts. Potential barriers to the widespread investment of Nature-based Solutions were considered, as well as transition costs, operational input costs and a change in risk profile. This research provided initial insights into the tools that would facilitate the adoption of Nature-based Solutions, such as the use of remote sensing tools for soil measurements to reduce the cost burden of Measurement, Reporting and Verification (MRV). Further research is required around the best metrics to measure natural and social capital to align these farm assets with the valuations of financial lending institutions.

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Substitution of Leguminous Forage for Oat Hay Mitigates Enteric Methane Emissions of Crossbred Simmental Calves

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Dietary manipulation has the potential to mitigate enteric methane emissions and to maintain or enhance livestock productivity. The present study is aimed to investigate the optimal levels of leguminous forages (alfalfa hay and common vetch hay) in the diet of male calves to mitigate enteric methane emission, improve energy utilization while maintaining nutrient digestibility and productivity. Forty-eight Crossbred Simmental calves' data from three studies with enteric methane emissions measured by respiration chamber were analyzed. The diets were iso-energetic and iso-nitrogenous, and the forage-to-concentrate ratio of all diets was at 60:40. The body weight gain and feed conversion efficiency were not affected by the levels of leguminous forages in the diet, whereas the nutrient digestibility was higher in the 16-20% than the 20-40% proportions. Although the ratio of methane energy to gross energy intake was lower in the 30-40% leguminous forage diets than the 20% diet, the ratio of fecal energy to gross energy intake was the opposite. In the quadratic regression ($R^2 = 0.697$) between normalized body weight gain and normalized methane emissions, the highest body weight gain of calves was observed at the value of 0.716 for methane emission, which equals to 2.65 g/kg BW^{0.75} methane production and corresponds to around 20% leguminous forage in the diet. In conclusion, the optimal levels of leguminous forages for mitigating methane emission per kg dry matter intake or per kg of metabolic body weight, while maintaining nutrient digestibility and feed conversion efficiency, in crossbred Simmental beef calves in the dryland environment were at 16-20%.

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Methane and Carbon Dioxide Emissions from Excreta of Beef Steers Fed a Tannin-Rich Legume

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Methane (CH₄) and carbon dioxide (CO₂) are important greenhouse gases (GHG) generated by livestock. The objective of this study was to determine the emission of CH₄ and CO₂ from excreta of beef steers fed a tannin-rich forage legume. Fifteen Brahman x Angus crossbred steers were fed one of three experimental diets: 0, 50, or 100% inclusion of 'AU Grazer' sericea lespedeza hay [SL; *Lespedeza cuneata* (Dum. Cours.) G. Don] into 'Tifton-85' bermudagrass hay (*Cynodon* spp.) diets. Emissions were evaluated using the static chamber (non-steady state) technique. Feces and urine were collected directly from each animal for two experimental periods. Gas sampling occurred between 0900 and 1100 h, on days 0, 1, 3, 5, 7, 14, 18, 25, and 32 after excreta application to the chambers. One subsample was taken per deployment time (0, 15, and 30 min) per chamber. Day was considered the repeated measurement. Data was analyzed using Glimmix procedure of SAS. Effect of the day after feces application was found for both gases ($P < 0.001$), while interactions of day and the level of inclusion of SL were observed for both gases in urine ($P < 0.001$). Peak CH₄ in feces occurred in the first day after application of excreta, regardless of tannin-inclusion. Peak CH₄ in urine was greatest for 0SL in the first day ($P < 0.001$). Reductions to background emissions were obtained three days after excreta application. Peaks CO₂ in urine and feces followed the same pattern as CH₄ but reductions to background levels were reached at d 18. The inclusion of a tannin-rich legume in the diets of beef steers was an important tool to mitigate the emission of CH₄ and CO₂ from ruminant excreta. Further studies are necessary to elucidate the role of dietary sources in GHG emissions from excreta.

Growing and Non-growing Season Nitrous Oxide Emissions from Irrigated Crops Utilizing Dairy Manure

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Information is limited on greenhouse gas emissions from irrigated cropping systems utilizing dairy manure, especially during the non-growing season. In semiarid southern Idaho, we measured nitrous oxide emissions at three different field sites where manure was used as fertilizer in dairy forage rotations. Daily nitrous oxide flux measurements were conducted using vented, non-steady-state, closed chambers. The chambers were manually deployed a few times a week, while another set of chambers was fully automated, allowing for continuous flux measurements. Nitrous oxide pulses during the growing season were highest after the first few irrigation events and greatest from the manure treatments versus synthetic fertilizer. During the non-growing season, in winter and early spring, freeze-thaw events dramatically increased nitrous oxide fluxes and strong diurnal pulses were noted. Cumulative nitrous oxide emissions during the non-growing season were found to be approximately equal to those during the growing season. Cumulative annual losses of nitrous oxide were positively correlated with manure application rate. Despite the high nitrous oxide emissions from the manure treatments, this only represents about one percent or less of the total applied nitrogen.

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Pasture Type under Continuous Stocking Had No Effect on Beef Cattle Performance or Enteric Methane Emissions

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We aimed to assess the effect of pasture type on beef cattle enteric methane emissions under continuous stocking. Since April 2021 Stabilizer beef steers and heifers (n=30) were allocated to graze the permanent pasture (PP) or the perennial ryegrass and white clover (G-WC) sward at the North Wyke Farm Platform, Rothamsted Research, UK. Herbage mass was estimated using a rising plate meter and G-WC herbage samples were taken to estimate white clover content. Individual enteric methane emissions (n=12) were estimated using the SF₆ technique during two 1-week periods in mid-June and mid-August. Live weight (LW) was measured before and after each period and average daily gain (ADG) was calculated. Data were analysed using repeated measures including pasture type, period and the interaction as fixed effects and time as a repeated measurement. The herbage mass in the PP and G-WC was 1801 and 2631 kg DM/ha in June and 2595 and 2142 kg DM/ha in August, respectively. The white clover content of the G-WC herbage was 3.3% and 29.7 DM% in June and August, respectively. Animals in both pastures had similar LW (531±0.8 kg), ADG (0.76±0.052 kg/d), daily enteric methane emissions (215±6.3 g methane/d) and methane emissions yields (0.39±0.011 g methane/kg LW; 340±46 g methane/kg ADG). While LW decreased from June to August (532 vs. 529±0.8; P=0.03), daily methane emissions and per kg ADG increased (201 vs 231±6.6 g methane/d; 0.36 vs 0.42±0.012 g methane/kg ADG; P<0.01), but no period effect was observed for ADG or methane emissions per kg LW. We conclude that the pasture types tested had thus no effect on beef cattle LW gain or methane emissions, but these variables were affected by the month when the measurements were undertaken.

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Impacts of Cow System on Greenhouse Gas Emissions of Beef Production

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A conventional (CONV) pasture-based cattle production system with cows wintered on corn residue and summer grazing of bromegrass pasture was compared to partial-confinement system (ALT) with cows and calves in a drylot during the summer and grazing cover crops and corn residue over the fall and winter. Four groups of 20 cows were in each system. Eddy covariance methods were used to measure emissions from grazed areas (corn residue and pasture) and pen chambers were used to measure emissions from feedlot pens (cows and calves). Cattle from the CONV system produced more CH₄ and CO₂, but produced more beef per cow exposed (321 and 303 kg HCW for CONV and ALT, respectively). Measured CH₄ and modeled N₂O emissions totaled 7.5 ± 0.3 and 7.4 ± 0.3 kg CO₂e kg⁻¹ HCW for CONV and ALT production, respectively. There was a measured uptake of 233 g C m⁻² and 98 g C m⁻² from bromegrass pasture and cover crop, respectively. Calves from the ALT treatment were 45 kg smaller at weaning ($P < 0.01$) and had compensatory growth (1.21 vs 1.38 kg ADG; $P < 0.01$) during the growing period. Similar CH₄ and CO₂ production animal⁻¹ and kg⁻¹ DMI resulted in lower CH₄ and CO₂ kg⁻¹ ADG ($P < 0.01$). During the finishing phase CONV calves had greater ADG (1.81 vs 1.52 kg ADG; $P < 0.01$) but similar DMI ($P = 0.25$). The ALT calves were fed 35 d longer to achieve similar backfat which resulted in greater total CH₄ animal⁻¹ across the entire feeding period ($P = 0.02$) and greater total CO₂e ($P = 0.02$) for ALT calves. Methane production was greater in ALT calves (2.1 vs 2.5 kg CO₂e per kg HCW, $P = 0.04$). Due to days to market, ALT calves showed more global warming potential post-weaning.

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Methane and Carbon Dioxide Production of Lactating Holsteins Cows with Different Crude Protein Feeding Strategies

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Although ruminal production of greenhouse gases (GHG) has been well-studied in relation to carbohydrate nutrition, limited research has studied the potential mediating effects of level of crude protein (CP) on ruminal carbohydrate fermentation and subsequent GHG emissions. Additionally, it is unclear how time-varying dietary composition affects GHG production. Our objective was to quantify the methane (CH₄) and carbon dioxide (CO₂) production of 8 non-cannulated, multiparous mid- to late-lactation Holsteins (mean = 133, SD = 12 days in milk) during a 2x2 factorial study of CP level (LP, 13.9%; HP, 15.4%) and feeding pattern (O = CP oscillating at 48 h interval, S = static). Cows were housed in tie stalls and fed total-mixed rations with a 60:40 forage-to-concentrate ratio. Each O feeding pattern consisted of one lower and one higher CP diet (O-HP 13.9-16.9%; O-LP 12.2-15.5%) alternated at 48 h intervals such that the average diet composition over time equaled that of the corresponding S diet (S-HP; S-LP respectively). Diets were formulated by linearly exchanging soy hulls and ground corn with solvent soybean meal, maintaining a constant NDF:starch ratio (1.18) and constant ratio of rumen-degradable to crude protein (0.61). Treatments (n = 4) were arranged in a replicated Latin Rectangle design across four 28-d periods. On d 25-28 of each period, we recorded feed intake and milk production and took samples oforts and milk to calculate dry matter intake (DMI) and production. From day 14-21 of each period, we sampled gas production 3x/day with a GreenFeed unit (C-Lock, Rapid City, SD) during balanced intervals (-2.5)-(-0.5), 1-3, 4-6, 6.5-8.5, and 11-13 h relative to 1x daily morning feeding. We fit linear mixed models with fixed effects of CP level, feeding pattern, and period, and a random intercept for cow and computed model-implied means and standard errors (SEM). Neither CP level, feeding pattern, nor their interaction influenced DMI (26.3 ± 0.8 kg/d; $p = 0.401-0.935$) or affected production of milk (36.7 ± 1.3 kg/d; $p = 0.335-0.745$) or fat-protein-corrected milk (FPCM; 36.6 ± 1.3 kg/d; $p = 0.353-0.910$). We observed no significant effects of CP level, feeding pattern, or their interaction on CH₄ production (474 ± 24 g/d; $p = 0.461-0.892$), intensity (13.1 ± 0.6 g/kg FPCM; $p = 0.500-0.943$), and yield (18.0 ± 0.7 g/kg DMI; $p = 0.280-0.917$). HP tended to increase CO₂ production ($p = 0.070$), feeding pattern had no effect ($p = 0.123$) and CP level tended to interact with feeding pattern ($p = 0.084$), with O-HP driving the interaction (O-HP = 15,597, S-HP = 14,890, O-LP = 14,827, S-LP = 14,870 g/d; SEM = 410.). Neither CP level, feeding pattern, nor their interaction affected CO₂ intensity (418 ± 16 g/kg FPCM; $p = 0.226-0.870$). HP increased CO₂ yield ($P = 0.022$) although neither feeding pattern ($p = 0.474$) nor the interaction ($p = 0.927$) influenced CO₂ yield (O-HP = 595, S-HP = 586, O-LP = 565, S-LP = 557 g/kg DMI; SEM = 16). Neither CP level, feeding pattern, nor their interaction affected the ratio of CH₄:CO₂ in daily emissions (0.095 ± 0.004 L/L; $p = 0.309-0.950$) or affected oxygen consumption ($10,362 \pm 296$ g/d; $p = 0.102-0.646$). Neither experimental factor nor their interaction influenced the respiratory quotient (1.05 ± 0.01 ; $p = 0.263-0.865$), which suggested positive energy balance. Overall, results showed minimal effects of CP level and feeding pattern on GHG emissions. Tendencies for greater CO₂ production and yield in cows fed O-HP relative to other treatments require further investigation of ruminal and post-absorptive metabolism.

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Fluxes of Greenhouse Gases in an Argentinian Beef Cattle Livestock System

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Pasture-based livestock production offer the potential of fixing CO₂ into soil organic matter, eventually offsetting the simultaneous emissions through other greenhouse gases associated with animal production like CH₄ and N₂O. The objective of this work was to measure and compare the fluxes of CO₂ and CH₄ in a beef cattle livestock system typical of the Flooding Pampas, Argentina. Study was performed on a long-established cow-calf plus backgrounding livestock system located in Balcarce. Climate is temperate, humid and soils are an intricate pattern of Natraquolls and Natraqualfs. The herd (Aberdeen angus) grazes continuously on tall wheatgrass (*Thinopyrum ponticum*) pastures from August through April and is fed tall wheatgrass hay between May and July. Net fluxes of CO₂ and CH₄ between January 2017 and January 2018 were assessed through the eddy covariance method, with a system based on a Gill WindMaster Solent R3-50 tridimensional sonic anemometer (Gill Instruments, Lymington, UK), a LI-7500A open path infrared CO₂/H₂O analyzer and a LI-7700 open-path CH₄ analyzer (LI-COR, Lincoln, NE, USA). The livestock system was a source of CH₄ throughout the year, with an average emission rate of $9.8 \times 10^{-3} \mu\text{mol CH}_4 \text{ m}^{-2} \text{ s}^{-1}$. The CO₂ flux, in turn, reflected the effects of air temperature and of variations in soil water availability on pasture growth and carbon uptake. Overall, the slightly negative net CO₂ flux accumulated during the period suggests that the system was a very weak sink, with system net CO₂ uptake allowing to offset just above 20% of its emissions as CH₄. The study continues in order to assess the effect of interannual climate variability on the fluxes of greenhouse gases of this livestock system.

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Positive Impacts of Using Natural Additives in Mitigation of Methane 10 Years Ago

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The growing concern about the increase in greenhouse gases emission in recent decades brings the need to develop new management models for livestock. This condition aims to make the production system more efficient, profitable, and sustainable, resulting in less impact to the environment. The presence of additives in animal diets, can modulate ruminal fermentation, improve productivity, and contribute to mitigation of enteric methane emission to the environment. Fator P is a natural additive elaborate by a blend of amino acids, minerals, probiotics and essential fatty acids, developed to meet the new tends of the current market. Research in the last 20 years with this additive has shown that Fator P improved 15% animal performance, decreased 17% methane emissions, and decreased methane emission intensity, through improved animal performance. In other studies, using quantitative PCR assay, this additive reduced 64.8% ($p=0.0009$) the population of total methanogenic bacteria in the rumen. The aim of this study was to summarize Fator P cattle data generated in the last 10 years (2012 to 2021) and evaluate its effect on the environment. Considering that during this period, around 2 million animals in pasture were supplemented with Fator P, and based on our previous research, it can be estimated that a total of 775,655 tons of carbons equivalent were mitigated, which corresponds to around 57,000 ha forest area preserved ($\pm 1,667$ trees/ha in the Atlantic Forest biome). These results showed that the use of additives in animal diets can improve animal productivity and sustainability of livestock production.

Key words: Sustainability, Livestock, Greenhouse Gases, Methane.

Nitrous Oxide Emissions of Overseeding Aeschynomene into Bahiagrass Pastures in Florida

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Aeschynomene (*Aeschynomene americana* L.) is a warm-season annual legume forage adapted to subtropical regions and commonly cultivated in southern Florida grazinglands; however, there is limited information about nitrous oxide (N₂O) emissions of aeschynomene-bahiagrass (*Paspalum notatum* Flügge) mixed swards. The objective of this study was to quantify the effects of overseeding aeschynomene on forage characteristics and N₂O emissions. The study was conducted at the University of Florida - Range Cattle Research and Education Center, Ona, FL, from June to May 2019-2020 and 2020-2021. Treatments were the split-plot arrangement of overseeding aeschynomene or control (no legume, main plot) and N fertilization levels, 60 kg N ha⁻¹ or control (no N fertilization, sub-plot) distributed in a randomized complete block design with four replicates. Plots were overseeded and/or fertilized into an established bahiagrass pasture in April 2019 and 2020. Forage characteristics were evaluated 8-wk after seeding and every 35 d thereafter. Greenhouse gas emissions were collected from closed static chambers and N₂O concentration was determined by gas chromatography and linear interpolation. There was an overseeding strategy × harvest interaction on nutritive value and aeschynomene inclusion increased crude protein concentration in the last three harvests. Fertilized plots had greater N₂O-N emissions than the control, with 16.4 vs. 13.5 g N₂O-N ha⁻¹ d⁻¹, respectively. There was no effect of overseeding aeschynomene on herbage accumulation and N₂O-N emissions. Overseeding aeschynomene into bahiagrass pastures is an effective management practice to increase nutritive value without increasing N₂O emissions in Florida.

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In Vivo Measurement of Enteric Methane Emission from Local West African Zebu Cattle Using the Greenfeed System

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In West Africa, domestic ruminants, kept mainly in pastoral and agro-pastoral systems, due to their low productivity are major contributors of enteric methane emissions in the agricultural sector. Mitigation has become important for the sustainability of these production systems; however, the Tiers 1 and 2 estimates do not give the actual values of methane emitted in this area. The objective of this study was to improve the Tiers estimates by measuring *in vivo* enteric methane emissions from Sudanese zebu cattle in West Africa. The study was conducted at the research station of CIRDES based at Bobo-Dioulasso in western Burkina Faso. Ten steers aged approximately 24 months with an average live weight (LW) of 140.0 ± 14.9 kg (i.e., 0.56 Tropical Livestock Unit-TLU) were used. The animals were kept in a well-ventilated barn and equipped with a Greenfeed (GF) system (ID: 252, C-Lock, SD, USA) for gas monitoring. They were fed either hay of *Brachiaria ruziziensis* or *Andropogon gayanus*, both harvested at seed maturity stage. For each forage, two offered dry matter (DM) intake levels, 3.5% and 2.5% of LW were tested. The experiment lasted six weeks for each forage: two weeks of adaptation to GF, two weeks of adaptation to feed, one week for data collecting on 3.5% of LW DM offered and one week for collecting data on 2.5% of LW DM offered. The animals were taken to the GF at 06:30 am, 10:00 am, 02:00 pm and 06:00 pm during the first six days. On the seventh day of each trial, an additional night measurement was performed at 00:00 am. The one-way analysis of variance (ANOVA) was performed with R software (Version 4.1.2) to determine statistically differences between treatments. Results showed that enteric methane emissions were as expected higher with 3.5% than with 2.5% of LW DM offered ($p = 0.038$). Between the two forages, there was highly significant difference ($p = 0.008$) in intake level for 3.5% of LW DM offered but not ($p = 0.446$) for 2.5% of LW DM offered. Methane emission (g/day) significantly differed ($p = 0.010$) between forages at 3.5% of LW DM offered and tended to differ ($p = 0.058$) at 2.5% of LW DM offered. The overall annual enteric methane emissions in these animals were 39.9 ± 5.9 kg/TLU/year.

Key words: Enteric methane emission, Zebu, Grass hay, Feed intake, West Africa

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Enteric Methane Emission of Cattle Farming in Agricultural Systems with Cover Crops

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Integrated cover crops farming, and livestock sequencing systems are diversified agroecosystems that can contribute to ecological intensification, increasing food production, maintaining or improving environmental quality and preserving natural biodiversity. The objective of this study was to quantify enteric methane (CH₄) emissions and dry matter intake (DMI) in beef steers under two rotational grazing systems: i) mixture of cover crops (vetch + ryegrass + forage radish) included in a soybean-maize sequence and ii) alfalfa and fescue pasture. The experiment was carried out in the Mixed Systems trial located at the Pergamino Experimental Station of the Instituto Nacional de Tecnología Agropecuaria. Fresh samples from each treatment were taken regularly to determine chemical composition: crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF). Eighteen Angus steers (251 ± 22 kg) were randomly assigned to the 2 treatments (9 steers/group) (cover crops or pasture). The live weight gain (LWG) of steers was recorded every 15d. Enteric CH₄ was measured using the sulphur hexafluoride tracer technique during 5 consecutive days. Steers consuming cover crops generated 28% less enteric methane than those consuming pasture ($p < 0.05$) (119.1 ± 17.1 and 164.9 ± 28.1 g d⁻¹ for cover crops and pasture, respectively), while no difference was observed in averaged daily gain (ADG) between treatments (mean 1.1 kg d⁻¹). There were also no differences in CH₄ intensity, and the mean value was 142 g CH₄ kg⁻¹ ADG. Cover crops had higher concentration of CP (24.3 vs 17.3%) and lower concentration of NDF and ADF (36.2 vs 52.6, 19.4 vs 31.2 and for NDF and ADF, respectively) than pasture. In conclusion, mixture of cover crops improves animal performance and reduced environmental impacts. The data recorded in this experiment will be useful to evaluate the sustainability of agroecosystems that include livestock.

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On the Use of SF₆ Technique in Latin America for Measuring Methane Emissions in Ruminants

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The sulfur hexafluoride (SF₆) gas-tracer technique is especially useful for measuring CH₄ methane emissions in ruminants under grazing conditions and it has been used for in Latin America for conducting scientific research, primarily nutritional evaluations. Although guidelines on how to properly implement this technique have been published, the use of the SF₆ method is still often criticized due to a greater variation within measurements, when compared to results obtained either from respiration chambers or by the GreenFeed method. This may suggest that detailed evaluation of the SF₆ technique is worth conducting in order to identify the main sources of variability and minimize inconsistencies. The aim of this study was to provide a summary of key variables in this matter, reported in studies with the SF₆ technique conducted in Latin America. Data were obtained by searching a wide range of databases, and only papers published in English language peer-reviewed journals were considered for analysis. The final database comprised 63 studies, of which 38 were in grazing systems and the rest were in confinement. The categories evaluated were: beef cattle (40); dairy cattle (14); and small ruminants (9). The variables considered were: i) the duration of collection period (12h = 3%; 24h = 68%; 5 days = 29%); ii) the collection vessel (PVC yokes = 68% and stainless-steel cylinders = 29%); iii) inflow restrictor type (capillary = 65% and ball bearing system = 24%); and iv) the mean permeation rate of the SF₆ tubes, in mg/day (≤ 2 = 17%; 2 to 5 = 44% and ≥ 5 = 10%). Additionally, calibration times reported were within 4 to 17 weeks. The minimum time between the insertion of the SF₆ tubes into the rumen and the beginning of the measurement period was 3 days, while the maximum was 21 days. Overall, it is concluded that studies like these are important to identify weaknesses and complement efforts among research institutions in Latin America to get more reliable results when using the SF₆ tracer technique.

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Effects of Dietary Pomegranate Peel on Dairy Cows' Nitrogen Balance, Antioxidant Status, and Methane Emission

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This study aimed to quantify the effect of dietary inclusion of pomegranate peel (PP), rich in polyphenolic compounds such as tannins, on palatability, performance, nitrogen and methane emission in dairy cows. Four multiparous, late-lactating Brown Swiss dairy cows (796 ± 30 kg body weight (mean \pm SEM)) were randomly allocated to three treatments in an incomplete Latin Square design with three periods (14 days adaptation, 7 days milk, urine, and feces collection, and 2 days methane measurements in respiration chambers). The cows were fed a basal mixed ration (BMR) composed of maize and grass silage, alfalfa, concentrate, straw, and hay. Treatments were formulated using PP, replacing 0% (control), 5%, and 10% of BMR on a dry matter (DM) basis, and were tested randomly in each cow in the three periods. Plasma was obtained from blood collected from the jugular vein on day 15 of each period. Experimental data were analyzed by fitting linear mixed models using R. The PP and BMR contained 218 and 3.5 g total extractable tannins per kg DM, respectively, and thereof 203 and 3.3 g hydrolysable tannins. Feed intake, nutrient digestibility, and ECM were not affected by the inclusion of PP. Proportions of fat, protein, and lactose in milk did not differ among the treatments. Urea concentrations in milk and plasma, and urinary nitrogen excretion were lower ($P < 0.05$) in animals fed 10% compared to 0% PP. Absolute and relative methane emissions did not differ among treatments. Total antioxidant capacity and polyphenol concentration in plasma and milk were not significantly affected by PP intake. In conclusion, including up to 10% PP in DM does not affect palatability and performance of late-lactating cows and alleviates metabolic and environmental nitrogen load but does not mitigate enteric methane emissions.

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PAMA: Management of Grazing with the Potential to Mitigate Methane Emissions in Dairy Farms in Colombia

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The Pasture Enhanced Grazing by Altitude (PAMA) is based on improved pasture structure that modifies the animal's intake behavior, as a strategy that promotes higher forage intake under grazing and increases milk production with lower enteric methane emissions. The purpose of the study was to test two grazing management strategies in Kikuyu (*Cenchrus clandestinus*) pastures with Holstein cows: traditional rotational grazing (RT) with target pre-grazing pasture heights of 40 cm (high grazing intensity (stocking rate) with low grazing frequency (rest), and PAMA with pre-grazing pasture height of 25 cm (medium intensity, high grazing frequency).

Lactating Holstein cows were used. The experiment was conducted in November 2021 in the east of the city of Medellín (Santa Elena), Colombia. It was carried out in the experimental farm Paysandú of the National University of Colombia, located at 2400 meters above sea level, with an average temperature of 14°C and an average rainfall of 2500 mm per year, belonging to an ecological zone of humid forest - low Montane (bh - MB). The experimental design was a crossover, with two periods of 12 days each (seven days of habituation and five days of measurement), with five experimental units for each treatment. Ten multiparous Holstein cows with an average live weight (BW) of 531 kg were used, which were 119±10 days in lactation and with an average production of 33.1 L/day (liters per day). The experimental area was 1.9 hectares, divided into nine equal paddocks (1350 m²). Grazing management was based on a grazing regime of one 1350 m² plot per 2 days of occupancy. Pasture management was according to the proposed pasture heights for both treatments (PAMA, 25 vs RT=40 centimeters). The amount of forage in the paddocks was defined by grass growth using the double sampling technique and height was measured using a sward stick with 50 height measurements per paddock. During the sampling days, grazing consumption per animal was determined using chromium oxide as external marker and indigestible NDF as internal marker. Milk production per animal/day and its compositional quality were measured. Daily CH₄ emission was measured using the sulfur hexafluoride (SF₆) tracer technique. The SF₆ permeation tubes used had average permeation rates of 5.9029 mg/day. The tubes were deployed in the animals' reticulum (oral dosing), 20 days before the start of the measurements.

Consumption was higher ($P<0.05$) in PAMA (19.9) vs RT (16.6 kg DM/cow/day). The nutritional quality of kikuyo (*Cenchrus clandestinus*) pasture forage in both treatments was similar ($P>0.05$) for NDF, ADF, crude protein, organic matter and *in vitro* digestibility. Milk production was higher ($P<0.05$) in PAMA (33.6) vs RT (31.5 kg/cow/day), but milk compositional quality was similar (protein, fat, total solids, lactose). Enteric methane emissions were lower ($P<0.05$), in the PAMA treatment (356) vs RT (514 g/cow/day), which implies an efficiency of enteric CH₄ emissions of 10.6 g CH₄/day/kg milk for PAMA and 16.3 for RT. In conclusion, this study showed that PAMA as a grazing management strategy in kikuyo in Colombia, is a technological alternative that increased milk production and mitigated enteric methane emissions in dairy cattle in the high-altitude regions of the Andes.

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Evaluation of Enteric Methane Emission in Grazing Cattle Supplemented with Oat Plus Common Vetch Hay in the High Andean Zone of Peruvian Highlands

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The Peruvian Andean region has an average population of 3.7 million cattle raised mainly in extensive production systems, which are characterized by scarce and low-quality forages to feed the animals, this is directly associated with higher enteric methane emissions. Therefore, the objective of this study was to evaluate the effect of energy and protein supplementation with oat (*Avena sativa* L.) plus common vetch (*Vicia sativa*) hay on methane emission and live weight (LW) gain in the high Andean zone. The study was carried out in the Junín region (Peru) with 20 *Brown swiss x criollo* heifers in the growth stage (18 months) and an average starting weight of 267 kg. All animals were grazing native grasses and only half of the group received the supplement (1% of their live weight). The experiment was conducted as a 2x2 Latin square with adaptation periods to the diet and equipment of 12 days, plus 7 days of measurements. Enteric methane emissions were quantified through sulfur hexafluoride (SF₆) methodology, while feed intake was estimated from titanium dioxide (TiO₂), feed chemical composition and body weight gain were also evaluated. On average, the final weight of the heifers was 259 kg. The animals consumed 2.55 kg of oat plus common vetch hay (1% of the LW). Net methane emissions for heifers with and without supplementation were 182.5 and 187.6 g/day, respectively (*P-value* ≥ 0.05).

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Yield Gap Analysis to Identify Attainable Productivities and GHG Emissions Reductions in Colombian Cattle Systems

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Identifying sustainable strategies to mitigate greenhouse gas emissions (GHGE) will help the Colombian government meet their ambitious goal of a 51% reduction in national GHGE by 2030. Estimation of yield gaps for identifying the potential to improve cattle farms productivity and efficiency in Colombia help on reducing the GHGE intensities from the cattle sector. This study aimed to: (1) calculate the gap between attainable and actual milk and meat yields for specialized dairy, dual-purpose, cow-calf, and fattening production systems in 3 agro-ecological zones (AEZ) in Colombia; (2) identify the main aspects that restrict the meat and milk yields in these production systems; and (3) analyze how closing yield gaps affect the carbon footprint (CF) of meat and milk. The most suitable AEZ for cattle activities were identified by considering environmental, climatic, edaphic, and land characteristics. From a dataset of 1505 surveyed farms, a yield gap benchmarking analysis for estimating potential meat and milk yields increases in each of the identified AEZ was applied. The most productive farms were included in the “best farms” category while the rest of the farms were grouped into the “farms operating below potential” category. A “cradle to farm-gate” Life Cycle Assessment was used to calculate the CF. Three scenarios were proposed for closing the yield gaps by 50, 75, and 100%, between the two groups of farms. Three AEZs likely to support cattle activities were identified. Average milk production from the farms operating below potential was 45-50% of potential production, and meat was 34-51% below potential production, indicating that a potential to achieve increases in milk and meat productivity exists already. The CF of 1 kg milk or meat were lower in the groups of best-performing farms. Yield gaps for milk and meat production can be closed by improving cattle management practices and use of better technologies. Closing yield gaps significantly decreases CF.

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Integrating Sub-Saharan African Environmental Research for Transforming Livestock Systems: Challenges, Progress, and Future Research Priorities

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Livestock are an important source of livelihoods in agricultural systems in sub-Saharan Africa (SSA), while also being the largest source of national GHG emissions in most African countries. There is therefore a critical need to develop climate-smart solutions for livestock-based systems in SSA based on baseline measurements and intervention testing to mitigate GHG emissions from these systems. Our objective was to compile a comprehensive review of studies measuring or estimating GHG emissions livestock systems in SSA to evaluate existing data and guide future research and policy. We compiled studies based on literature searches and interviews with experts working on livestock emissions in SSA. Data on livestock emissions were divided into enteric methane and manure based on IPCC guidelines. Despite the importance of livestock emissions, we found that there has been limited research measuring livestock GHG emissions in SSA. The results indicated that enteric methane emission factors (EFs) in low productivity cattle systems were consistently lower than IPCC Tier 1 default EFs, which could lead to overestimation of enteric emissions for cattle in SSA using IPCC Tier 1 methods. For small ruminants, EFs were higher than IPCC Tier 1 EFs, but this may be due to inclusion of high productivity systems in South Africa. Manure EFs were substantially lower than IPCC Tier 1 default EFs for all three categories of manure emissions evaluated (manure deposited while grazing, manure applied as fertilizer, and manure management). For enteric methane, there were large data gaps for small ruminants, agropastoral/pastoralist systems, and West and Southern Africa. We found very few studies on manure in general and only one study on emissions from manure management. In addition to filling these data gaps, future research should focus on locally appropriate GHG mitigation interventions and improving livestock activity data for developing Tier 2 GHG inventories in SSA.

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Understanding the Diverse Impacts of Methane Inhibitors on the Rumen Microbial Community

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It is essential to understand the impact that different methane inhibitors have on the rumen microbial community. Here we have examined the effects of supplementing a high-forage diet with the investigational methane (CH₄) inhibitor 3-nitrooxypropanol (3-NOP) and canola oil (OIL) on the rumen microbiome of beef cattle, enteric CH₄ emissions, and ruminal fermentation. 3-NOP and OIL individually reduced enteric CH₄ emission (-28.2% and -24.0%, respectively), and the effects were additive when used in combination (-51.3%). 3-NOP increased H₂ emissions 37 fold, while co-administering 3-NOP and OIL increased H₂ in the rumen 20-fold relative to the control diet. The inclusion of 3-NOP or OIL reduced the microbial diversity of the rumen microbiome. 3-NOP resulted in targeted changes in the microbiome decreasing the relative abundance of *Methanobrevibacter* and increasing the relative abundance of *Bacteroidetes*. The inclusion of OIL resulted in large scale changes to the microbial community, ruminal volatile fatty acid concentration and gas production. OIL significantly reduced the abundance of protozoa and fiber-degrading microbes in the rumen but it did not selectively alter the abundance of rumen methanogens. The addition of OIL to the diet resulted in a large decrease in the number sequences attributable to *Fibrobacter* (41-243 fold decrease; $P < 0.001$) in both rumen fluid and digesta. These data show that 3-NOP specifically targeted rumen methanogens inhibiting the hydrogenotrophic methanogenesis pathway and increased H₂ emissions and propionate production. OIL caused large scale changes in the rumen microbial community by indiscriminately altering the abundance of a range of rumen microbes, reducing the abundance of fibrolytic bacteria and altering rumen fermentation. Our data suggests that co-administering CH₄ inhibitors with distinct mechanisms of action can enhance CH₄ inhibition and provide alternative sinks to reduce the accumulation of ruminal H₂.

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Forage Intake and Methane Emissions of Romosinuano Steers in the Caribbean Savannah of Colombia

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Beef production systems located in Colombian humid Caribbean region (Córdoba) are mostly based on tropical grasslands. Both dry matter intake (DMI) and methane (CH₄) emissions are important variables to be considered for improving grazing management and consequently enhance animal productivity. The aim of this descriptive study was to measure DMI, daily weight gain (DWG), total CH₄ emissions (CH₄, g/day), CH₄ yield (CH₄/DMI, g/kg), CH₄ intensity (CH₄/DWG, g/kg), and gross energy losses as CH₄ (Y_M) of eight beef creole Romosinuano steers (297 ± 26.1 kg BW), under a rotational grazing in a silvopastoral system with arboreal species for shadow. Grazing paddocks were characterized by heterogeneous grass species, mainly *Dichanthium aristatum*, *Brachiaria arrecta* x *Brachiaria mutica*, and *Panicum maximum* cv Mombasa. Animals had an adaptation period of 30 days to get used to manipulation, and an experimental period lasting for 17 days comprising 12 days for dosage of chromium oxide (10 g/day), as an external marker, and the last five days of fecal sample collection taken directly from the rectum. Enteric CH₄ emissions were measured during the last three days of the experimental period by using a hand-held laser CH₄ detector, six times/day (from 05:00h to 21:00h), with five-minute measurements for each animal. Dry matter intake was 7.8 ± 1.43 kg/day, representing 2.63% of BW. Total CH₄ production was 118 ± 49.1 g/day; CH₄ yield 15.7 g/kg; CH₄ intensity 233 ± 134.3 g/kg; and Y_M 3.87 ± 1.86 %. There was a positive correlation between CH₄ yield and CH₄ intensity (0.46; $P < 0.02$). Calculated emission factor according to IPCC (2016) was 53.4 ± 23.7 kg of CH₄/head/year. This is the first study measuring CH₄ emissions of Romosinuano cattle in their native ecosystem. Overall, values are lower than expected for beef cattle in these conditions.

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Methane Emissions from Alpacas under Grazing Conditions during the Dry Season in the Peruvian Andes

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Although methane (CH₄) emissions is a major issue in domestic ruminants due to their contribution to the greenhouse effect and to represent a significant dietary energy loss, much less is known related to South American camelids, especially when considering nowadays climate change scenarios. The objective of this study was to compare enteric CH₄ production from two varieties of alpacas (Huacaya and Suri; n= 14), in a late pregnancy stage with an average body weight (BW) of 56.4 ± 6.0, maintained under grazing conditions at high altitude in the Peruvian Andes. The pasture offered to the animals was a mixture of approximately 70% perennial ryegrass, 20% of legumes (mainly alfalfa and red clover) and 10% herbs (chicory and plantain). Forage availability was on average 200 g/kg DM per square meter. Dry matter intake (DMI) was estimated from total fecal production assessed by fecal collection bags at an individual animal level. Diet indigestibility was obtained by *in vitro* DM incubation. Diet selectivity on pasture was evaluated by using the hand-plucking method. Collected forage was taken to the laboratory for macroscopic analysis of the functional groups (e.g. grasses, legumes, others), part plants and phenological stage. Samples were dried and grounded for further chemical composition analyses. Methane emissions were assessed by the SF₆ tracer technique alongside with DMI estimates during the last five days of the experimental period (21 days). Data were analyzed as a completely randomized designed with varieties considered as treatments. No differences were detected among the tested variables ($P > 0.10$). Mean DMI was 1418 ± 402 g/d, total CH₄ 32.0 ± 7.26 g/d, and CH₄ yield 24.9 ± 9.47 g/kg DMI. To the best of our knowledge, the present study is one of the first attempts to measure enteric CH₄ emissions in alpacas under cultivated pasture at the Andean highlands.

Key words: methane emissions, SF₆ technique, South American camelids.

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Six Macroalgae Harvested Along the California Coast and Their Ability to Mitigate Methane Emission by a Forage Diet

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Because enteric methane emission is of the utmost concern to purveyors of sustainable ruminant livestock systems, marine scientists in California selected and harvested 6 macroalgae that might mitigate enteric methane emission when supplemented to forage-fed cattle. *Asparagopsis taxiformis*, was harvested near Santa Catalina Island, and *Gracilaria parvispora*, *Gracilaria andersonii*, *Codium fragile*, *Acrosiphonia coalita*, and *Devaleraea mollis* were harvested along the central coast, freeze dried, ground to pass a 2-mm screen, and added to a timothy hay diet at 2% of dry matter in a randomized complete block design (2 rep/block and 4 blocks). Timothy hay and the 6 hay/macroalgae diet combinations were incubated (39°C) in an *in vitro* gas production system to measure enteric methane production with the bottles containing a solution of 3.44:1 of artificial saliva and ruminal fluid. Methane production was measured for 48 hours, logging the time of each 2-mL emission and then data were fit to a nonlinear equation, $G = A_0(1 - e^{-k_e(t-T_{Lag})})$ where A_0 is the asymptote (g), k is the rate constant (g/hour), and T_{Lag} is the time (hours) to initial emission. Equations developed for the timothy hay and each diet combination were used to predict the total enteric methane emission for a 321-kg steer consuming 3.3 kg of timothy hay/day with a ruminal retention time of 11.4 hours. When the steer consumed only hay, it produced 96 g of methane/day ($A_0 = 21.5$ g, $k = 0.0951$ g/hour, and $T_{Lag} = 5.7$ hours). In contrast, when the steer consumed the diet with 2% *A. taxiformis* ($A_0 = 9.2$ g, $k = 0.0845$ g/hour, and $T_{Lag} = 10.0$ hours), it produced 81% less ($P < 0.05$) methane (18 g/day) compared to hay alone. Methane production from the diets containing *G. parvispora* ($A_0 = 20.1$ g, $k = 0.0954$ g/hour, and $T_{Lag} = 6.1$ hours), *G. andersonii* ($A_0 = 19.6$ g, $k = 0.0970$ g/hour, and $T_{Lag} = 6.3$ hours), *C. fragile* ($A_0 = 19.4$ g, $k = 0.0935$ g/hour, and $T_{Lag} = 6.4$ hours), and *A. coalita* ($A_0 = 19.6$ g, $k = 0.0949$ g/hour, and $T_{Lag} = 6.8$ hours) did not differ ($P > 0.05$) from the hay diet. The diet with 2% *D. mollis* ($A_0 = 19.4$ g, $k = 0.0964$ g/hour, and $T_{Lag} = 6.8$ hours) tended ($P = 0.09$) to produce 23% less methane (73 g/d) compared to the hay diet. In this experiment, a wild source of *A. taxiformis* was effective at mitigating enteric methane emissions, but *D. mollis*, as a fast-growing farmed species, showed promise as a moderate enteric methane mitigator.

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Frequent Removal of Slurry Reduces Methane Emission from Pig Houses

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Frequent removal of slurry from pig houses is one of the simplest ways to reduce methane emissions from pig production. Combining with anaerobic digestion or other mitigation strategies in the outside storage, a substantial effect on methane emission can be achieved. The objective of the current study was to investigate the effect of different slurry systems for frequent removal of slurry on the methane emission from pig houses. Four experimental units with 30 growing-finishing pigs (30-110 kg) and fully slatted floor were equipped with one of four slurry systems. 1) Control: slurry channel with vacuum flushing two times during the production period, 2) Frequent vacuum flushing: slurry channel with vacuum flushing once a week, 3) Slurry funnels: funnel based slurry system with flushing three times a week, and 4) Slurry trays: slurry trays with back flushing and removal of slurry once a week. Methane was measured continuously over four periods of 11 weeks using cavity-ring-down spectroscopy. Furthermore, air exchange and climatic conditions inside and outside were measured. The results showed that the total methane emission, including contributions from both slurry and enteric fermentation, was reduced by ca. 40% in the pig house with frequent vacuum flushing compared to the control pig house. In the pig houses with slurry funnels and slurry trays, the methane emission was reduced by ca. 60%. Because enteric methane is not affected by removal frequency, the effect on methane originating from the slurry is considerably higher. In conclusion, frequent removal of slurry has a significant effect on the methane emission from pig houses. For slurry systems with low amount of residual slurry after its removal, such as slurry funnels and slurry trays, the main part of the remaining methane emission originates from enteric fermentation.

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Differences in Enteric Methane Emissions Across Lactations

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A total of 105,701 methane (CH₄) spot measurements were obtained from 2,436 dairy cows on 18 farms in the UK. Eleven farms fed a partial mixed ration (PMR) and 7 farms fed a PMR with grazing. Cows were mostly Holstein-Friesian with an average milk yield of 30.4 kg/d from 2.4 milking visits to robotic milking stations per cow. Methane concentrations in breath (ppm) were measured at 1s intervals during each milking for seven days using an infrared CH₄ analyser (Guardian Plus; Edinburgh Instruments, Livingston, UK). Methane data were extracted from the time-series signal using Signal Processing in MatLab to identify and measure eructation peaks. Emission rate (g/min) for each milking was derived from maximum peak amplitude (ppm)/ [1- EXP(-(peak rise for amplitude in seconds /60))] x 60 x 0.706 x 10⁻⁶. Data were analysed using GenStat 20th Edition, (VSN, Hemel Hempstead, UK). A Spearman rank correlation and a multiple experiment meta-analysis model were used to assess differences in CH₄ emissions among cows, week of lactation (1 to 70) and parity (1 to ≥5). Herd average CH₄ emission rate differed among the 18 farms (overall mean 0.37 ± 0.12 g/min; range 0.20 to 0.63). Methane emission rate increase to 0.40 g/min at week 10 of lactation, and was steady until week 70. Methane emission rate declined with increasing parity. Rank correlation for CH₄ emission rate among weeks of lactation was generally high, with 83% of rank correlations being >0.5. Profile of CH₄ emission rate during a lactation appears consistent among herds. We conclude that a high CH₄ herd is consistent across lactation, which is useful for evaluating spot measurements.

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Screening of Candidate Metabolite Ion-features in *Moringa oleifera* Associated with High and Low Enteric Methane Inhibition from Ruminants

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Moringa oleifera accessions were characterized in terms of bioactive plant secondary metabolite m/z ion-features (MIFs) and evaluated the relationship with anti-methanogenesis to identify potential MIFs that were responsible for high and low methane inhibition from ruminants. Plant extracts from 12 *Moringa oleifera* accessions were evaluated at 50 mg/kg DM feed for gas production and methane inhibition. Subsequently, the accessions were classified into low and high enteric methane inhibition groups. The MIFs of four out of 12 accessions (two lowest and two highest methane inhibitors), were characterized. A total of 24 samples were selected according to their methane reduction potential, which ranged from 18% to 29%. Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) and untargeted metabolomics with univariate and multivariate statistical analysis with MetaboAnalyst were used in the study. Although 86 MIFs showed ($P < 0.05$) variation between higher and lower methane inhibition groups and lay within the detection ranges of the UPLC-MS column, only 14 were significant with a volcano plot. But Bonferroni correction reduced the candidate MIFs to 10, and their R^2 -value with methane production ranged from 0.39 to 0.64. Eventually, MIFs 4.44_609.1462 and MIF 4.53_433.1112 were identified as bioactive MIFs associated with high methane inhibition, whereas MIF 9.06_443.2317 and 15.00_487.2319 were associated with low methane inhibition with no significant effect on *in vitro* organic matter digestibility of the feed. These MIFs could be used by plant breeders as potential markers to develop new *Moringa oleifera* varieties with high methane inhibition characteristics or for standardisation to develop a consistent product. However, further investigation into identifying the name, structure and detailed biological activities of these bioactive metabolites needs to be carried out for future standardization, commercialization and application as dietary methane mitigation additives.

Keywords: methane inhibition, plant secondary metabolite, m/z ion-features

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Experimental Facility to Study Climate-House-Animal-Manure Interactions

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Sustainable improvement of animal housing requires simultaneous control of animal performances, gaseous emissions and manure production. We considered that for long-term use of datasets, minimal stoichiometry data should include knowledge of mass and speciation of carbon, nitrogen, hydrogen and oxygen: optimal feed index; optimal carbon output as CO₂ and soil storage; optimal nitrogen output as N₂ and plant fertilizer; optimal hydrogen output for energy production; optimal oxygen output as H₂O and CO₂. A pilot-scale laboratory was designed and built to measure accurately the mass budget, the energy consumption and gaseous emissions of animals reared in commercial conditions with repeatable climate, animal housing, breeding equipment, rearing practices, and manure management. Six “houses” with 5 m² rearing area were installed in a controlled climate environment. Temperature outside the houses can be selected between -10 °C and +40 °C. Hygrometry outside the houses can range between 30 and 80% when temperature is above 12°C. Houses are naturally ventilated depending on opening size and air density difference between inside and outside climate (pressure differences less than 5 Pa). Therefore, house fluxes only result from animal and manure sensible heat and water vapor production to minimize house energy input. A small heating can be required to compensate for wall heat losses. Ventilation can vary between 3 and 200 m³ h⁻¹ m⁻². Energy flux detection level and measuring accuracy was around 1 W per house (0.2 W m⁻²). Measurement variability of water content in manure was above 3% because of difficulties to get representative samples and because of non-water volatile compounds lost during sample drying. Mass budget of C, hydrogen, oxygen and nitrogen including the gaseous emissions showed the oxygen input. Energy budget showed the importance of taking into account separately the specific heat of dry air and water vapor in inlet and outlet air.

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How to Define the Quality of Measured Emissions Values

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The last years, new databases of international manure emission values have been developed as ELFE (VIGAN et al, 2019) and DATAMAN (Beltran et al., 2020). When collecting these values and all metadata, two main questions rapidly emerged: 1) the definition of data quality, and 2) how to evaluate and improve them. The definition of the quality of the emission values goes far beyond the uncertainty associated with the measurements itself. The quality must be evaluated by considering the objectives of measurement and the different steps of the quantification (measurements, data processing, calculations) and reporting processes. We will present the analysis of emission values from livestock housings that we have carried out for illustration purposes. We will first discuss the minimum accuracy requirements for the different kind of measurement objectives. Then we will present the checkpoints for the different steps, which relevant for the quality of the final emission value reported and we propose recommendations to optimize the quality. The final objective is to propose an evaluation method based on information concerning these checkpoints given in papers and weight attributed to checkpoints in function of objectives and context.

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Effects of Feeding Biochar to Finishing Beef Cattle on Enteric Methane Production

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Two experiments were conducted to evaluate impacts of biochar on methane (CH₄) and carbon dioxide (CO₂) emissions, performance, and carcass characteristics of finishing beef cattle. A food use authorization (FDA) was granted as biochar is not approved for feeding to beef cattle. Experiment 1 (initial BW = 329 kg ± 19 kg) utilized biochar sourced from pistachio shells (VGrid Energy Systems) in a 70% corn, 20% distillers grains, and 5% corn residue diet. Experiment 2 (initial BW = 386 kg ± 19 kg) utilized biochar sourced from ponderosa pine wood waste (Vital Ag) in a 40% corn, 40% Sweet Bran, and 15% corn silage diet. Both experiments evaluated 2 treatments: control (CON) diet containing no biochar and biochar (BIO) replacing 1% of dietary corn. Pen was the experimental unit with 8 steers per pen and 8 replications. Four replications rotated through a two-chamber emissions barn in 5-day cycles to measure CH₄ and CO₂ emissions. Cattle performance data were analyzed using the MIXED procedure of SAS with treatment and body weight block as fixed effects and emissions data analyzed as repeated measure. Feeding biochar did not impact either CH₄, in g/day ($P = 0.75$) and g/kg of dry matter intake (DMI; $P = 0.99$) in experiment 1 or CO₂ as g/day ($P = 0.94$) and g/kg DMI ($P = 0.88$). No impact on cattle performance or carcass characteristics was observed ($P \geq 0.21$). In experiment 2, there were no statistical differences between treatments for CH₄, g/day ($P = 0.78$) and g/kg DMI ($P = 0.84$) or CO₂, g/day ($P = 0.50$) and g/kg DMI ($P = 0.52$). Supplementing biochar at 1% of diet DM did not impact eructed CH₄ or respired CO₂ by finishing beef cattle. Cattle performance and carcass characteristics were not impacted by feeding biochar.

Keywords: biochar, carbon dioxide, methane

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CO₂ and CH₄ Emissions from Manure in Respiration Chambers are Negligible Compared to Cow's Emissions

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Carbon dioxide and methane measured in respiration chamber include the emissions from manure deposited in the chamber. The aim was to quantify emissions of CO₂ and CH₄ from manure left in respiration chambers for the first 24 hours after cows left the chambers. Manure from dairy cows in our four identical respiration chambers is removed twice daily, and for the current experiment manure deposited during the last 12 h before exit was left in the chambers. Air flow rate was 2000 L/min, equivalent to the flow used in preceding cow experiments. The CO₂ and CH₄ emissions were measured in two experiments (A and B). In experiment A, emissions related to feeding 2 diets were measured 4 times and in experiment B, emissions related to feeding 6 diets were measured 3-5 times. Data from experiments were analyzed separately. The model included diet, period and chamber as fixed effects (PROC GLM, SAS). Carbon dioxide and CH₄ emissions solely from manure were 26.7±11.0 (mean ± sd) and 0.22±0.09 L/day in experiment A, and 16.2±10.6 and -0.13±0.31 L/day in experiment B. There was no effect of diet on emissions. There was an effect of chamber except for CH₄ in experiment A. While cows were in the chambers, total CO₂ and CH₄ emissions were 7566±848 and 544±67 L/day for experiment A, and 7389±1163 and 490±88 L/day for experiment B. Effect of chamber could be caused by small differences in background concentration of CO₂ and CH₄ between chambers. For each PPM of difference in background air between chambers cause a difference of 2.8 L at a flow of 2000 L/min per 24 hour. In conclusion, emission of CO₂ from manure in chambers is minor, and emissions of both CO₂ and CH₄ are negligible compared with those from cows.

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Effects of a Mixture of Tannins and Saponins on Methane Production, Intake, and Nutrient Digestibility

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A replicated complete randomized designed was used to determine the effects of different doses of a mixture of tannins and saponins on performance, dry matter intake (DMI), and enteric methane emissions. It was hypothesized that as the dose of tannins increases within treatment, CH₄ emissions will be decreased without negatively affecting dry matter intake (DMI). Eighteen angus-crossbred steers (235 ± 18 kg BW) were assigned to 1 of 3 treatments: 1) no additives (0 g of tannins and saponins; CTRL), 2) low concentration of tannins (7.5 g of tannins and saponins additive; LOW-TANN); and 3) high concentration of tannins (15 g of tannins and saponins additive; HIGH-TANN). Cattle were adapted to treatments for 14-d followed by 6-d of collections. During the collection period, CH₄ emissions were measured using the SF₆ tracer technique. During the trial intake and orts were recorded daily to measure DMI. After collection of samples, a 35-d washout period followed, and treatments were reassigned for a second period. Data were analyzed with the MIXED procedure of SAS, with the fixed effect of treatment and random effect animal within treatment. Animal was considered the experimental unit. Preliminary data from the first period showed no significant effects ($P \geq 0.207$) of treatment on DMI, performance (final BW and ADG), and enteric CH₄ emissions. Although no significant results were observed for the measured parameters, DMI and CH₄ emissions (g/day) had low standard error of the mean (0.191 kg and 6.800 g/day, respectively). Therefore, there is the possibility that the HIGH-TANN treatment can decrease DMI and CH₄ emissions when compared to CTRL and LOW-TANN. Data from the second trial is needed to elucidate the effects of the HIGH-TANN dose on reducing enteric CH₄ emissions.

Keywords: methane, protein digestibility, tannins

Gas Exchanges and Dry Matter Intake When Lactating Dairy Cows Are Fed 3-NOP and Fat

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It is well-known that fat and 3-NOP (Bovaer) reduce enteric methane production from ruminants. The aim of the study was to investigate the interaction effect between fat (33 g or 64 g crude fat kg⁻¹ DM) and 3-NOP (0 or 80 mg kg⁻¹ DM) in a 2×2 factorial arrangement. Four lactating multiparous Danish Holstein dairy cows were used in a balanced 4×4 Latin square design. Gas exchanges were measured in respiration chambers. All four cows were multi-cannulated with a rumen-, duodenum- and ileum cannula, as digestibility measures were collected as well. Whole-cracked rapeseeds were used as the fat source. Cows were fed the TMR ad libitum, and dry matter intake (DMI) was measured. The NDF content was 293 g kg⁻¹ DM and 282 g kg⁻¹ DM for TMR low and high in fat content, respectively. The DMI was reduced when cows were fed the 3-NOP diets (P=0.008), but increased fat content did not reduce the DMI (P=0.54). Increased fat content did not reduce methane production in L kg⁻¹ DMI (P=0.29), while 3-NOP reduced the methane production in L kg⁻¹ DMI by 25 % (P<0.001). No interaction between fat and 3-NOP was found on methane production in L kg⁻¹ DMI (P=0.45). Hydrogen production in L kg⁻¹ DMI increased when 3-NOP was fed (P<0.001) by 3300 %. No significant effect of fat was found on hydrogen production (P=0.98), and neither on the interaction of fat and 3-NOP (P=0.90). 3-NOP increased the carbon dioxide production (P=0.009) and oxygen consumption (P=0.005) in L kg⁻¹ DMI. In conclusion, there was no interaction of fat and 3-NOP on the methane production. The effects of fat were only numeric, while 80 mg 3-NOP kg⁻¹ DM reduced the methane production, reduced the dry matter intake and increased the hydrogen production.

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Predicting CO₂ Production from Lactating Dairy Cows Based on an International Dataset

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Large scale quantitative measurement of enteric CH₄ production from cattle is difficult, time consuming and costly with respect to equipment needed. However, combining measured breath CO₂:CH₄ concentration ratio and predicted CO₂ production is a potential proxy for CH₄ production. In the current study, predictions of CO₂ production were developed using an international data set of 1502 individual lactating cow observations of CO₂ production and associated traits. Production of CO₂ was recorded using either GreenFeed or respiration chambers, but method was confounded with research location, and therefore, no distinction was made. Variables for model development were as follows; continuous variables were dry matter intake (DMI), energy corrected milk yield (ECM), body weight (BW) and days in milk (DIM). Parity was a categorical variable. Stepwise regression with CO₂ production as the dependent variable was used to identify which variables contributed the most to the variation in CO₂ production. Based on the results from stepwise regression, retained variables were used in a mixed model with research location as random effect. The resulting equation: $\text{CO}_2 \text{ (L/day)} = -0.30 \times \text{DIM} + 23.6 \times \text{BW}_{(\text{kg})}^{0.75} + 176 \times \text{DMI}_{(\text{kg/day})} + b$, where b is -106 , -39.8 , -26.0 for 1st, 2nd and greater than 2nd parity cows, respectively ($R^2_{\text{marginal}} = 0.59$, $R^2_{\text{adjusted}} = 0.76$). Simultaneously a model that can be used at farm level was developed, with predicting variables reasonable for a farmer to obtain: $\text{CO}_2 \text{ (L/day)} = 0.92 \times \text{DIM} + 62.2 \times \text{ECM}_{(\text{kg/day})} + 35.4 \times \text{BW}_{(\text{kg})}^{0.75} + b$, where b is -171 , 11.8 and -33.7 for 1st, 2nd and greater than 2nd parity cows, respectively ($R^2_{\text{marginal}} = 0.41$, $R^2_{\text{adjusted}} = 0.68$). In conclusion, DMI described most of the variation in CO₂ production; however, farmers typically do not have this information. Therefore, metabolic body weight, ECM, DIM and parity are more practical variables for predicting individual CO₂ and potentially CH₄ production from lactating dairy cows on commercial farms.

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Evaluation of *Cymbopogon winterianus* (Lemongrass) for its Potential to Mitigate Enteric Methane Emissions

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The current experiment assessed the anti-methanogenic potential of a species of lemongrass, *Cymbopogon winterianus* (CW), sourced from California's Central Valley as a feed additive for beef steers. Lemongrasses contain tannins and essential oils, which have been investigated for their broad effect on rumen function and potential to suppress enteric methane (CH₄) emissions. A randomized complete block design was employed using 20 Angus x Hereford steers that were blocked in pairs based on initial bodyweight and randomly assigned to one of the two treatments: 1) basal diet only (n=10; CO) or 2) basal diet + CW included at a rate of 2% DM (n=10; TR) for 10 weeks. Baseline CH₄, carbon dioxide (CO₂) and hydrogen (H₂) emissions were measured using GreenFeed technology before lemongrass was added to the diet of the TR group followed by gas readings at 6, 8, and 10 weeks of treatment inclusion. Data was tested for normality and analyzed using the 'nlme' package in R statistical software. Steer nested in block was used as the random effect in the model and fixed effects included treatment, week, and the baseline response (CH₄) data where appropriate. Lemongrass inclusion did not affect DMI, CH₄ production, yield, or intensity at weeks 6, 8, or 10 ($P > 0.05$). However, the CO group had an overall tendency to emit less emissions compared to the CW group on a production, yield, and intensity basis during the trial which might be due to fiber content differences between groups. The lemongrass used for this experiment did not contain adequate levels of secondary compounds compared to other lemongrass species to elicit a reduction in CH₄ but had a high-cellulose content, which encouraged methanogenesis. This study highlights the importance of growth conditions for naturally grown additives and establishing thresholds for secondary compound concentrations.

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Enteric Methane Emissions of Tibetan Sheep Vary with Level of *Ligularia virgaurea* Supplementation

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Ligularia is the most widely distributed undesirable plant in the alpine grassland. Therefore, there is a high chance of this plant being consumed by livestock. The aim of the study was to determine the effect of *L. virgaurea* on methane (CH₄) emissions. In Qinghai-Tibet Plateau, thirty-two Tibetan sheep lambs (29 ± 1.37 kg body weight (BW)) were fed freshly native pasture, cut and weighed each day from 30 ha of *L. virgaurea*-free fenced alpine meadow. The treatments contained 0, 100, 200 and 300 mg/kg BW per day *L. virgaurea*. Individual daily CH₄ emissions were estimated using the sulfur hexafluoride (SF₆) tracer gas technique together with measurements of daily dry matter intake (DMI). Addition of *L. virgaurea* to the diet of Tibetan sheep was found to influence DMI ($P = 0.026$), CH₄ emissions ($P = 0.017$), and CH₄ emissions of per unit of feed DM ($P = 0.009$). A 100 mg/kg BW addition of *L. virgaurea* to the diet decreased CH₄ emission rates without reducing DMI. In conclusion, this result investigated the potential of *L. virgaurea* as a natural addition agent for the mitigation of CH₄ emissions for the Tibetan sheep industry.

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Grazing Management in Salinized Meadow: Enteric Methane Emissions of Crossbred Simmental Calves

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Salinized meadow is a natural grassland with low productivity of cultivated crops in the world. Therefore, improving the productivity of salinized meadow is a major problem. A reasonable stocking rate is the main means to promote the coupling of forage-livestock systems and optimize the production mode of the grassland system. Therefore, the research aimed to explore the greenhouse gas emissions of livestock from grazing in salinized meadows. Twenty-four 3 months male calves (115 ± 20 kg BW) (Simmental) were assigned to 3 grazing rates that contained 0.44 calves/ha, 0.67 calves/ha, and 1.33 calves/ha to determine the effect of salinized meadow on calves' methane emissions. The methane (CH_4) emissions were measured by infrared absorption-based gas analyzer. Compared with the other groups, the grazing rate of 1.33 calves/ha increased CH_4/DMI . The CH_4 emission factors (Y_m) of 0.44 calves/ha and 0.67 calves/ha were significantly lower than 1.33 calves/ha. These results provide information about ruminant production in salinized meadow, and can help in establishing appropriate grazing and management strategies. Appropriate grazing rate can reduce CH_4 emissions per unit intake. Reasonable grazing methods can increase the utilization rate and balance grassland development, and promote the sustainable development of grassland resources.

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Effective Nutritional Strategies to Mitigate Enteric Methane in Dairy Cattle

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Intensive research in the past decade has resulted in a better understanding of factors driving enteric CH₄ emissions in ruminants. Methane inhibitors, alternative electron sinks, vegetable oils and oilseeds, and tanniferous forages are among the recommended strategies for mitigating CH₄ emissions from dairy and beef cattle and small ruminants. These strategies were also effective in decreasing CH₄ emissions yield and intensity. Higher inclusion rate of oils may negatively affect feed intake, rumen function, and animal performance, specifically milk components in dairy cows. In the case of nitrates (electron sinks), concerns with animal health may be impeding their adoption in practice. Tannins and tanniferous forages may also have a negative impact on nutrient digestibility and more research is needed to confirm their effects in long-term experiments with high-producing animals. A meta-analysis of studies with dairy cows fed the CH₄ inhibitor 3-nitrooxypropanol (3-NOP) at The Pennsylvania State University showed: (1) a consistent 28 to 32% decrease in daily CH₄ emissions or emissions yield and intensity; (2) no effect on dry matter intake, milk production, and body weight and body weight change and a slight increase in milk fat concentration and yield (0.19%-units and 90 g/d, respectively). 3-NOP also appears to increase milk urea nitrogen concentration; (3) an exponential decrease in the mitigation effect of the inhibitor with increasing dose (40 to 200 mg/kg feed dry matter, corresponding to 3-NOP intake of 1 to 4.8 g/cow/d); and (4) potential decrease of the mitigation effect of 3-NOP over time, which needs to be further investigated in long-term, full lactation or multiple-lactation studies. It is concluded that widespread adoption of mitigation strategies with proven effectiveness by the livestock industries will depend on cost, government policies and incentives, and willingness of consumers to pay a higher price for animal products with decreased carbon footprint.

Key words: enteric methane, mitigation, 3-nitrooxypropanol, dairy cow

Synergistic Effects of Natural Hydrogen Acceptors and a Methanogenesis Inhibitor on *In Vitro* Rumen Fermentation

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Inhibiting rumen methanogenesis often causes hydrogen accumulation, potentially negatively affecting the rumen fermentation process. We hypothesized that the use of hydrogen acceptors when methanogenesis is inhibited would reduce hydrogen accumulation and improve production of useful fermentation end-products. In this study, phenol, catechol, resorcinol, hydroquinone, pyrogallol, phloroglucinol, and gallic acid were examined as hydrogen acceptors by *in vitro* batch culture. Three experiments were performed: in experiment 1 (Exp.1), 0, 2, 4, and 6 mM of each phenolic compounds were examined to find their optimum concentration; in experiment 2 (Exp.2), each phenolic compound at its optimum concentration obtained from Exp.1 was combined with 2-bromoethanesulfonate (BES) at 3 μ M as a methanogenesis inhibitor; in experiment 3 (Exp.3), the long-term effects of phloroglucinol (36mM) with or without BES (3 μ M) were examined by sequential incubation. The use of 6 mM phenolic compounds in Exp.1 did not negatively affect volatile fatty acid (VFA) production, ammonia production, and total gas production. Hence, 6 mM of each phenolic compound was used in Exp.2. In Exp.2, phloroglucinol, gallic acid, and pyrogallol combined with BES significantly increased acetate production by 47%, 39%, and 36%, respectively. Whereas methane production was reduced by 75%, none of the phenolic compounds decreased hydrogen accumulation in the early stages of fermentation. In Exp.3, after three sequential incubations, phloroglucinol combined with BES decreased hydrogen accumulation by 72% and further inhibited methanogenesis as compared to BES alone. Interestingly, phloroglucinol alone significantly decreased methane production by 99% with just a numerical increase in hydrogen accumulation compared to control. Also, phloroglucinol with or without BES increased total VFA, acetate, and total gas productions and decreased ammonia production. Our results confirmed the potential of phloroglucinol to capture excess hydrogen and redirect it towards VFA production. These findings will further be evaluated *in vivo*.

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Accounting for Agricultural Greenhouse Gas Emission Reductions in National Inventories: Lessons from the Ammonia World

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The Paris Agreement obliges the reporting of Nationally Determined Contributions to reductions in greenhouse gas emissions. In the European Union, indirect emissions associated with ammonia emission have been regulated for many years. Agriculture, and especially manure management, accounts for most European ammonia emissions and exceedance of emission limits can result in legal enforcement. A range of measures to reduce ammonia emissions have been developed and are increasingly being implemented. International acknowledgement of reductions achieved requires documentation in national emission inventories. The documentation must show the effectiveness of measures on commercial farms, the implementation rate for different livestock categories and the continued use/maintenance of measures. The experience gathered reducing ammonia emissions can be considered a pilot for the mitigation of agricultural greenhouse gases. The experience indicates that gaining acceptance for specific abatement measures in emission inventories requires the development of international mechanisms for agreeing the effectiveness of abatement measures and how measures should be incorporated into emission inventories. For ammonia, this is achieved respectively via the UN Task Force on Reactive Nitrogen and the UN Task Force on Emissions, Inventories and Projection. The availability and accessibility of data concerning implementation and maintenance varies depending on national legislation, the extent of digitalization in agriculture and relationship between agriculture and government. The approximately 25% reduction in European ammonia emissions since 1990 is far lower than for gases from non-agricultural sources (e.g. SO₂, about 80%), largely due to the difficulty in passing abatement costs on to the consumer. Measures are adopted mainly in situations where there are economies of scale i.e. on larger farms or through the use of contractors. The lesson from the ammonia world is that emission inventories that can account for reduction measures are essential for successful emission reductions but risk documenting failure, unless costs are shared more equitably.

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Emission of Enteric Methane in Dairy Cows with Different Genetic Profiles in the Humid Tropics of Costa Rica

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The objective of this study was to know the enteric methane emission in milk-producing cows with different genetic profiles. Sixteen cows were selected, who were distributed in the following 3 genetic groups: 7 F1 (50% Jersey x 50% Gyr), 5 Triple cross (50% Jersey x 31% Holstein x 19% Sahiwal) and 4 Jersey. In this group of cows, enteric methane was measured on the mean of each month using the SF₆ technique for 12 months. The enteric methane emission did not show a significant difference ($p > 0.05$) between racial groups, although it did vary depending on the lactation phase, attributable to milk production and dry matter (DM) consumption. The average daily emission (including the lactation and dry periods) was 274.49, 322.69 and 297.77 g cow⁻¹ for the F1, Triple cross and Jersey, respectively. While the annual emission was 91.22, 111.82 and 111.42 kg cow⁻¹ for each of the respective racial groups. When the cows are dry they are less efficient in the use of energy since they presented a higher methane conversion factor (MCF), while it varied according to the lactation phase, being lower in the period <76 days, increasing between 76 to 150 days and tending to decrease at the end of this phase (> 150 days). Considering the annual average, the cows with the highest proportion of *Bos taurus* genetics presented a higher FCM of 5.90, 7.22 and 7.05% for F1, Triple cross and Jersey respectively. In conclusion, the racial groups did not show significant difference in the emission of enteric methane and FCM. Although, according to the trends, the F1 cows showed a lower emission of enteric methane and FCM compared to the cows with greater European genetics (*B. taurus*).

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Herd Structure Management Strategies to Improve Productivity and Reduce Methane Emissions in Tropical Dairy Farms

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In tropical milk production systems, the main greenhouse gas emission (GHG) is methane, reason why nearly 14.5% of all anthropogenic GHG emissions are attributable to the livestock sector. There is much interest in reducing emissions with climate smart practices to increase animal productivity while reducing emission intensity. A potential strategy to achieve this dual objective is optimizing the herd structure, which is very lacking in the tropics. Thus, the paper aims at exploring the effect methane emissions of 1) a higher percentage of milking cows in the herd and 2) an optimal replacement rate of milking cows. The data was obtained from 87 farms, classified as specialized dairy (80% of farms) and dual-purpose (20%). Furthermore, these farms were classified as traditional farms and improved production systems. Milk productivity and methane emission were estimated for both scenarios. In the scenario with improved production systems, the ratio of milking cow to herd population was increased to 75-80% while the replacement rate was set at 20%. Compared to the traditional farms, the size of the herd was reduced by 2% and 11% in the specialized dairy farms and dual-purpose farms, respectively, without compromising total milk production. Also, methane emissions were reduced by 3% in specialized dairy farms compared to 14% in dual-purpose farms. Furthermore, the emission intensity was reduced significantly in both systems due to a higher proportion of productive animals in the herd. In conclusion, the optimization of the herd structure by increasing the percentage of cows in production and improving the replacement has positive effects on the productivity and reduction of methane in dairy farms.

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Transforming the Honduran Livestock Sector to Contribute to a Low-Carbon Economy

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Livestock is one of the most relevant economic sectors in Honduras. It contributes 13% to GDP, generates 400,000 annual jobs, and supports 180,000 families. Unfortunately, the sector is a significant Greenhouse Gas emitter, representing about 9% of total emissions, which is why it needs to be transformed. However, migrating this activity toward a low carbon pathway while maximizing the economic welfare will require 1) Capacity-building to strengthen actors across the value chain, 2) financial support to catalyze the transformation, and 3) changes in the political arena to achieve an irreversible process of decarbonization.

With the financial support of NAMA FACILITY, the Honduran's Ministries of Agriculture and Environment are leading the NAMA Support Project (NSP) "Transforming the Honduran livestock sector to contribute to a low-carbon economy". This five-year program seeks to align national extension programs to promote innovations that improve productivity, profitability, climate resilience while maximizing carbon sequestration at the farm level. The technical assistance is complemented with climate financing under market schemes, which seek to mobilize public and private funding. In addition, the NSP, jointly with national organizations, will create a Knowledge Management Platform tailored to different audiences to improve understanding of low-carbon solutions.

The NSP will strengthen the sector's national governance creating the necessary alignments to guarantee the continuity of the livestock NAMA. The reduction of emissions and co-benefits at the farm level will be monitored through a digital MRV platform integrated at the national level with an independent public institution, which will report the sector's contribution to the national mitigation commitments (NDC).

The NSP will directly support the transformation of 1,200 farms, following a gender-inclusive approach, benefiting 13,500 people (i.e., employees, family members, extension officers, and loan officers). Altogether, the NSP expects to directly mitigate 762 ktCO₂e during its implementation and 5,328,250 tCO₂e over ten years.

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Fecal archaeal Diversity in Holstein Dairy Calves from Birth through Weaning

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Ruminant livestock are major contributors to anthropogenic methane emissions worldwide. Enteric methane is generated by methanogenic archaea residing in ruminant digestive tracts. While there have been several studies looking at the bacterial diversity in the early-life microbiome of dairy calves, our knowledge of methanogen diversity at birth through maturity is limited. The objective of this study was to investigate the composition of the methanogenic archaeal community at birth and through the weaning transition in dairy calves. In this study, fecal samples were obtained from ten female Holstein calves at 6 timepoints (TP) between 2–13 weeks of age (TP1: 16–20 days; TP2: 30–34 days; TP3: 44–48 days; TP4: 58–62 days; TP5: 72–76 days; TP6: 86–90 days). Calves were fed acidified milk until weaning at 8 weeks old and had access to starter grain throughout the study. Fecal samples were extracted for genomic DNA, PCR-amplified for the V6–V8 region of the 16S rRNA archaeal gene, sequenced on the Illumina MiSeq platform, and analyzed using the QIIME pipeline. The occurrence of methanogens in the feces of calves was further validated with quantitative real-time PCR (RT-qPCR). The archaeal richness, estimated by number of observed species, and archaeal diversity, estimated by Shannon diversity index, both differed significantly between time points and both increased over time ($P < 0.05$), with the largest increases occurring during weaning. Weighted and unweighted UniFrac analysis showed significant differences ($P < 0.05$) between archaeal communities across timepoints. Throughout the study, *Methanobrevibacter* (66.1 %) was the dominant genus, followed by *Methanosphaera* (33.7 %). *Methanobrevibacter* was the most prevalent genus at timepoints 1, 2, and 3 (95.9%), but its observed abundance decreased at timepoints 4, 5 and 6 (36.3%). In contrast, *Methanosphaera* increased gradually with time and was most abundant at timepoints 4, 5, and 6. Further, RT-qPCR analysis also agreed with 16S rRNA sequencing findings where *Methanobrevibacter* was the most dominant genus at time points 1, 2 and 3, but decreased at timepoint 4. *Methanosphaera* was less dominant at timepoints 1, and 2 and then increased at timepoints 3, and 4. Our results indicate that there is considerable variation in the calf methanogenic archaea pre-weaning, but how these changes compare with those of the adult microbiome warrants further investigations. Such knowledge will help inhibit methanogenic colonization early in life to reduce enteric methane emissions without perturbing ruminal function later in life of dairy cattle.

Keywords: dairy calves, gastrointestinal tract, methanogen

Grass-legume Mixtures and Nitrogen Fertilizers Can Affect Trace Gas Emission and Soil Microbial Biomass

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Grass-legume mixtures can reduce greenhouse gas emissions from soil compared to nitrogen fertilizers. An experiment was conducted for two years at the University of Wyoming Sheridan Research and Extension Center to compare nitrous oxide and carbon dioxide gas emissions, soil microbial biomass, and mineral-nitrogen for meadow brome grass (*Bromus riparius* Rehm.) receiving three nitrogen rates (0, 56, and 112 kg ha⁻¹), alfalfa (*Medicago sativa* L.) monoculture, and 50-50% and 70-30% meadow brome grass-alfalfa mixtures. Measurements were conducted in mid-May, August, and October each year. Treatments significantly affected ($P=0.012$) nitrous oxide fluxes. In the first year, nitrous oxide fluxes from alfalfa monocrop (150 $\mu\text{g nitrogen m}^{-2} \text{ h}^{-1}$) and 50-50% mixture of meadow brome grass with alfalfa (125 $\mu\text{g nitrogen m}^{-2} \text{ h}^{-1}$) were highest. Meadow brome grass significantly ($P < 0.05$) suppressed nitrous oxide fluxes in monocultures differentially fertilized with nitrogen (59 and 80 $\mu\text{g nitrogen m}^{-2} \text{ h}^{-1}$) and in 70-30% mixtures with alfalfa (92 $\mu\text{g nitrogen m}^{-2} \text{ h}^{-1}$). Similarly, in the second year, the 50-50% mixture of meadow brome grass with alfalfa (162 $\mu\text{g nitrogen m}^{-2} \text{ h}^{-1}$) and alfalfa monoculture (139 $\mu\text{g N m}^{-2} \text{ h}^{-1}$) had highest nitrous oxide fluxes. Lower ($P < 0.05$) fluxes were recorded in the 70-30% mixture of meadow brome grass with alfalfa (92 $\mu\text{g nitrogen m}^{-2} \text{ h}^{-1}$) and grass monocultures (59 to 80 $\mu\text{g nitrogen m}^{-2} \text{ h}^{-1}$). There were poor correlations between soil mineral-nitrogen and nitrous oxide flux ($R^2=0.3$, $P<0.05$) in both years. Although there was no significant treatment effect, the range of carbon dioxide flux (15 to 67 mg carbon m⁻² h⁻¹) were 10 times lower than those found in undisturbed forage crops. Treatments had similar soil microbial biomass in August and October of both years. Mineral-nitrogen demand by grass, therefore, underpins the suitability of meadow brome grass-alfalfa mixtures in reducing greenhouse gas emissions and sustaining soil microbes.

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Agricultural Catchments Programme in the Era of Climate Change

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The Agricultural Catchments Programme (ACP) was established in 2008 with a remit to evaluate the Nitrates Action Programme (NAP) under the EU Nitrates Directive. More than a decade later, the ACP has expanded its baseline data collection to include the monitoring of greenhouse gas emissions (GHG) and soil carbon sequestration to align with national and European policy. Agriculture accounts for 35% of Ireland's GHG emissions and a 22-30% reduction in GHGs is required by the agricultural sector by 2030 as outlined in the Climate Act. The objective of this research is to quantify GHG emissions for a range of agricultural land uses and understand how Ireland can reach its environmental targets, including water quality, without compromising food production. We used the eddy covariance (EC) technique to monitor fluxes of carbon dioxide, water vapor and methane at high resolution (10 Hz) frequencies, with the aim of providing spatiotemporally integrated fluxes of net ecosystem exchange of each gas. Five EC flux towers were established on farms within the agricultural catchments which also included instrumentation to monitor meteorological variables. Each catchment varies by its dominant land use type (e.g., arable and grassland) and soil drainage class. We observed temporal variation in GHG fluxes according to management and land use. Longer-term measurements will enable us to assess the impact of different agricultural practices on GHG emissions and determine the rate of soil C sequestration for each system.

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Methane Emissions from Swiss x Creole Steers in Two Grazing Agroecological Conditions in Chiapas, México

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In tropical regions of Mexico, cattle are typically grazed in extensive systems, which produce greater amounts of greenhouse gases, especially enteric methane. This research was carried out in a livestock farm of Central Valley of Chiapas, Mexico, to determine the enteric methane emission of Swiss Brown x Creole steers grazing in different agro ecological zones. Two treatments were evaluated: silvopastoral system (T1) with *Pennisetum purpureum* and mature scattered trees of *Enterolobium cyclocarpum* (Jacq) and *Ceiba pentandra*, and a grazing system in a monoculture of *Pennisetum purpureum* (T2). Eight Swiss Brown x Creole steers (228 ± 12.8 kg) were used. Temperature of grazing areas, nutritional value of the pastures and intake were determined; and for enteric methane emissions, the sulfur hexafluoride (SF₆) technique was used to measure it. The effects of the treatments were evaluated using analysis of variance for a completely randomized design including three measurement periods of 21 days each one. Temperature in grazing areas differed ($P > 0.05$) among treatments, with lower values in silvopastoral system (26.6 °C) and high values in the T2 (26.6 °C). Daily enteric methane emissions per animal was observed to be similar ($P < 0.05$) among treatments, with an average of 213 and 209 g/steer for T1 and T2, respectively. According to estimated intake levels, differences were detected in the amount of methane emitted per unit of dry matter intake (DMI), being higher ($P < 0.01$) in T2 (25.0 g CH₄ kg⁻¹ DMI) compared to T1 (19.98g CH₄ kg⁻¹ DMI). The conversion factor (Y_m) differed ($P < 0.05$) among treatments (6.9% and 7.3%) for T1 and T2, respectively. It is concluded that for both agroecological conditions, as the trial progressed, grass consumption increased, energy efficiency improved and Y_m values decreased, especially for the silvopastoral system (T1).

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Potential of Orange Essential Oil for Enteric Methane Mitigation in Crossbred Heifers

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This study examined the effects of orange essential oil (OEO) as a feed additive to reduce methane emissions in crossbred heifers fed tropical grass. For the *in vitro* evaluation of rumen fermentation pattern and gas production, the experiment was under a completely randomized design using the following treatments: control (CTL, no additive); OEO1 (0.25%) and OEO2 (0.5%) in a substrate of forage:concentrate at 70:30 ratio on a dry matter basis. The *in vivo* study was designed with the same OEO treatments. Six crossbred heifers (*Bos indicus* × *Bos taurus*) fitted with rumen cannulas were assigned in a 3 × 3 replicated Latin square design (21-day periods). Heifers were randomly assigned to the OEO levels in the TMR at a fixed DM intake of 2.8 % of body weight and CH₄ emission measurements were carried out in open-circuit respiration chambers. For the *in vitro* experiment, no change was observed in pH, volatile fatty acid proportions and the acetate:propionate ratio ($P > 0.05$). A significant decrease in methane production was observed with the inclusion of OEO1 ($P < 0.05$). For the *in vivo* trial, a reduction in gross energy intake, apparent digestibility, and valerate concentration was observed with the OEO2 treatment ($P < 0.05$). However, a decrease was recorded in daily CH₄ production (g/d) ($P < 0.05$) as well as in energy lost as CH₄ (MJ GEI/d) and emission factor (CH₄/head per yr) at 0.5% OEO ($P < 0.05$). It is concluded that the inclusion of OEO as a feed additive at 0.5% in a TMR, reduces 12% enteric methane emissions without altering rumen fermentation pattern or dry matter intake in crossbred heifers fed a basal ration of tropical grass.

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Methane Emissions from Cattle Measured Using the SF₆ Tracer Technique and Respiration Chambers: Meta-Analysis

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The SF₆ tracer technique is widely used to determine methane emissions from cattle. Some research has suggested that emission estimates using this technique might be less than when measured using 'gold standard' respiration chambers, which would have consequences for the generation of national inventory methane yield (g/kg dry matter intake; DMI) factors. The objective of this analysis was to determine the agreement and relationship of methane emissions from cattle measured using the SF₆ tracer technique and respiration chambers from direct method comparison studies. Data was extracted from nine publications that presented 26 pairs of methane data from cattle using SF₆ and chamber methods. For methane production (g/d) measured using SF₆ vs chambers using Orthogonal (i.e., Deming) regression, the 95% confidence interval (CI) for the intercept was -35.89 to 13.92 and 0.931 to 1.132 for the slope indicating that two methods provide similar methane results. For methane yield determined using the two methods, the 95% CI was -33.22 to 5.98 for the constant and 0.756 to 2.501 for the slope, again indicating that the two methods provide similar results. However, the Lin's concordance coefficient (CCC) was only moderate for SF₆ vs chamber methane yield (CCC = 0.58), while being strong for the comparison of methane production (CCC = 0.97). The moderate CCC of the methane yield comparison was mainly due to moderate precision as indicated by the Pearson correlation of 0.61, while the relationship had little slope bias (bias correction factor of 0.95). In conclusion, the SF₆ tracer technique resulted in similar cattle methane production and yield estimates to those measured using respiration chambers, suggesting that both techniques can be used interchangeably to generate methane emission factors without the need to correct for technique.

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Variation in Urinary Nitrogen Composition of Sheep Fed Fresh Pasture and Alternative Forages

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Animal urinary nitrogen (N) concentration and composition affects nitrous oxide (N₂O) emissions from urine patches deposited on soil during grazing. The objective of this study was to summarize individual sheep data ($n = 154$) on N concentration and composition from seven trials with sheep fed fresh ryegrass based-pasture or alternative forages including white clover, chicory, fodder beet and various leafy and bulb brassicas (29 treatments). Urinary N concentration varied nearly 10-fold across individual urine samples and correlated positively with forage N concentration ($r = 0.56$) and negatively with urine volume ($r = -0.46$). The urine volume was on average greater in sheep fed alternative forages (4.9 ± 1.92 kg/d) than those fed pasture (2.8 ± 0.92 kg/d) and the range in urine N concentration was smaller in sheep fed alternative forages (0.11 to 0.52 %) than the range of 0.25 to 1.06 % in those fed pasture. In other research, hippuric acid N has been associated with reduced urine N₂O emissions. Urinary hippuric acid N proportion ranged from 0.07 to 8.36 % of total urine N. There was no clear distinction of hippuric acid N proportion of urine N between sheep fed pasture or alternative forages and in some trials there was a large between animal variation (CV > 40%). Purine derivatives N ranged from 0.60 to 5.45% of urine N and creatinine N from 0.34 to 2.54% of urine N. Hippuric acid N, purine derivatives N and creatinine N proportions correlated negatively with urine N concentration. In conclusion, urinary N concentration was on average less in sheep fed alternative forages due to a greater urine volume excreted (more diluted urine) while urinary N composition as a proportion of urine N was mainly driven by forage N concentration (N excess in the diet) independent of forage type eaten.

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Environmental Impact of Changes in the Component Traits of Beef Cow Productivity in South Africa

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The environmental impact (carbon footprint) of changes that occurred in cow productivity of cowherds in four South African landrace breeds over a period of 25 - 30 years was investigated. Cow productivity, defined as kilogram calf weaned per Large Stock Unit (LSU) mated, increased between 10.4% and 18.3%. This resulted in a decrease in the carbon footprint, as defined by the enteric methane emissions factor (a LSU is estimated to produce 94 kg methane/year (Tier 2)), of between 6.6% and 12.0%. The three component traits used to estimate cow productivity are: (1) weaning weight of the calf, (2) feed requirements to produce the calf (for this purpose each cow's productivity was expressed in Large Stock Units as it is linked to daily feed intake) and (3) the frequency at which a calf is produced (inter-calving period was used to estimate calving percentage). It is important to note that the LSU equivalent of cows with the same body weight, but different frame sizes, differs. Furthermore, the relationship between cow weight and LSU is not linear. The relative contribution of the three traits towards cow productivity for breeds of different frame sizes are reported. This was investigated by changing each component with 5% while keeping the other two constant for the different frame sizes. The results indicated that inter-calving period is by far the biggest contributor to cow productivity and thus the carbon footprint, varying from 44% in the case of small frame breeds to 51% in the case of large frame breeds. The contribution of the weaning weight of the calf showed very little difference between the breeds and varied between 32 and 33%. Cow weight (higher cow weight is undesirable when feed is a limiting factor) had the smallest contribution, viz. between 17 and 24%.

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Seasonal Variations in GHG Emissions from Norwegian Dairy Cattle

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Norwegian dairy cows are typically housed in insulated barns with mechanical ventilation and a high level of mechanization. After installation of Greenfeed units in commercial dairy barns, emission data are now available for research. The aim of this study was to investigate effects of low barn air temperature on background and dairy cow CH₄ emissions in Norwegian dairy farms. Temperature and GHG data over the year 2021 was obtained from permanently installed Greenfeed units on 12 modern dairy barns in mid Norway. Number of dairy cows per Greenfeed unit varied from 20-76. Methane measurements were obtained from 579 Norwegian Red cows. In addition, detailed information and production data was gathered on farm and via interviews with the farmer for three case study farms. Mean CH₄ across all 12 herds and months was 427 g/day (\pm 117 SD). We found a seasonal variation of methane production with lower emissions during summer months of June (401 \pm 113.2 g/day) and July (395 \pm 115.0 g/day) and higher emissions during winter (e.g. February: 444 \pm 114.5 g/day). These differences were not statistically significant but could be explained by reduced feed intake during the grazing period. More data from October – December and milk production will be included in later analyses. In conclusion, the first systematic measurements of methane emission from Norwegian dairy cows in commercial production correspond with data from other countries. The seasonal variation observed may be a phenomenon related to the sudden change in animal environment from closed insulated barns to open doors with access to pasture during June and July. The effect of temperature and access to grazing on GHG emissions in a cold climate should be further investigated.

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Effect of Six Macroalgal Species on *In Vitro* Methane Production in a Lactating Dairy Cow Diet

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Reducing enteric methane emissions from ruminants is an important goal in producing animal-sourced products such as milk and meat from sustainable ruminant livestock systems. Six species of macroalgae collected along the California coast were evaluated for their potential to mitigate *in vitro* methane emission when supplemented to a lactating dairy cow diet. *Asparagopsis taxiformis*, collected near Santa Catalina Island, and *Gracilaria parvispora*, *Gracilaria andersonii*, *Codium fragile*, *Acrosiphonia coalita*, and *Devaleraea mollis*, collected from the central coast, were freeze-dried, ground to pass a 2-mm screen, and added to a lactating dairy cow total mixed ration (TMR) at 2% of the dry matter. The TMR was composed of 30.8% corn silage, 30.0% alfalfa silage, and 29.2% concentrate feedstuffs. The TMR (control) and the 6 treatment combinations were placed in a bottle with a solution of 3.44:1 of artificial saliva and ruminal fluid in an *in vitro* gas production system to measure methane production in a randomized complete block design (2 rep/block and 4 blocks). Methane production (G) was measured for 48 hours, logging the time of each 2-mL aliquot produced and then data was fit to a nonlinear equation, $G = A_0(1 - e^{-ke(t-T_{Lag})})$ where A_0 is the asymptote (g), k is the rate constant (g/hour), and T_{Lag} is the time (hour) to initial emission. Equations developed for each treatment combination were then used to predict total enteric methane emission for a 722-kg dairy cow consuming 27.8 kg/day of TMR. When the cow consumed only TMR, it produced 635 g of methane/day ($A_0=25.9$ g, $k=0.148$ g/hour, and $T_{Lag}=4.02$ hours). However, when the cow consumed the TMR with 2% *A. taxiformis* ($A_0=16.2$ g, $k=0.0995$ g/hour, and $T_{Lag}=5.5$ hours), it produced 48.3% less ($P < 0.05$) methane (328 g/day) compared to the TMR. The diets containing *G. parvispora* ($A_0=23.1$ g, $k=0.152$ g/hour, and $T_{Lag}=4.39$ hours) and *G. andersonii* ($A_0=23.5$ g, $k=0.148$ g/hour, and $T_{Lag}=4.21$ hours) produced 10.9% (566 g/day) and 9.8% (573 g/day) less ($P < 0.05$) methane compared to the TMR. For TMR containing *C. fragile* ($A_0=24.1$ g, $k=0.151$ g/hour, and $T_{Lag}=4.21$ hours), *A. coalita* ($A_0=24.1$ g, $k=0.148$ g/hour, and $T_{Lag}=4.10$ hours), and *D. mollis* ($A_0=25.1$ g, $k=0.146$ g/hour, and $T_{Lag}=4.02$ hours) methane production did not differ ($P > 0.05$) from the TMR. In this experiment, *A. taxiformis* was the most effective at mitigating enteric methane production, whereas *G. parvispora* and *G. andersonii* showed moderate reductions in enteric methane production.

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Covering Manure Piles: Effect on Ammonia and Methane Emissions

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Emissions of methane and ammonia from solid manure piles have been investigated with different covering practices. The concentrations of ammonia and methane were measured with two cavity ring-down spectroscopy instruments that provide online measurements up- and downwind of the piles. The inverse dispersion model, backward Lagrangian Stochastic model is used to derive emissions from measurements of the concentrations and wind statistics. Manure piles were built on a field in three experiments and different ways of covering were investigated to determine the consequence of non-perfect covering after being built up in terms of ammonia and methane emissions. The experiments show small differences in methane emissions depending on the covering procedure even though the temperatures inside the piles were different. In contrast, ammonia emissions were highly influenced by the covering. The lowest total emissions were observed from a manure pile that was completely covered by plastic tarp immediately after being built. During the first five days, an uncovered pile emitted 3-6 times more ammonia than a covered pile over 40 days. Over the same period without cover, the ammonia emissions were approximately 8 times higher than from a pile with cover. Removing the plastic from a covered pile after the storing period initiates ammonia emissions at the same rate as an uncovered pile after being built. The thoroughness of the coverage appears to be of minor importance compared to covering immediately after building the pile. Overall, the effect of covering solid manure piles does not have a significant effect on methane emissions, but a huge influence on ammonia emissions. Covering should be conducted as soon as possible to reduce ammonia emissions from solid manure storage.

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Large Potential for Mitigation of Methane Emissions from Slurry Tanks with Early Tank Acidification

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The effect of early acidification of slurry tanks has been investigated on four full-scale slurry tanks. Ammonia and methane concentrations were measured online with cavity ring-down spectroscopy up- and downwind of the source. The concentrations in combination with wind statistics were used to estimate emissions with the backward Lagrangian Stochastic model, which is an inverse dispersion model. Measurements were conducted in rotation of approximately one week at four different slurry tanks (two with cattle and two with pig manure) before and after acidification. The tanks were acidified to approximately pH 5 with Sulphuric acid. The measurements were conducted from July to October 2020 with an additional measurement in March 2021. The use and addition of slurry to the tanks have in part been subjected to the farmer's needs, which have been beyond the control of this project, especially after October 2020. The results show a very clear reduction (over 90%; t-test $P < 0.05$) in methane emissions after acidification, which was retained throughout the measurement period even though pH increased during the storage period. The emissions of ammonia were comparable or lower than when a natural crust is present. The presence of a natural crust itself reduces the ammonia emissions, but it is a challenge to determine low emissions where the measured concentration is close to the background level. The tanks are not completely representative of a normal production cycle, but the permanent reduction of methane emissions after acidification is above 90%, which shows a huge potential for mitigation of methane from the livestock manure chain with early tank acidification.

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Effects of Dietary Biochar Supplementation on Enteric Methane Emissions in Feedlot Cattle

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Ruminant production contributes to anthropogenic greenhouse gas emissions, mostly in the form of enteric methane production. One strategy to reduce enteric methane emissions is through supplementation of feed additives to the diet. Biochar, an organic matter that has undergone gasification, has been effective at decreasing methane production *in vitro*, and thus shows promise as a potential feed additive in livestock diets to mitigate methane production. The objective of this study was to investigate the effects of softwood biochar, when added to a traditional feedlot diet, on methane production and nutrient utilization. Twenty-four Angus-influenced crossbred steers were randomly allocated to one of three treatment groups: 0% (Control), 1% (Low) and 2% (High) biochar inclusion based on dry matter intake. After a 2-week baseline period and every 2-weeks thereafter, methane production was measured using the GreenFeed system (C-Lock Inc., Rapid City, SD). During the study, steers were fed 3 diets according to their life stage. Average daily gain (kg/day) and dry matter intake (kg/day) were not affected ($p \leq 0.05$) by treatment group. Methane production (g/day), yield (g/kg DMI) and intensity (g/kg ADG) were also not affected ($p \leq 0.05$) by treatment group. Although biochar was shown to reduce methane emissions *in vitro*, there was no significant effect when tried *in vivo* in this study. However, biochar made from other sources of organic matter may yield a different result and should be investigated further.

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Effect of Feeding *Ascophyllum nodosum*, a Novel Brown Seaweed Extract, and Soya Oil on Daily Dry Matter Intake and Enteric Methane Emissions in Ewes

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Enteric methane from ruminant livestock production is responsible for 5.5% and 19% of global and Irish total greenhouse gas emissions, respectively. While a number of studies supplementing marine derived seaweeds have demonstrated reductions in enteric methane *in vitro* and, in small and large ruminants, the emphasis has been on non-native red seaweeds that aren't readily available in northern Europe. The objective of this study was to quantify the effect of dietary supplementation with an indigenous brown seaweed, *A. nodosum* and a novel brown seaweed extract on enteric methane emissions in sheep. These diets were compared with an unsupplemented control diet as well as a diet containing soya oil, previously shown to suppress methane emissions. Two 8 week experiments were conducted, animals were fed a basal diet of *ad libitum* grass silage (~1 kg DM), 0.4 kg barley based concentrates and 0.1 kg maize meal (with additives included for experimental diet). In experiment 1, 60 ewes (n=20) were blocked by body weight (77 ± 1.6 kg), individually penned and randomly assigned to one of three dietary treatments (no additive (control), *A. nodosum* (BSW), a brown derived seaweed extract (BSWex)). At the end of experiment 1, there was a 2 day wash-out period where ewes received the unsupplemented basal diet. In experiment 2, eighty ewes (n=20) were re-randomized and blocked by body weight (88 ± 1.5 kg), and assigned a new dietary treatment (no additive (control), BSWex, soya oil (SO), soya oil plus the seaweed extract (SO+BSWex)). All additives were supplemented at 4% of daily DMI, except for BSW (2% DMI). Methane was measured during both experiments using portable accumulation chambers, feeding was staggered so that animals were fed 3 hours prior to each measurement. Data were analysed using mixed models ANOVA (PROC MIXED, SAS v9.4.). Statistically significant differences were considered when $P < 0.05$. DMI was not affected by diet in either experiment ($P > 0.05$). In experiment 1, there was no difference ($P > 0.05$) observed in methane emissions expressed as either g/day or g/kg DMI between control and treatment diets. Similarly in experiment 2, there was no effect of any of the dietary supplements employed on methane emissions. Dietary supplementation with BSW or BSWex had no effective reduction on methanogenesis in ewes. Similarly, including SO in the diet in isolation or combined with BSWex, had no significant anti-methanogenic effect.

Risk Characterization Based on Qualitative Data on Cameroon National Strategy for Climate-Smart Livestock Development

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Resilience is perceived as people's ability to prepare, adapt and cope with difficulties or challenges, with regards to climate-change related stresses. This study is aimed at developing a National strategy for Climate-Smart Livestock for Cameroon based on prospective empirical data. This report is based on rainy season data collected from livestock farmers from the five agro-ecological zones in Cameroon involving all livestock categories. This arm of the study was centered on vulnerability and resilience and used essentially a qualitative survey approach whereby data was collected through interviews with 496 farmers. The classification of information integrated three dimensions in the analytical matrix. The first dimension was the classification of vulnerability into four classes, social, ecological, physical, economic and institutional; the second dimension classified coping mechanisms into adsorptive, adaptive and transformative capacities; and the third-dimension dealt with degrees of structural change ranging from low, high and very high. Following the thorough thematic analysis of the data with the support of Atlas Ti software, it can be concluded that the paradigmatic frame can now be structured as three-level dynamic components: adsorptive/coping, adaptive and transformative capacities as pillars of resilience framework. As we move from adsorptive to transformative, the magnitude of change impacted in the community to improve resilience increases but the target is always to strike a mixed-balance solution such that the change does not cause social and ecological shocks beyond the absorbance capability of the farmers. In fact, the targeted people should be able to adsorb the shock caused by the change without altering their functioning or socio-cultural equilibrium. Resilience is more successful when it does not cause further dysfunction within the system be it social or ecological. Thus, changes should be introduced gradually, properly monitored and evaluated while considering the social and ecological diversity. This vision is considered in our endeavor to develop Climate-Smart Livestock strategy for Cameroon.

Key words: Resilience, climate-smart livestock, risk characterization, Cameroon

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Performance and Methane Emission of Tropical Cattle Supplemented with Either Commercial Concentrate or Tannin-Rich Forage

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In Africa, cattle are often fed low quality tropical roughages resulting in low animal production and high methane (CH₄) emission per unit feed intake. The objective of this study was to determine if partially replacing Brachiaria hay and molasses (CON) with a leguminous forage; *Desmodium intortum* hay (CON+DES; high in protein and tannins) or commercial concentrate (CON+CUBES; high in protein and energy) would increase animal performance and reduce the environmental impact of low yielding cattle (3.9±1.1 kg milk/d). Twelve lactating crossbred (Friesian × Boran) cows (liveweight: 351 kg; Days in milk: 119) were used in a 3×3 Latin square design with 28-d periods. Compared with CON, CON+DES diet decreased ($P<0.05$) DMI (-14%), nutrient [organic matter (-10%), crude protein (-16%) and fibre (-13%)] apparent total tract digestibility (ATTD), animal performance [milk production (-24%) and average daily gain (-41%)] and shifted nitrogen (N) excreted to feces resulting in a decreased urinary N: fecal N ratio (-33%). Reduced DMI was likely a result of higher lignin and presence of tannins in CON+DES diet, suppressing nutrient digestibility and ammonia production in the rumen compared with CON. Compared with CON, CON+CUBES diet increased DMI (+26%), CP ATTD (+17%) and animal performance [milk production (+54%) and average daily gain (+106%)]. This was most likely a result of reduced dietary fiber content (reducing rumen fill), and greater digestible protein and energy intake. Both supplementation options (CON+DES and CON+CUBES) reduced CH₄ yield (CH₄ per kg DMI) (by 15% and 9% respectively) compared to CON. Based our results, supplementation with commercial dairy concentrates increase animal performance and reduce CH₄ yield. In contrast, *Desmodium intortum* hay despite showing environmental gains would not be profitable for farmers in the absence of a carbon price incentive. Further research on studies with lower inclusion rates and/or better-quality leguminous forage are warranted.

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Effects on Rumen Microbiome and Milk Quality of Dairy Cows Supplemented the Macroalga *Asparagopsis taxiformis* in a Grass Silage-Based Diet

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The objective was to determine the effects on rumen microbiome and milk quality while reducing enteric methane (CH₄) by the addition of *Asparagopsis taxiformis* (AT) to diets of dairy cows. Six Nordic Red cows at 122 ± 13.7 (mean ± SD) days in milk, parity 2.7 ± 0.52 and producing 36 ± 2.5 kg milk/d at the start of the trial were blocked by milk yield, and assigned to an extra period Latin square change-over design comprising two dietary treatments. The dietary treatments were either a diet consisting of grass silage and a commercial concentrate mixture (50:50) not supplemented or supplemented with 0.5% of AT on organic matter intake basis. Recordings of methane and hydrogen production (using the GreenFeed system), feed intake, milk yield and composition was made the last week of every 3-week period. Milk samples was subjected to sensory test, and analysis of milk fatty acid, iodine, bromine and bromoform. Rumen fluid samples was collected from all cows by *esophageal* stomach tube d 19 in each of the experimental periods for volatile fatty acid composition and molecular analysis. Total feed intake decreased 2.9 kg/d, but milk yield was not affected for cows supplemented with AT. Daily CH₄ production, CH₄ yield, and CH₄ intensity decreased by 60%, 54%, and 58%, respectively for cows fed the diet supplemented with AT. Further, acetate:propionate ratio was altered from 3.1 to 2.2 when cows were supplemented with AT. At genus level *Methanobrevibacter* had highest relative abundance (56.9%) in rumen fluid in diets not supplemented with AT, while genus belonging to family *Methanomethylophilaceae* was more abundant (55.7%) in rumen fluid when diets were supplemented with AT. The most prominent change in milk quality was an increase in iodine from 154 to 2089 µg/L for cows fed AT.

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Long-Term Carbon Balance in Managed Pasture Systems

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Temperate grasslands constitute over 30% of the Earth's naturally-occurring biomes and make an important contribution towards the abatement of anthropogenic greenhouse gas emissions. The accumulation of carbon (C) in grassland systems predominantly takes place in below-ground repositories, enhanced by the presence of a stable soil environment with low carbon turnover rates, active rhizodeposition and high levels of inputs. The size of the C sink activity in managed pastures can be influenced by management, such as altered forage type, fertilizer inputs or grazing intensity. This impact of management on net sink activity is further complicated by the impact of abiotic factors, such as inter-annual variation in rainfall and temperature and a mechanistic understanding of ecosystem response to such changes is still lacking. In the present study, the net C balance of a managed grazed grassland system in south-east Ireland was measured over an 18 year period. Eddy covariance measurements of net ecosystem exchange of C were complemented by regular assessment of standing biomass, leaf cover, harvest and dissolved organic C exports and organic amendment inputs. The lowest Net Ecosystem Productivity values were observed for two years where the grassland was ploughed and converted to an alternative (winter kale) forage. The impact of weather anomalies in 2012 and 2018 were studied. Cool, wet conditions in 2012 facilitated net carbon uptake for more than ten months of the year, while the grassland was a net carbon source in 2018 due to a) low spring Gross Primary Productivity due to abnormally low temperatures and b) an unusual and extended summer drought. In total, the grassland was a net carbon sink over the 18-year period, with a Net Biome Productivity of $1.02 \pm 0.43 \text{ tC ha}^{-1} \text{ yr}^{-1}$. This research highlights both the impact of management and climatic factors on net carbon sink activity.

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Regenerative Ranching to Mitigate Climate Change: Drivers and Barriers for Adoption in Chile

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Livestock production is currently under heavy scrutiny, as it is perceived as one of the leading causes of climate change. Regenerative livestock production has emerged as a way to produce goods and services, but increasing biodiversity, strengthening communities and enriching soils. Many of the key principles and practices that regenerative farmers and ranchers use are related to increasing soil organic matter, thus, sequestering carbon. Evidence in the literature indicates that C balance can be reduced, even up to zero emissions. However, adoption of regenerative practices is limited, so the goal of this study was to understand the main drivers and barriers to its implementation by producers in Chile. We asked 15 stakeholders (including producers, consultants and others) what were the main drivers that motivated producers to change to regenerative management, and what were the main barriers they perceive limiting the adoption of these practices. Then, we grouped the barriers in 13 topics and asked 29 stakeholders to value how relevant they thought they were. We used a likert scale (from 1 to 5) where (1) was least relevant and (5) was very relevant. The main driver for adoption was the perception of a crisis associated to the conventional production methods that the producers were using. Producers indicated that the crisis could be financial, environmental, emotional, or a combination of them. The main perceived barriers for adoption were a) lack of education on regenerative farming/ranching; b) the need of changing cultural paradigms about what is right or wrong when managing the farm/ranch; and c) lack of quantitative data at a local level, about the effects of regenerative farming/ranching in production, profits, etc. The main barriers are complex to tackle and interrelated, which suggest that successful strategies to increase adoption of regenerative practices will need close collaboration among educators, researchers, and producers.

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Comparing the GreenFeed System with the Tracer Ratio Method using Sainfoin as Methane Mitigation Model

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Increasing dietary forage proportion potentially increases ruminal methane (CH₄) emissions. Including the tanniferous legume sainfoin may counteract this. Other CH₄ sources such as aisles soiled with excreta can contribute to the CH₄ emissions from the housing, too. The aims of this study were (i) to compare two measurement approaches: one based on individual animal level the other on housing level, and (ii) to quantify the effect of sainfoin on CH₄ emissions. A herd of 20 lactating cows past lactation peak were kept in a naturally ventilated cubicle housing with solids floors. Two silage-based total mixed rations were fed in a case-time-control design of two consecutive experimental phases. The cows received either a grass silage-based diet or a sainfoin-rich silage-based diet, being similar in supply with crude protein and energy. Each diet was fed for at least 8 days. Individual enteric CH₄ emissions were measured using the GreenFeed system (C-Lock Inc., Rapid City, SD). Emissions of CH₄ at housing level were determined using a tracer ratio method with SF₆. The tracer gas was dosed continuously via a tube system with critical capillaries next to the aisles. Composite air samples were taken using a sampling system consisting of PTFE tubes and critical capillaries. The tracer gas concentration was measured by gas chromatography and CH₄ concentration by cavity ring-down spectroscopy. Milk yield, feed supply and leftovers were recorded daily. Both measurement approaches registered that the sainfoin-based diet lowered CH₄ emissions by around 9%, compared to the grass silage-based diet (GreenFeed : 415 vs. 458 g CH₄ cow⁻¹ d⁻¹; housing level measurements: 459 vs. 504 g CH₄ cow⁻¹ d⁻¹, respectively; both p < 0.001). In conclusion, both methods show nearly the same CH₄ emission reduction rate in the sainfoin-based diet. The slightly greater emissions detected with the tracer ratio method may reflect the part of the CH₄ emissions not registered by GreenFeed, such as hindgut or manure-derived emissions.

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Carbon Footprint for Different Intensification of Beef Cattle Systems in Argentinean Flooding Pampas

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The Argentine beef cattle sector faces the challenge of meeting the increasing meat demand through eco-friendly, intensified systems. Under this context, cow-calf systems' externalities such as carbon footprint (CF) play a major role. The study focused on assessing CF through a life cycle assessment in contrasting modal cow-calf farms for the Southeastern Flooding Pampas, with equations proposed by IPCC in 2019. Systems selection was based on high (H), medium (M) and low (L) levels of technification, with differences in diet composition, stocking rate, and herd parameters. Cows were fed mainly with grassland (100% for L, 75% for M and 55% for H), with additional sources such as annual grasses and pastures in the M and H systems (up to 45% of the diet). The stocking rate was 0.5, 0.75 and 1.0 cows ha⁻¹ for L, M and H respectively, and the age at first calving 36, 31 and 24 months in the L, M and H, while weaning percentage was 70% for L, 82% for M and 83% for H. The results showed that the CF were 21.44, 18.25 and 16.24 kg CO₂eq kg LW⁻¹ for the L, M and H systems respectively, showing a reduction of CF reaching 24%. The total emission (kg CO₂eq) increased 18% between the L and H system. The methane emission represented, on average, 86.7%, nitrous oxide 12.5% and carbon dioxide 0.8% for the three systems. The meat production efficiency increased from 70 kg LW ha⁻¹ for L, to 120 kg LW ha⁻¹ for M, and 190 kg LW ha⁻¹ for H system. The improved management practices can contribute to increasing the efficiency of cow-calf farms in Argentinean Pampas and reduce CF of this activity, leading the country to a sustainable production.

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Preliminary Measurements On Diel CH₄ and N₂O Fluxes on Brachiaria Pasture

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Brazil has extensive pasture areas for beef cattle grazing. An experiment was carried out in summer in a Brachiaria pasture in Southeastern Brazil, characterized by hot and humid climate in summer and dry in winter, with the objective of measuring hourly fluxes of methane and nitrous oxide from soil and identifying a possible relationship with environmental parameters, including air temperature, soil temperature, solar radiation, photosynthetically active radiation (PAR), and soil moisture. Temperature and radiation data were obtained from a climatological station on February 13 and 14, 2020, starting at 9:00 am and ending at the same hour of the next day. Soil moisture data were recorded by humidity sensors. The gas collection method used was the static closed chamber. Five chambers were used at the same time. Methane and nitrous oxide concentrations were sampled every 2 hours, in intervals of 0, 10, 20, 30, and 40 minutes. The gas samples were extracted by BD 60 mL syringes with a three-way luer lock valve and then transferred to 12 mL vacuum vials of the Labco Exetainer brand. A Thermo Scientific chromatograph model, Trace 1310, was used to determine the concentrations of gases. The statistical analysis was performed using the PROC CORR procedure of the SAS program. Preliminary results showed that N₂O fluxes peaked at 3 p.m. with around 10 µg N-N₂O m⁻² h⁻¹ and a minimum value of -2 µg N-N₂O m⁻² h⁻¹ at 1 a.m. N₂O fluxes were positively correlated with air temperature (0.70939 /0.0066) and with PAR (0.59037/0.0337), while they were negatively correlated with relative humidity (-.69266/0.0087). Methane correlated negatively with soil temperature (-0.88646/<0.001), although the concentrations were very low throughout the day. Other diel measurements are going to be carried out and the experiment will be repeated in the summer season.

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Micro- and Macroalgae Additives to Mitigate Enteric Methane Emissions from Norwegian Sheep

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Worldwide there is a growing interest for using micro- and macroalgae as feed additives to mitigate enteric methane emissions in ruminants. The only algae reported so far to have this effect are the red macroalga *Asparagopsis taxiformis* and *A. armata*. However, the potential negative effects of *Asparagopsis* on feed intake and milk production in dairy cows have called its use into question. The microalgae *Chlorella vulgaris* and the macroalgae *Porphyra* sp and *Laminaria hyperborea* have been investigated as potential species to mitigate enteric methane emissions from sheep in Norway.

Chlorella vulgaris was included in a concentrate pellet and fed to castrated rams. Microbiotas of sheep fed a control diet or a diet including a *C. vulgaris* concentrate were compared. Although there was a trend towards a decrease of methanogenic microbes in both groups after adding concentrate to the diets, no differences in the microbiotas between the two diets were observed.

Two macroalgae species (*Porphyra* sp., *Laminaria hyperborea*) were tested for their mitigating effect on enteric methane production in sheep using open-circuit respiration chambers at the NIBIO research station in Tjøtta, Norway. Dried and powdered *Porphyra* sp. were fed to ewes and methane emissions were compared with sheep fed diets including white clover silage or soybean meal. We found no differences in the methane production (L CH₄/kg DMI) between the groups. The residue of *Laminaria hyperborea* was chosen for a long-term *in vivo* experiment with sheep as *in vitro* measurements showed this macroalgae was promising for mitigating enteric methane. The experiment was performed with lambs from August to December of 2021. Data collected were daily feed intake, growth rate, nitrogen digestibility, iodine content (in feed, feces, urine, and blood) and enteric GHG emissions. Rumen fluid samples were taken for DNA, VFA and ammonia profiles.

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Methane and Nitrous Oxide Emissions from Manure Stockpiles in a Beef Feedlot Establishment

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In a typical Argentinian feedlot, beef cattle are held in pens, and the dry solid manure is often stockpiled for later spreading onto nearby fields. Stockpiles heap manure into stacks with no active management, thus they are expected to emit greenhouse gases (GHG) like methane (CH₄) and nitrous oxide (N₂O). The purpose of this study was to quantify CH₄ and N₂O emissions from two stockpiles coming from pens with different diets (starting vs. finishing). Gas fluxes were measured over 564 days, since deposition until spreading, completing 13 sampling occasions (every 45 days approximately) using the static chamber technique. Five chambers were randomly inserted onto the surface of each pile and other two chambers were placed on the surrounding soil without manure as control. Manure stockpiles were a significant direct source of CH₄ and N₂O emissions compared with control soil, and fluxes were significantly influenced by the time passed across sampling occasions. No differences among GHG emissions between stockpiles were found. Whilst cumulative CH₄ emissions from the starting and finishing stockpiles were 10.5 ± 6.3 and 9.7 ± 5.5 kg CH₄ m⁻² (mean ± SD), respectively, the control had a negative cumulative CH₄ value (-0.06 ± 0.5 kg CH₄ m⁻²) acting as a CH₄ sink. Cumulative N₂O emissions from the starting and finishing stockpiles were 51.1 ± 11.6 and 78.4 ± 26.8 g N₂O m⁻², respectively, and from the control soil was 2.3 ± 2.2 g N₂O m⁻². At the end of the trial, the day before manure spreading, manure stockpiles were still moist, with undecomposed organic matter inside, and with fluxes of CH₄ and N₂O significantly higher (P<0.05) than those from surrounding soil. Management practices such as stocking time, rather than animal diet, appear as critical factors controlling nutrient decomposition and GHG emissions from stockpiles on intensive production systems.

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Nitrous Oxide Emissions from Combining Cattle Dung and Urine Patches in a Temperate Grassland

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Excreta from grazing animals contain large amounts of nutrients, which can result in nitrous oxide (N₂O) emissions from small areas on grasslands. Sites where animals congregate are likely to overlap dung with urine patches. The purpose of this study was to investigate the effect of combining cattle dung and urine depositions on N₂O emissions in comparison with individual depositions in two different seasons (dry and wet) and to develop local N₂O emission factors. Evaluations were performed in Tandil, Argentina, during two separate 98-day trials; a winter-spring (dry) season in 2019 and a summer-autumn season (wet) in 2020. Fresh excreta were applied on the surface of the pasture within circular bases simulating natural cattle deposition which included the following four treatments: dung (2.50 kg), urine (0.75 L), dung+urine (2.50 kg + 0.75 L), and control (without excreta). Nitrous oxide fluxes were measured using the static chamber technique with a total of 21 and 23 sampling occasions in dry and wet seasons, respectively. Daily N₂O fluxes revealed a distinct pattern depending on the treatment and time after application. The addition of urine onto dung patches under hot and wet weather conditions had a synergistic effect (threefold increase) on cumulative N₂O emissions compared to the sum of the individual N₂O emissions from the separate excreta patches (P=0.007). Alternatively, during cold and dry conditions, no differences were found between the N₂O emissions from the combined dung+urine and the sum of the individual patches (P=0.35). Thus, preventing the overlap of dung and urine patches under wet conditions can serve as an alternative mitigation strategy for N₂O emissions in temperate managed grazed pastures. Moreover, the N₂O emission factor mean value, considering the three types of excreta applied, was 0.14 ± 0.08%; and was lower than the mean 0.4% aggregated default emission factor established by IPCC.

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Carbon Balance Analysis of a Sown Pasture in Inland Arid Area, China

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In arid regions, which occupy about 41% of the global terrestrial area, sown pasture is one of the most important food production systems. Based on life cycle analysis of carbon balance, the sown pasture of wheat and alfalfa-fescue mixture rotationally grazed by sheep was the carbon sink, whilst the harvested pasture was the carbon source. The carbon emissions per food equivalent unit (a food production units calculated based on protein and energy content) in the grazing pasture was 78.84% lower than that of the hay pasture. In grazing annual pasture and perennial pastures, the carbon emissions from livestock, processing and allocation of forage products accounted for 3.95%, 96.05% and 2.01%, 97.99% of the whole carbon emissions, respectively. The carbon emissions of sown pasture which harvesting hay mainly came from fertilization, irrigation and the processing and transportation of forage products. Therefore, strategies of carbon mitigation should focus on the greenhouse gas emissions of livestock production in grazing systems, and the processing and circulation of fertilization, irrigation inputs, and forage products in hay-harvesting pasture.

Key words: Food equivalent unit; Grazing; Greenhouse gases

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The Effect of Obligative Anaerobic Lactic Acid Bacteria Isolated from Sheep Caecum and Their Effect on *In Vitro* Methane Mitigation in the Rumen

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Methane production in the rumen can be inhibited by various mechanistic reactions. Lactic acid bacteria (LAB) is one solution to reduce methane production from the rumen. Isolation and Identification of LAB obtained from sheep caecum were conducted. Obligative anaerobic conditions were used for LAB isolation. The Molecular identification procedure used the 16S rRNA gene sequence amplification method. Isolates that had been identified were then further studied to evaluate the potential to reduce methane production in the rumen using... (can you describe the *in vitro* method?). A control (without LAB) and a positive group (with LAB Isolated) were included in an *in vitro* experiment. The (concentrations?) of methane and volatile fatty acids (VFA) were determined on minimal media or added with rumen liquid inoculated with pure isolates of LAB. The result showed that three LAB, BAL-D26, BAL-D32, and BAL-D33 were isolated from the sheep caecum. The species of LAB that were isolated could decrease? the (concentration?) of methane resulting from substrate fermented with rumen liquid. The LAB also can increase the total VFA from rumen fermentation. These results suggest that the LAB isolated from sheep caecum has the potential as a methanogenesis inhibitor.

Keyword: Methane, Mitigation, LAB, Sheep Caecum

Effect of Supplementation of *Bacillus* Spp. on *In Vitro* Fermentation, Methane Production and Nutrient Digestibility

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Probiotics are used in animal nutrition for improving ruminal fermentation and to balance ruminal microflora to enhance health and production. Moreover, they are considered a promising approach for methane mitigation. Although *Bacillus* species have demonstrated a potential to reduce methane production, their dose response in ruminal fermentation parameters remains unclear. The objective of this study was to evaluate the effects of increasing doses of a *Bacillus* spp. probiotic on enteric methane production, gas production kinetics, ruminal fermentation profile, and nutrient digestibility *in vitro*, using a sorghum silage-based substrate. Incubations were conducted in sixteen separate days (replicates) during 12 h and 24 h. Treatments included: Sorghum-silage only (Control) or sorghum silage + one of three doses of a *Bacillus* spp. probiotic included at **1**, **5**, or **10** times the recommended dose (8×10^4 CFU/mL). Ruminal fluid was collected from two ruminally cannulated Angus-crossbred steers fed an ad libitum silage-based diet. Gas production kinetics, pH, *in vitro* true digestibility, digestibility of neutral and acid detergent fiber, methane (CH₄) production and concentration of volatile fatty acids (VFA) and ammonia nitrogen (NH₃-N) were measured after 24 h of incubation. Digestibility of starch was measured at 12 h of incubation. Inclusion of *Bacillus* spp. did not affect ($P > 0.05$) nutrient digestibility or pH. Concentration of NH₃-N and total VFA concentration increased quadratically ($P < 0.05$) with the probiotic inclusion, reaching a maximum with the 5× dose. Total gas production and CH₄ emissions were not affected ($P > 0.05$) by the probiotic inclusion, while rate of gas production increased quadratically as probiotic dose increased ($P < 0.01$). These dose-dependent effects on ruminal fermentation show promising results when the probiotic was included at 5 times the recommended dose. Considering the effects on VFA, studies should be conducted to assess potential *in vivo* CH₄ mitigation capabilities.

Methane Emission in Holstein Animals and Mitigation with Shading from Photovoltaics Panel

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In this study, we measured the methane emission in the exhaled air (CH_4 , kg year^{-1}) of Holstein heifers, with access to a shading structure provided by photovoltaic panels (used as a resource to reduce heat stress and to generate electrical energy), using an indirect calorimetry system with a facial mask adjusted to the muzzle of the animals. Two experiments were conducted at the Biometeorology Laboratory at São Paulo State University (UNESP), Jaboticabal, Brazil ($21^{\circ}10' \text{ S}$, $47^{\circ}48' \text{ W}$, 546 m altitude). In the first experiment, twelve animals (six heifers weighing $220 \pm 12 \text{ kg}$ and six calves weighing $353 \pm 21 \text{ kg}$) were assigned to two 12×12 Latin Square designs (12 animals through 12 hours of the day). In the first Latin Square design, data were collected from 8:00h to 19:00h. In the second Latin Square design, data were collected from 20:00h to 7:00h. In the second experiment, the same animals were managed in paddocks to observe, using the instantaneous scan sampling method each 10 min, the time spent in the artificial shade from the photovoltaic panels (10 panels with 325 W each, projecting a total of 19.3 m^2 of shade) from 07:00 to 17:00h. Measured meteorological variables were solar irradiance, air temperature, relative humidity and wind speed. In the first hour of the day, heifers spent 90% of the time exposed to the solar radiation. As the solar radiation increased, the time spent in the artificial shade also increased. Between 09:00 to 13:00h, the animals spent about 80% of the time in the shade. During one year, total methane emission in the exhaled air was measured as 1.33 ton- CH_4 and the artificial shading structure using photovoltaic panels produced 5.19 MWh (monthly average of 432.33 kWh), saving US\$ 740 per year, and reducing the emission of 2.77 ton- CO_2 or 0.13 ton- CH_4 . In conclusion, the combination of livestock production and photovoltaic panels has the advantages of 1) improving animal welfare through reducing heat stress and 2) reducing the total methane emission of the facilities through generating electrical energy through renewable sources instead of fossil fuels.

Photovoltaic Panels as Shading Resources Enhance Environmental Sustainability for Feedlot Cattle

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Photovoltaic panels have been employed as shading resources for livestock, which in turn improve the thermal comfort and welfare of animals, while they can generate additional income to the farmers and enhancing environmental sustainability. In this study, we determined the enteric methane emission of feedlot cattle shaded with photovoltaic panels and environmental benefits in terms of reduction in CO₂eq emissions. The experiment was carried out in the Laboratory of Animal Biometeorology, at the São Paulo State University, Brazil. Thirty crossbred beef cattle (Nelore x Angus), with an average of 370 ± 22kg of body mass, and 17 months of age, were finished over 117 days (from 10/03/2021 to 01/28/2022), and randomly assigned in one pen without protection against solar radiation (S1; n=15), and in a second one shaded (S2; n=15) with photovoltaic panels (set of 10 panels with 300 W + 11 panels with 500 W) that provided shaded area of 2.8 m² animal⁻¹. The enteric CH₄ emission was measured by employing a flow-through indirect calorimetry system, with a non-ventilated facial mask. Six animals from each group were measured in four Latin square designs of six hours, over six days, by completing 24 hours of assessments for each animal. The S1 system had average emissions of 100g of CH₄ animal⁻¹ day⁻¹ or 176 kg of CH₄ animal⁻¹ for the entire period of confinement, against 92.75g CH₄ animal⁻¹ day⁻¹ or 162.75 kg of CH₄ animal⁻¹ for the S2 pen. Over the same period, 4.7 MWh of electricity was generated by the photovoltaic panels. Based on the CO₂ emission factor from the Brazilian National Integrated Electricity System of 0.6001 tCO₂/MWh, 2.8 tons of CO₂ were not emitted to the atmosphere. By converting the methane emission to CO₂eq, we have 316.8 kg of CO₂eq animal⁻¹ emitted in S1. On the other hand, for the S2, by subtracting the CO₂eq that were not emitted due to the photovoltaic panels, only 104.28 kg of CO₂eq animal⁻¹ were emitted, which represents reduction close to 70% in the CO₂eq emissions. In conclusion, artificial shading provided by photovoltaic panels in feedlot cattle appears to be a promising environmental management strategy to enhance sustainability and mitigating carbon emissions.

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Combined Effects of Dietary Fat, Nitrate, and 3-NOP on Dairy Cows' Enteric Methane Emission

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The objective was to investigate the combined effects of fat, nitrate, and 3-NOP on enteric methane production and production performance. 24 primi- and 24 multiparous lactating Holstein cows were included in an incomplete 8×8 Latin square design with six 21 d periods. Cows were blocked according to parity and days in milk. Diets were arranged in a 2×2×2 factorial design: 2 levels of fat (whole cracked rapeseeds) (low fat; 30 g crude fat (CF)/kg DM, or high fat; 63 g CF/kg DM), 2 levels of nitrate (calcium ammonium nitrate; Silvair) (0 g/kg DM or 10 g/kg DM; diets were isonitrogenous using urea) and 2 levels of 3-NOP (3-nitrooxypropanol; Bovaer) (0 mg/kg DM or 80 mg/kg DM). Methane emission was measured using GreenFeed. Data from the last 7 d of each period was averaged and included in the analysis, using a mixed procedure of R. For methane yield (g CH₄/kg DMI) there was no 3-way interaction between additives, however significant 2-way interactions were observed between fat, nitrate and 3-NOP. All three additives reduced methane yield, but effects were not additive and effect of combining additives did not exceed their individual effects. Methane yield varied from 16.7 for cows on low fat/no nitrate/no 3-NOP to 12.9 g/kg DMI for cows on high fat/nitrate/3-NOP. DMI was reduced by dietary supplementation and a 2-way interaction between fat and nitrate was observed. Addition of fat did not affect DMI, whereas addition of nitrate reduced DMI by 5%. However, combining fat and nitrate reduced DMI by 13%. 3-NOP reduced DMI by 13%. In conclusion, fat, nitrate, and 3-NOP are effective methane mitigation additives, but their individual effects were not additive, despite different modes of action. Potential DMI reductions may be a challenge for the implementation of otherwise effective methane mitigation additives, when feeding a Danish ration.

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Methane Potential of Manure from Dairy Cows Supplemented with Dietary Fat, Nitrate, and 3-NOP

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Potent feed additives reduce enteric methane emission from dairy cows. The aim of this study was to investigate if biogas potential of manure from methane mitigated dairy cows was also affected. As part of a larger experiment, the methane potential of manure was investigated, when dairy cows were supplemented with fat, nitrate, and 3-NOP as enteric methane mitigation additives. 24 primi- and 24 multiparous lactating Danish Holstein cows were included in an incomplete 8×8 Latin square design with six 21-day periods. Every cow received six of eight diets. Diets were arranged in a 2×2×2 factorial design: 2 levels of fat (whole cracked rapeseeds) (low fat; 30 g crude fat (CF)/kg DM, or high fat; 63 g CF/kg DM), 2 levels of nitrate (calcium ammonium nitrate; Silvair) (0 or 10 g/kg DM; diets were isonitrogenous using urea) and 2 levels of 3-NOP (3-nitrooxypropanol; Bovaer) (0 or 80 mg/kg DM). All treatments reduced enteric methane. Manure was sampled from individual cows during the last day in period two and five. Accumulated methane production was determined *in vitro* in a batch assay after 90 days of incubation and methane yield was expressed at standard conditions (STP). A total of 94 observations were analyzed, using a mixed procedure of R. In cows fed low fat diets, methane yield in manure was 231, 247, 242 and 214 L CH₄/kg volatile solids (VS) when no additives, nitrate, 3-NOP and nitrate+3-NOP were fed, respectively. However, in manure from cows fed high fat diets, the yield was 286, 282, 269 and 266 L CH₄/kg VS, when no additives, nitrate, 3-NOP and nitrate+3-NOP were fed, respectively. In conclusion, dietary fat was the only factor significantly affecting the methane yield in manure, which was increased by 18% for cows fed high fat diets compared to cows fed low fat diets.

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Low N₂O Emission Associated with Deposition of Sheep Excreta in Irish Grasslands

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Grazing ruminants deposit urine and feces onto pasture soils, which can cause emissions of a potent greenhouse gas, nitrous oxide (N₂O). IPCC provides default (Tier 1) emission factors (EF; proportion of deposited nitrogen emitted as N₂O) associated with ruminant excreta deposition. However, due to great spatial and temporal variation, development of country-specific (Tier 2) EFs is encouraged. Ireland has already generated a Tier 2 EF for cattle but no data are available for sheep. Therefore, the aim of this study was to generate Tier 2 EFs for N₂O emissions from sheep excreta deposition in Irish grasslands, as well as disaggregate emissions by type of excreta, type of grassland and season of application. An experiment was carried out on two sites in the west of Ireland: a well-managed lowland grassland on mineral soil and a rough-grazed hill-land pasture on acid peat soil. Four treatments were applied to the soil in a fully randomized block design: control (C), sheep urine (U), dung (D), and artificial urine (AU) in spring, summer and autumn. Static chambers were used to measure N₂O for full year after each application. On the lowland site, a peak of N₂O was observed within the first days after U and AU application for each season, and there was no significant effect of treatment and season on N₂O emissions. Average EFs were close to zero: -0.003 and -0.007 % for urine and dung, respectively, thus lower than the 2019 IPCC Tier 1 EFs. Initial results from hill-land showed no peak of N₂O following treatments application and significantly lower emissions compared to lowland. These findings suggest very low N₂O emissions from sheep excreta deposited in Irish grasslands and highlight the importance to develop Tier 2 EFs. Further research is required to explore the fate of nitrogen in these soils.

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Correlations between Methane Emissions and Production Traits in Australian Merino Sheep

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Variability of methane (CH_4) emission in sheep and impact of selecting low emitting individuals are investigated as part of greenhouse gas mitigation strategies. This study aimed to estimate the correlations between CH_4 and different production traits in Australian Merino. Using portable accumulation chambers, data of CH_4 emissions of 863 animals born between 2018 and 2020, sired by 19 rams was collected. Animal solutions for CH_4 (g/d) were generated using a repeated measure model including sex-pen-trial as fixed effect, and animal and date-hour of the measure fitted as random effects. For estimating correlations with CH_4 , the traits selected were related to feed efficiency (feed intake, residual feed intake - RFI), growth (average daily gain - ADG, metabolic weight - MWT, yearling body weight - BW), carcass quality (rib-eye area - REA, fat thickness - FT), feeding behavior (number of meals); fecal egg count (Log_e FEC), and wool production (staple length - SL, greasy fleece weight - GFW, fiber diameter - FD). Residuals of the mentioned traits were estimated using a model including age, type of birth and sex-pen-trial as fixed effects, with the exception of RFI. The coefficients of correlation indicated that CH_4 was not associated with GFW and FD residuals ($p > 0.05$). Significant ($p < 0.05$) but low correlations were estimated for SL, Log_e FEC traits, RFI, FT and number of meals, with values ranging from 0.09 to 0.15. Higher correlations were found between CH_4 and REA and BW (0.23 and 0.29, respectively). The strongest associations were with ADG (0.36), feed intake (0.45) and MWT (0.46). In agreement with the correlations with growth and feed intake, a positive correlation (0.14) between CH_4 and RFI, indicates that high emitters may present lower feed efficiency.

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Methane Contrasting Groups in Three Sheep Breeds in Uruguay

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Breeding sheep for low methane (CH₄) emissions is an attractive mitigation strategy which implementation requires exploring the impact on other traits. This study aimed to explore these associations by comparing the performance of animals classified by CH₄ emission. Data of CH₄ emissions of 494 animals (218 Corriedale; 68 Texel; 208; Dohne Merino) born between 2018 and 2020, sired by 29 rams was collected using portable accumulation chambers. With the objective to allocate animals to contrasting CH₄ groups, CH₄ (g/d) was analyzed with a repeated measure model including sex-pen-trial as fixed effect, and animal and date-hour of the measure fitted as random effects. Then, adjusted CH₄ emission (animal solutions) was used to define three groups: low (<25%), medium and high (>25%). The effect of CH₄ group on different traits was estimated with a linear model that included age, type of birth and sex-pen-trial as fixed effects. The analyzed traits in this trial were related to feed efficiency (feed intake, residual feed intake - RFI), growth (average daily gain - ADG, metabolic weight - MWT), carcass quality (rib-eye area - REA, fat thickness - FT), feeding behavior (number of meals) and wool (staple growth - SG). CH₄ group had a significant effect ($p < 0.05$) on feed intake, ADG and MWT in all breeds. High emitters were heavier, had higher ADG, and ate more. A significant effect on number of meals was reported in Texel, showing that low CH₄ emitters had lower number of meals compared to high emitters. Non-significant differences ($p > 0.05$) were observed on RFI, REA, FT and SG in any breed. More research will contribute to increasing the number of animals tested, leading to a more comprehensive description of associations, including genetic and phenotypic correlations, being also the basis for economic impact studies.

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Microbiome-Driven Breeding Strategy to Mitigate Methane Emissions in Beef

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The aim of this study was to explore the possibility of using specific microbial gene abundances in the rumen to predict individual breeding values for emissions of the potent greenhouse gas methane (CH₄). Our approach provides an efficient strategy for integrating microbiome information into future breeding programs to mitigate CH₄ and predicts its expected response to selection when this microbiome-driven breeding strategy is applied on data of our Beef Research Centre. Our data included 359 animals with genomic (37K SNPs), metagenomic (1,107/225/1,141 rumen microbial genera/metagenome-assembled uncultured genomes (RUGs)/genes identified), and CH₄ emission information collected in respiration chambers. We found that substantial variation in the microbiome is under the influence of the host genome by estimating significant host genomic effects for 194/14/337 rumen microbial genera/RUGs/gene abundances with heritabilities ranging from 0.13 to 0.61. It was also revealed that 29/22/115 microbial genera/RUGs/genes were host-genomically correlated (from |0.59| to |0.93|) with CH₄ emissions, highlighting the strength of a common host genomic control of specific microbial processes and CH₄. Only one of these microbial genes was directly involved in methanogenesis (*cofG*), whereas others were involved in providing substrates for archaea (e.g. *bcd* and *pccB*), important microbial interspecies communication mechanisms (*ABC.PE.P*), host-microbiome interaction (*TSTA3*), and genetic information processes (*RP-L35*). In our research population, selection based on the abundance of the 30 most informative microbial genes provided a mitigation potential of 17% of average CH₄ emissions per generation. This is higher than selection based on CH₄ measurements using respiration chambers (13%) and indicates the high potential of microbiome-driven breeding to cumulatively reduce CH₄ emissions and mitigate climate change.

A Combination of *Capsicum oleoresin* and Botanicals Decreased Enteric Methane Emission in Dairy Cows

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The objective of this study was to investigate the effects of a combination of capsicum oleoresin (CAP) and botanicals (BTC) on lactational performance and enteric methane emissions in dairy cows. Twenty Holstein cows (days in milk 77 ± 28 at the beginning of the experiment; 12 multiparous and 8 primiparous) were used in a replicated 4×4 Latin square design experiment with four 28-d periods. Cows were randomly assigned to 1 of 4 treatments: control (CON) or 150, 300, or 600 mg/cow/d BTC. Cows received the same basal diet and BTC was top-dressed on the TMR. Enteric methane emission was measured using the GreenFeed System. Data were analyzed using the MIXED procedure of SAS with square and cow within square as random effects. Polynomial contrasts were used to test for linear and quadratic effects of BTC dose. Compared to CON, BTC linearly decreased daily methane emission ($P = 0.04$; average for CON and BTC treatments: 389 vs. 364 g/d, respectively; SEM = 11.8), yield ($P = 0.05$; 14.0 vs. 13.3; SEM = 0.53) and tended ($P = 0.08$) to decrease emission intensity (10.5 vs. 9.96 g/kg energy corrected milk; SEM = 0.57). Dry matter intake (average 27.8 kg/d; SEM = 1.25) was not affected by BTC supplementation and consequently, lactational performance (average 37.2 kg/d energy corrected milk; SEM = 1.49) did not differ among treatments. Supplementation with BTC tended ($P = 0.06$) to increase milk fat concentration (3.80 vs. 3.95; SEM = 0.19; average for CON and BTC treatments, respectively) and milk urea nitrogen ($P = 0.08$; 10.6 vs. 11.0 mg/dL; SEM = 0.33, respectively) and had no other effects on milk composition. In this study, methane yield and intensity were decreased or tended to be decreased by 5 to 6% by BTC compared to CON, suggesting a potential moderate mitigation effect of BTC on carbon footprint of milk.

Keywords: plant extract, enteric methane, dairy cow.

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Carbon Footprint of Specialized Dairy Farms in Cundinamarca, Colombia, using a Life Cycle Assessment Approach

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In Colombia, cattle production is responsible for 55% of greenhouse gas emissions (GHGE) from the Agriculture, Forestry, and Other Land Use sector. Specialized dairy farms account for 15% of the Colombian cattle inventory and contribute to 29% of national milk production. Cundinamarca belongs to the most productive Colombian dairy basin, which contributes 39% of total milk production from specialized dairy systems in the country. Life Cycle Assessment (LCA) studies of GHGE from specialized dairy farms have not been widely performed, restricting the establishment of climate change mitigation actions for the cattle sector in Colombia. This study calculated the carbon footprints (CF) of 83 specialized dairy farms in Cundinamarca, Colombia, by using the LCA methodology. Farms were located in 3 cattle regions: Ubaté Province (n=33), Sabana Centro (n=32), and Sabana Occidente (n=18). In a “cradle to farm gate” perspective, GHGE were calculated using the 2019 Refinement to the 2006 IPCC Guidelines, and locally estimated emission factors. A biophysical allocation method was used for handling co-products leaving the farm; one kg fat and protein corrected milk (FPCM) and one kg live weight gain (LWG) corresponded to the functional units for milk, and LWG, respectively. The CF (kg CO₂-eq kg⁻¹FPCM) for Sabana Centro (1.4 FPCM) and Ubaté Province (1.3 FPCM) were the lowest among cattle regions. Sabana Occidente showed the highest CF (1.8 FPCM). Most of the farms in the three cattle regions were characterized by implementing good pasture and herd management practices. Although milk production was higher in Sabana Occidente (6208 kg FPCM cow⁻¹yr⁻¹), its higher percentage of unproductive animals in the herd could have negatively affected the CF. The CF of milk could be reduced by: increasing productivity by using strategies supplementation with local resources, introducing silvopastoral systems, adopting good agricultural practices, and reducing the share of unproductive animals in the herd.

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Modelling the Interaction of Nutritional and Bioactive Compounds of Forages on Gas and Methanogenesis Kinetics

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Livestock are strongly dependent on the nutritional chemistry of forage plants. Methanogenesis is the main biochemical pathway for the removal of metabolic hydrogen released from fermentation in the rumen. A natural mitigation alternative in animal nutrition is assessed disponible nutrients in conjunction with biological activity of compounds such as saponins, tannins, essential oils, alkaloids, flavonoids, saponins and other metabolites that would have potential to inhibit methane (CH₄) production in the rumen. Inferring the relationships between nutritional quality and active compounds is crucial to understand this complex system and to establish if their interaction plays a role in methane production. The aim of this study was to develop mathematical models related to kinetics of gas production and methanogenesis affected by bioactivities of secondary compounds and chemical composition of forages from dairy farms of Cundinamarca high tropic of Colombia. Graphical models will be a suitable tool to accomplish the aim; they represent the system through a graph where nodes correspond to random variables and edges to relationships among them. In a partial correlation graph or network (PCN), relationships between pairs of variables exist if the corresponding partial correlation is not null. The forages were collected from 83 dairy farms. In total, 1318 forages were sampled and 200 samples were statistically selected by using K-Medias, Ward, and DBSCAN methods. Chemical composition was analysed by NIRS. The fermentations were carried out by using wireless, fully automated ANKOM gas production system over 72 hrs. The gas samples were collected at 4, 8, 12, 24, 48 and 72 h to determine methanogenesis kinetics and liquid phase samples were also collected to evaluate soluble carbohydrates, ammonia, and volatile fat acids. The study expects that modelling forages with methane-suppressing potential will allow us to understand their role to play in mitigation of enteric CH₄ for smallholder's conditions in Cundinamarca high tropic.

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Phenotypic Factors Affecting Methane Output Measured in Irish Sheep Production Systems

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Maximizing production per hectare is paramount to the profitability of pasture based production systems. However, pressures on ruminant agricultural enterprises to become more sustainable and reduce overall methane output is at a crescendo. In order for farmers to maintain efficient production while becoming more environmentally sustainable, a deeper understanding of methane (CH₄) emissions within their system is required. Therefore, the objective of this study was to investigate the phenotypic factors affecting enteric CH₄ output from Irish pasture based sheep production systems. Individual sheep methane output was measured using portable accumulation chambers (PAC) whereby each animal entered the chamber for a 50 minute period during which time CH₄ concentration was recorded at 0, 25 and 50 minutes from entry. All animals were removed from feed at least one hour prior to entering the PAC and had their live-weight recorded. A total of 7,027 CH₄ measurements were collected from 2,404 animals across four flocks from 2019-2021. Animals were classified as lambs (<12 months; n= 2,151 records), nulliparous hoggets (12 to 24 months; n=763 records) and ewes (primiparous or greater; 24 to 174 months; n= 3455) which were further classified as lactating or dry (non-pregnant and non-lactating). Factors associated with animal production and CH₄ output were determined using linear mixed models. A multiple regression model was also built up using stepwise forward-backward regression, including interactions of biological interest; the significance threshold for entry and exit of variables into/from the model was set at 1%. Factors associated with CH₄ production were animal life stage, diet type, breed, time from feed removal, atmospheric pressure, relative humidity, PAC chamber number, age and live-weight (P<0.001). Results highlight the multiple animal and environmental factors affecting CH₄ production and will enable generation of more accurate genetic indexes for CH₄ output in sheep systems.

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Delivering Sustainability and Resilience in Agriculture and Food Systems (Food Futures)

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Tools measuring sustainability are essential to deliver positive behavioral change, address climate change and build resilience. Focused on agricultural systems, Food Futures has identified appropriate metrics across key areas of social, environmental and economic sustainability; delivering a SMART tool that measures sustainability and supports farm management/decision making – using livestock farms in Northern Ireland as a model system. Food Futures integrates digital platforms, for example, Geographical Positioning Systems, Light Detection and Ranging (LiDAR) and Life Cycle Analysis to stream various data layers from multiple sources to improve soil health, water quality (catchment areas), carbon sequestration, precision nutrient applications and on-farm habitat areas, for example, areas of special scientific interest and special protection areas. More specifically, building upon literature/legislative reviews and expert input, Food Futures has developed multiple sustainability-wide metrics (i.e. carbon footprint, gross margin per-hectare, farm-family well-being) with each composed of a sustainability index. Subsequent performance reports and artificial intelligence modelling facilitate data provision to farmers of their performance against these metrics – with methods to improve on-farm sustainability credentials. These analyses have informed and co-created the Food Futures digital SMART tool; a dashboard composed of a suite of climate-smart mitigation measures and a narrative for on-farm sustainability to enable continuous knowledge-exchange (measure, report and verification) with farmers on their sustainability accreditation.

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Evaluation of Two Essential Oil Blends to Reduce Enteric Methane Emissions in Feedlot Steers

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Twenty-four Angus cross steers (bodyweight; BW = 360 ± 10 kg) were used in a randomized complete block design experiment, with the objective of evaluating the efficacy of 2 essential oil (EO) blends to reduce enteric CH₄ emissions from feedlot steers. Steers were blocked by BW and allocated in 3 pens (8 steers/pen) where treatments were randomly assigned. Treatments were control (CT) receiving a basal feedlot diet, basal diet supplemented with either Agolin® at 1 g (AG) or Mootral® at 23.5 g (MT). Treatments were delivered twice a day as top dress after feeding. Ground corn was used as a treatment carrier. Baseline and on-trial CH₄, CO₂ and H₂ measurements were collected with the GreenFeed machine. Dry matter intake (DMI) was recorded daily using the Roughage Intake Control system. Data were analyzed with R statistical software (4.1.1). The linear mixed effect models procedure within the “lme4” package was used with steer as the experimental unit ($n = 8/\text{treatment}$) and block as the random variable. There were no treatment \times time interactions ($P > 0.10$), nor treatment effects ($P > 0.10$) for CH₄ variables. Initial and final BW were not affected by treatment ($P > 0.10$). There was a treatment \times week interaction ($P = 0.039$) for DMI which disappeared when a pairwise comparison was done ($P > 0.10$). There were no interactions of treatment \times week ($P > 0.10$) nor an effect of treatment ($P > 0.10$) on BW, average daily gain, and gain:feed. No effects were observed on hot carcass weight, dressing percentage, and fat thickness ($P > 0.10$); however, a tendency ($P = 0.066$) for MT to reduce marbling score was observed. More research, especially long term trial is warranted to elucidate the efficacy of EO as feed additives to reduce enteric CH₄ emissions from feedlot cattle.

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Replacing Grains with Potato Waste in Feedlot Cattle: Greenhouse Gas Emissions and Land Use Requirements

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This study evaluated greenhouse gas emissions (GHG) and land use requirements associated with standard grain-based feedlot diets with or without potato waste. A whole farm model, Holos (www.agr.gc.ca/holos-ghg), was used to estimate emissions from animals and crops grown on simulated feedlot farms located in western (Manitoba) and eastern (Ontario) regions of Canada. Feeding scenarios examined were for weaned calves: i) sent directly to feedlot; ii) backgrounded in confinement, followed by feedlot finishing; or iii) backgrounded in confinement, grazed on pasture, and then sent to feedlot. Standard feedlot diets included barley grain and barley silage in the west, and corn grain, corn silage and soybean meal in the east. Potato waste replaced barley grain and corn grain at 15% and 30% on dry matter basis during the feedlot finishing phase. Across cattle categories in the west, total GHG emission intensity was reduced by 2.1-4.8% with 2.36, 2.31, and 2.24 kg CO₂e/kg live weight, for the standard diet, 15% and 30% potato inclusion, respectively. Similarly, reduction in the east was 2.2-4.6% with 1.65, 1.61, 1.57 kg CO₂e/kg live weight, for the standard diet, 15% and 30% potato inclusion, respectively. Although potato waste inclusion reduced net farm emissions due to decreases in direct, indirect and energy emissions mainly from cropping, it also resulted in higher methane emissions as a result of lower dietary total digestible nutrients. Using potato waste also reduced land required for feed production by 16% and 31% (west) and 14% and 28% (east) at 15% and 30% inclusion, respectively. Results suggest that feeding potato waste could reduce GHG emissions through significant reductions in land required to produce grain in standard feedlot diets. Furthermore, utilization of potato waste in cattle diets re-directs waste away from landfills, mitigating emissions and eliminating disposal cost, while producing high quality animal protein.

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Can an Automated In-paddock Feeder Successfully Deliver a Methane Inhibitor to Grazing Dairy Cattle?

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Feed additives that reduce methane emissions from ruminants (inhibitors) have been shown to be effective in feedlot systems. This is because inhibitors often have a temporal effect in the rumen (~ 1-6 hours) and are most efficacious when consumed regularly. So how can inhibitor technology be integrated into grazing systems? In-paddock automated feeders can precisely allocate feed to individual animals and thus may be suitable for delivering inhibitors to grazing livestock. The objective of this study was to determine the ability of an automated feeder to deliver a small amount of supplement six times per day to grazing dairy cows. 150 lactating dairy cows were divided into three groups, balanced for age and live weight. Each group had access to one automated feeder, but with differing number of feed stations accessible per feeder. One group had access to all four feed stations, the second had three and the third had only two feed stations accessible, thus increasing the competition for feeder access by the cows. For two five-week periods during mid and late lactation, feeder access, supplement intake and behaviour was monitored. Results are forthcoming and will determine the proportion of a herd of dairy cows that can successfully access an automated feeder delivering a supplement multiple times per day, informing how many units a farm of a given size would require. The study will report on effect on the cows' daily time balance, behaviour, and pasture intake. These results will inform whether an in-paddock automated feeder could potentially be used to deliver a methane inhibitor in a pellet form in grazing systems.

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Effect of Substituting a Ryegrass-based Diet with Plantain (*Plantago lanceolata*) on Methane Emissions from Dairy Cattle

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The forage herb, plantain (*Plantago lanceolata*) has become increasingly common as a pasture species as it has been identified as a tool to reduce the risk of freshwater pollution from livestock grazing systems. Little is known, however, of the effect of diets containing plantain on enteric methane (CH₄) emissions from ruminants. The objective of the studies reported was to assess CH₄ emissions from dairy cattle fed ryegrass (*Lolium perenne* L.)-based diets supplemented with varying proportions of plantain. In both studies, dairy cattle were housed and individually fed to determine feed intake. CH₄ measurements were performed using GreenFeed units (C-Lock Inc., Rapid City, SD, USA). Experiment 1 offered Holstein Friesian heifers ryegrass-based silage substituted with 0, 15 and 45% fresh plantain for 42 days beginning March 2018. Relative to cows fed no plantain, total daily CH₄ (DMP; g/d) increased when plantain comprised 15% of the diet, associated with greater DMI. When plantain comprised 45% of the diet DMI increased, however, DMP was similar, but methane yield (g CH₄/kg DMI) was reduced by 13% ($P < 0.001$) relative to heifers not fed plantain. Experiment 2 offered lactating Friesian X Jersey dairy cows' fresh ryegrass-based diets substituted with 0, 40 and 80% fresh plantain in early and late lactation for 49 days. Methane yield was not affected by plantain substitution in early lactation, but in late lactation diets of 80% plantain reduced CH₄ yield by 18% and CH₄ intensity (g/kg milk) by 24% ($P < 0.001$). These studies show that there is potential to reduce CH₄ emissions from dairy cattle by including plantain in the diet, but the levels required might pose a challenge for integration into the farm system and further work is required to understand the variation in seasonal response.

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Rethinking Methane from Animal Agriculture

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As the global community actively works to keep temperatures from rising beyond 1.5°C, predicting greenhouse gases (GHGs) by how they warm the planet – and not their CO₂-equivalence – provides information critical to developing short- and long-term climate solutions. Livestock, and in particular cattle, have been broadly branded as major emitters of methane (CH₄) and significant drivers of climate change. Livestock production has been growing to meet the global food demand, however, increasing demand for production does not necessarily result in the proportional increase of CH₄ production. The present work intends to evaluate the actual effects of the CH₄ emission from U.S. dairy and beef production on temperature and initiate a rethinking of CH₄ associated with animal agriculture to clarify long-standing misunderstandings and uncover the potential role of animal agriculture in fighting climate change. Two climate metrics, the standard 100-year Global Warming Potential (GWP₁₀₀) and the recently proposed GWP*, were applied to the CH₄ emission from the U.S. cattle industry to assess and compare its climate contribution. Using GWP*, calculations show that CH₄ emissions from the U.S. cattle industry have not contributed to warming since 1986. The projected climate impacts show that the California dairy industry will approach climate neutrality in the next ten years if the current cow inventory holds constant, with the possibility to decrease warming if there are further reductions of emissions. GWP* should be used in combination with GWP to provide informative strategical suggestions on fighting SLCPs-induced climate change. By continuously improving production efficiency and management practices, animal agriculture can be a short-term solution to fight climate warming that the global community can leverage while developing long-term solutions for fossil fuel carbon emissions.

Greenhouse Gases Mitigation Through the Insertion of Forestry Component in Forage-Based Beef Cattle Production Systems

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It is necessary to establish sustainable strategies that increase livestock productivity and concomitantly reduce greenhouse gas (GHG) emissions. The objective of this study was to estimate the net carbon balance in two tropical forage-based beef cattle production systems. The study was carried out at the Embrapa Agrossilvipastoril in Sinop, MT, Brazil. Two systems were evaluated: livestock (L), with *Urochloa brizantha* cv. Marandu and livestock-forestry (LF) with Marandu intercropped with eucalyptus trees (*Eucalyptus urograndis* clone H13), in triple rows, with an east-west orientation and a 30 x 3.5 m spacing (30 m between rows and 3.5 m between trees within rows), resulting in a population density of 135 trees/ha. Twenty-four Nelore steers were evaluated in each system. The GHG emissions were estimated using: CH₄ (enteric fermentation, dung, and soil); N₂O (soil and dung), and fossil CO₂. Enteric CH₄ emissions were measured with the GreenFeed system. For C accumulation the C stocks from trees and soil were considered. The C footprints were 10.7 and 11.2 kg CO₂eq/kg carcass for L and LF systems, respectively. In both systems, the largest GHG emission was from enteric CH₄ (55% in L and 60% in LF). The L and LF system had a negative C balance of -27.0 and -1.5 Mg CO₂eq, respectively. With these results, we conclude that the insertion of the forestry component in the forage-livestock systems potentially contributes to mitigating GHG emission, supporting Brazilian public policies, such as 'Low Carbon Agriculture' for the adoption of sustainable production technologies in response to the country's commitment to reduce GHG emissions in the agricultural sector.

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Ammonia and Greenhouse Gas Emissions from Broiler Chickens can be Reduced by Growing Younger Birds

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Emissions of ammonia (NH₃) and greenhouse gases (GHG) from poultry farms have become of increasing interest during the past decade. The objectives of this research were to: (1) quantify emissions of NH₃, nitrous oxide (N₂O), methane (CH₄), and carbon dioxide (CO₂) from broiler houses, and (2) estimate the amount of gases produced per kilogram of bird grown for flocks of various duration to determine if emissions per kg vary with age. Two broiler houses in NW Arkansas were used. Houses were equipped with gas sampling ports connected to an Innova multi-gas analyzer for continuous monitoring of the gases. Ventilation from all fans in both houses was also continuously monitored. Concentrations and emissions of the four gases were measured during 4-week flocks (n=12), 7-week flocks (n=3) and 8-week flocks (n=4). Average emissions per kg bird for the 4-week flocks were 2.9 g NH₃/kg, 0.3 g N₂O/kg, 3.5 g CH₄/kg, and 658 g CO₂/kg. Ammonia emissions per kg increased by 179 and 293% for 7- and 8-week-old birds, respectively, compared to the 4-week old birds. Nitrous oxide emissions per kg increased by 164 and 387% for 7- and 8-week old birds, respectively. Methane emissions increased by 35 and 225% for 7- and 8-week birds, respectively. Carbon dioxide emissions increased by 185 and 351% for 7- and 8-week birds, respectively. Higher emissions from older birds are probably due to less efficient feed conversion as birds age. When emissions were put on a protein basis, significant differences were still present between all ages for CO₂ emissions, however, NH₃, N₂O and CH₄ emissions were not different between 7- and 8-week-old birds. These results strongly indicate that growing younger (i.e. - smaller) chickens may be more sustainable, since it results in much lower emissions of NH₃ and GHGs per kg of bird produced, while utilizing less feed.

Conductive Materials Stimulate Ruminal Methanogenesis and Induce Microbial Changes Indicative of Improved Electron Transfer

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Methane production in the rumen is characterized by microbial interspecies electron transfer. The dominant process involves the electron shuttles dihydrogen and formate produced by bacteria and protozoa. These electron shuttles are then used by their syntrophic archaeal partners to produce methane. In the rumen, however, the extent and importance of shuttle-free electron transfers, described in other methanogenic environments, is not known. We tested the effect of conductive materials for highlighting possible direct interspecies electron transfer (DIET) in the rumen ecosystem. We used *in vitro* and in sacco experiments to evaluate rumen fermentation and shifts in microbial community composition, respectively. The *in vitro* batch culture technique used four cannulated sheep as donors of ruminal fluid. Treatments were substrate alone (Control, 400 mg alfalfa:wheat; 3:1 ratio in 40 mL buffer-rumen fluid mixture) or supplemented with 5 and 10% (substrate basis) of granular activated charcoal (GAC), graphene (GPH) or magnetite (MAG) powders. For the in sacco experiment, membranes of the inert polymer polydimethylsiloxane containing conductive materials were incubated in the rumen of four sheep for 1, 7, and 28 days followed by amplicon sequencing. *In vitro* methane production increased by 8.2% with GPH_10% and by ~7.4% with MAG_5% or MAG_10% as compared to control ($P=0.002$). In contrast, total gas and VFA production and proportions of VFA were unaffected by conductive materials ($P>0.05$). For microbes adhering to membranes following rumen incubation, main differences were observed for the Euryarchaeota phylum with higher relative abundance for GAC and GPH compared to control. Concurrently, major discriminant taxa were mainly associated to the Euryarchaeota. The increase in methane production without other fermentation changes and the shift in microbes associated to conductive materials may suggest a DIET mechanism. DIET should be validated with additional tools and its extent in the rumen assessed as it could be another mechanism to consider for modulating methanogenesis.

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Interaction between Concentrate Type and Pasture Mass on Methane Emission of Grazing Dairy Cows

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Supplementation with wheat-based concentrates and grazing a pasture mass of ~2200 kg dry matter/ha are feeding strategies that can decrease enteric methane emissions of dairy cows. The objective of this study was to evaluate the effects of combining these methane mitigation strategies to enhance their mitigation potential. We randomly assigned 48 Holstein Friesian grazing dairy cows to one of four treatments arranged in a 2 × 2 factorial design: low (2000 kg DM/ha) or high (3500 kg DM/ha) pregrazing herbage mass, crossed with supplementation with 6 kg/d of a wheat- or corn-based concentrate (composing 61% of concentrate DM) i.e. high herbage mass and corn (HPC), high herbage mass and wheat (HPW), low herbage mass and corn (LPC), and low herbage mass and wheat (LPW). The low herbage mass pasture treatments had higher crude protein ($P < 0.01$) and a tendency to lower ADF contents ($P = 0.06$). Milk production was higher ($P < 0.05$, +2.9 kg/d) with the LPW than with the HPW treatments, but did not differ from the LPC or HPC treatments. Total methane emissions were 9.7% lower for the low herbage mass than for the high herbage mass pasture treatments ($P < 0.01$). Methane emissions intensity was lower ($P < 0.05$, 26.0%) with the LPW than with the HPW treatments, differences that were not observed between the corn treatments. In conclusion, a lower herbage mass decreased methane production and intensity compared to a higher herbage mass, but supplying wheat-based concentrates did not decrease methane compared to corn-based concentrates, although methane intensity levels with wheat were affected by pasture mass. At the levels applied in this study, the combination of lower pasture mass pregrazing and wheat supplementation applied together to decrease methane emissions does not offer additional benefits than their separate effects.

Nitrous Oxide Emissions from a Grazed Grassland: Using Eddy Covariance and Static Chambers in Tandem

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Quantifying nitrous oxide emissions from grazed pastures can be problematic due to the presence of hotspots and hot moments of nitrous oxide from animal excreta and synthetic fertilizers. In this study, we quantified field scale nitrous oxide emissions from a temperate Irish grassland under a grazing management using eddy covariance and static chamber techniques in tandem. Measurements of nitrous oxide by static chambers were made for four out of nine grazing events for a control, calcium ammonium nitrate, synthetic urine + calcium ammonium nitrate, and dung + calcium ammonium nitrate treatments. Nitrous oxide flux measurements made by static chambers were upscaled to the field scale for comparability with eddy covariance flux measurements using site specific emission factors from the treatments outlined above. Mean nitrous oxide emission factors were greatest from the calcium ammonium nitrate treatment while synthetic urine + calcium ammonium nitrate and dung + calcium ammonium nitrate emitted similar nitrous oxide emissions. Upscaled cumulative nitrous oxide emissions were derived mainly from animal excreta (dung and urine) accounting for 50% of total emissions, while emissions from calcium ammonium nitrate and background accounted for 36 and 14%, respectively. Cumulative nitrous oxide emissions measured by upscaled static chamber measurements were lower than gap-filled EC measurements. The EC technique was most accurate in quantifying nitrous oxide emissions, showing a range of uncertainty that was seven times lower relative to static chamber measurements. High uncertainties in static chamber flux measurements were due to the small chamber sample size per treatment (n = 5) and the high spatial and temporal variability in measured nitrous oxide fluxes. This study highlights the advantage of using both the eddy covariance and static chamber techniques in tandem to better quantify total nitrous oxide losses from grazed pastures while also constraining the contribution of individual nitrogen sources.

Quantifying Nitrous Oxide Emissions in Time and Space Using Static Chambers and Eddy Covariance

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Where nitrogen input from fertilizer application exceeds plant demands, hotspots of microbially produced nitrous oxide can exhibit disproportionately high rates of emissions relative to longer periods of time, known as hot moments. Hotspots and hot moments of nitrous oxide are sensitive to changes in agricultural management and weather, making it difficult to accurately quantify nitrous oxide emissions in space and time. This study investigates the spatial and temporal variability of nitrous oxide emissions using both static chambers and eddy covariance techniques, measured at a temperate managed grassland site in south-east Ireland. The field site received four fertilizer applications of calcium ammonium nitrate in 2019. Due to the inherently log-normal distribution of static chamber nitrous oxide flux measurements, Bayesian statistics were used to calculate daily mean flux values in addition to arithmetic statistics. Our results showed that nitrous oxide fluxes measured by static chamber and eddy covariance were most comparable when (1) flux measurements were high (i.e. greater than $115 \text{ N}_2\text{O-N } \mu\text{g m}^{-2} \text{ hr}^{-1}$), (2) when eddy covariance and static chamber measurements showed spatial and temporal alignment, and (3) when the static chamber sample size was large ($n \geq 15$). Where the static chamber sample size was small ($n \leq 5$), the Bayesian method produced large uncertainties due to the difficulty of fitting an arithmetic mean from a log-normally distributed data set with few flux measurements. Cumulative nitrous oxide emissions by gap-filled eddy covariance measurements were higher than static chambers fluxes calculated by the arithmetic and Bayesian method, and with lower uncertainties. This study implies that a large sample size and frequent static chamber flux measurements are necessary for comparison with eddy covariance fluxes and that Bayesian statistics are an appropriate method for estimating realistic means and ranges of uncertainty for static chamber flux data sets.

Impact of *Haemonchus contortus* Infection of Red Maasai and Dorper Lambs on Enteric Methane Emissions

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There is growing evidence about the impact of animal health on animal productivity and GHG emissions. It is well known that diseases lead to increased greenhouse gas emission intensities. However, recent studies indicate that diseases such as gastrointestinal parasitism increase methane yield. An animal experiment was conducted with two sheep breeds (Red Maasai and Dorper) to evaluate the effects of infection with the gastrointestinal worm, *Haemonchus contortus* on feed intake, liveweight gain, feed energy and nitrogen partitioning, rumen fermentation and enteric methane emission. Six- to seven-month-old lambs were used in the experiment, they were fed chopped Rhodes grass (*Chloris gayana*) hay and provided with water and a mineral lick. The experiment was a change-over design with three experimental treatments and feeding regimes, within each breed (ad libitum uninfected (AL-uninf); ad libitum infected (AL-infec); restricted uninfected (RE-uninf)). Four lambs from each breed were trickle infected with 1000 L₃ stage larvae of *H. contortus* for 4 consecutive days (AL-infec), two other treatment groups (AL-uninf and RE-uninf) were kept worm free. The lambs were kept in metabolic crates for 5 days after a two-day adaptation period for total faeces and urine collection while methane measurement was conducted in respiration chambers for 3 consecutive days. Liveweight (LW), faecal egg counts, and packed cell volume measurements were carried out at the start of the experiment and weekly thereafter. Contrary to our expectations, preliminary results show that infection with *H. contortus* did not reduce the lamb's dry matter intake (DMI) or increase methane yield (emission per unit of DMI, g CH₄/kg DMI). AL-Infec Dorper lambs lost 22 g/day while their Red Maasai counterparts gained 6 g/day. Furthermore, infection did not influence methane production, the AL-uninf Dorper lambs had the highest methane production at 18.7g/day while the RE-uninf Red Maasai had the lowest at 13.2 g/day. The AL-uninf Dorper had a significantly higher methane yield (31.4 g CH₄/Kg DMI) than all the other treatments, uninfected lambs had a significantly higher methane yield than infected lambs ($P < 0.05$). Energy and N balance data together with rumen function data are yet to be analyzed and may give more insight into the study results. The data collected will provide information to design interventions to reduce environmental footprint of extensive sheep farming.

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Greenhouse Gas Emissions from South Dakota Dairy Production

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Livestock production contributes to greenhouse gas (GHG) emissions. However, there is a considerable variability in the carbon footprint associated with livestock production. Site specific estimates of GHG emissions are needed to accurately focus GHG emission reduction efforts. A holistic approach must be taken in order to assess the full environmental impact of livestock production using appropriate geographical scale. The objective of this study was to determine baseline GHG emissions from dairy production in South Dakota using a life cycle assessment (LCA) approach. A cradle-to-farm gate LCA was used to estimate the GHG emissions to produce 1 kg of energy-and-protein corrected milk (ECM) in South Dakota. The system boundary was divided into feed production, farm management, enteric methane emissions, and manure management as these activities are the main contributors to the overall GHG emissions. The production of 1 kg ECM in South Dakota dairies was estimated to emit 1.22 kg CO₂ equivalents. The major contributors were enteric methane emissions (46.1%) and manure management (32.9%). Feed production and farm management made up 13.8 and 7.2 %, respectively. The estimate was close to the national average but slightly higher than those estimated for the California dairy system. The source of corn used in the dairies was also investigated to determine whether South Dakota grain production generated fewer GHG compared to production in Iowa. Corn sourced in South Dakota had about 10% lower emissions per kilogram mostly due to fertilizer application. Therefore, locally and more sustainably sourced feed input will contribute to further reducing the environmental impacts. Improvements in efficiency of milk production through better genetics, nutrition, animal welfare and feed production are expected to further reduce the carbon footprint of South Dakota dairies. Furthermore, the use of feed additives and anaerobic digesters will reduce emissions from enteric and manure sources, respectively.

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Improved Emission Factors and Intensities for African Livestock Systems for GHG Accounting and Mitigation

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GHG emissions need to be decreased to limit global warming to 2°C. Livestock is the largest contributor to GHG emissions from the agriculture sector globally. Africa contributes 14% of global livestock-value-chain GHG emissions. Many African countries are parties to the Paris Agreement and name livestock in their National Determined Contributions (**NDCs**); yet many of them still use Tier 1 methodology, while Tier 2 methodology will be needed to capture the effects of livestock development or GHG mitigation policies in their national GHG inventories. Accordingly, countries that have livestock in their NDCs need to move to Tier 2 methodology, which requires the collection of animal activity data. Under various third-party funded projects, we collected activity data for large and small ruminants in four countries (Ethiopia, Kenya, Tanzania, and Uganda) with the objective to provide IPCC activity data, estimate Tier 2 GHG emission factors (EFs; GHG emissions/animal/year), and estimate emission intensities (**EIs**; GHG emissions/kg meat and milk protein) at farm-level. To date, data from smallholder mixed-crop livestock systems in three Kenyan counties (Bomet, Nandi, and Nyando) suggests that IPCC 2019 Tier 1 EFs overestimate enteric methane emissions from cattle, sheep, and goats by, on average, 41% (95% Confidence interval (**95%CI**) = 32 to 49%), 12% (95%CI = 0 to 12%), and 31% (95%CI = 24 to 39%), respectively. Estimated EIs for cattle within the same geographical region varied widely (23 to >1,000 kg CO₂-equivalent/kg milk and meat protein). Farm characteristics that were attributed to farms with low EIs were 1) increased milk yield per cow, 2) higher sale of animals for meat, and 3) a higher proportion of productive than unproductive animals in the herd. Findings suggest that low GHG EIs can be achieved by using relatively simple and low-cost interventions.

Longitudinal Modelling of Methane Emission in Dairy Cattle: Genetic Variation & Associations with Breeding Goal Traits

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In dairy cows, genetic variation in feed intake and milk yield varies with the stages of lactation and the same would be expected for the genetic variation in methane (CH_4). However, genetic variation in CH_4 emission during different lactation stages is not known, particularly for the Nordic Red Cattle. Understanding this variation and CH_4 's genetic associations (r_g) with some of dairy cattle breeding goal traits during lactation is essential to assess avenues for its mitigation. Besides, estimates of parameters obtained from direct individual animal emission measurements are scantily reported. The objective was to model longitudinal CH_4 emission data to estimate its genetic variation and associations with dairy breeding goal traits. Data was collected from 310 cows from Finnish dairy research farm including 13,500 weekly average records. Production traits included were energy corrected milk (ECM), dry matter intake (DMI), metabolic body weight and residual feed intake (RFI). Methane phenotypes included were: CH_4 production ($\text{MeP} = \text{CH}_4 \text{ g/day}$), CH_4 yield ($\text{MeY} = \text{g CH}_4/\text{kg DMI}$), CH_4 intensity ($\text{MeI} = \text{g CH}_4/\text{kg ECM}$) and residual CH_4 production (RMP). Univariate and bivariate random regression models that fitted Legendre polynomials of 2nd order on days in milk (DIM) were used to model genetic and permanent environmental variations during lactation. Models included fixed effects of age, sampling point, recording year-month and random herd-test-date, permanent environmental and animal-genetic and residual effects. Mean MeP, MeI, MeY and RMP were $400.1 \pm 32.7 \text{ g/d}$, $13.9 \pm 3.5 \text{ g/kg ECM}$, $20.6 \pm 4.3 \text{ g/kg DMI}$ and $0 \pm 49.5 \text{ g/d}$, respectively. Across different lactation stages, heritabilities for CH_4 traits: MeP, MeY, MeI and RMP ranged from 0.029 to 0.05, 0.04 to 0.08, 0.05 to 0.11, and 0.02 to 0.05, respectively. Genetic correlations between MeP at different DIM were less than unity and varied between -0.35 to 0.99. Genetic correlation of MeP with RFI, ECM or DMI ranged from 0.10 to 0.53, 0.20 to 0.47 and 0.18 to 0.67, respectively. The positive and high r_g between MeP and RFI during early to mid-lactation indicates that feed efficient animals have also the predisposition for lower CH_4 emission.

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Estimating N₂ Emission by Comparing Direct (NH₃, N₂O, NO_x) and Indirect (N Mass Balance) Methods

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The main objective of this study was to estimate N₂ emission from poultry manure by comparing direct and indirect methods of measuring N compounds. For 8 vessels during a 7 day trial, total direct N losses were measured directly as: NH₃-N, N₂O-N and NO_x-N (NO-N and NO₂-N) emissions and summed to a total N loss. NH₃-N, N₂O-N and NO_x-N emissions were measured simultaneously through Impinger method, chemiluminescence method and photoacoustic NO_x analyser, respectively. The total indirect N losses were calculated through N mass balance within the same period of time per-vessel. Results will be further analyzed in the coming months. Further investigations are recommended to design and construct a set-up to measure N₂ gas losses directly as well as other N compounds simultaneously to be able to compare direct and indirect N losses in order to establish ratio between the N compounds.

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Incorporation of Forage Quality into Equations Calculating Enteric Methane Emissions from Dairy Cows in Norway

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Grass silage is the dominant roughage in Norwegian cattle production, but there are only a few studies showing the effect of its digestibility on the production of enteric methane. The main goals of this study were to create a database and to develop basic and operational equations using variables that are accessible at farm level to predict methane production, using digestibility of organic matter (OMD) as the main input parameter for forage quality. A comprehensive literature search with a focus on studies feeding grass silage to dairy cows, resulted in a database of 260 treatment means from 66 studies. In addition to OMD, the database contains information of dry matter intake (DMI), energy-corrected milk (ECM), dietary composition, and methane production. Two subsets from the database were created, each containing complete information of variables required for the development of the basic and operational equations. Methane production (MJ/day) was predicted using the lmer procedure of R. Selection of variables was based on lowest Bayesian information criterion (BIC) scores. The results show that DMI and diet concentrations of crude fat and starch were the most important variables in predicting methane production with the basic equation. The variables used in the basic equation are not accessible at farm level. Thus, operational equations to predict methane production were developed using variables available at Norwegian dairy farms such as concentrate intake, nutrient contents in concentrate, ECM, and OMD of the silage. The best equation was obtained using concentrate intake, crude fat in concentrate, and ECM as predictor variables for methane production. The predictive accuracy of the developed models was evaluated using k-fold cross-validation method. Both basic and operational models were comparable to recently published equations in terms of low root mean square prediction error (RMSPE) and high concordance correlation coefficient (CCC). For the basic model, the RMSPE and CCC were 10.8% and 0.767, and for the operational model, the RMSPE and CCC were 11.5% and 0.512. Adding OMD to the operational model slightly compromised its performance (RMSPE=11.7%; CCC=0.477). In conclusion, the developed operational model containing OMD as an indicator of grass silage quality can be used in practice to predict enteric methane emissions from dairy cows.

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Methane Yield and Microbial Metagenome in Low and High Emitting Cows after Rumen Content Exchange

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This study aimed to examine methane emission and reconstitution of the rumen microbial community after total exchange of rumen content between low and high methane yielding Norwegian Red dairy cows. Two Low and two high emitters in their first lactation were selected for rumen cannulation from a herd of 60 cows ranging in methane yield from 14.4 to 26.6 g/kg DMI. Methane yields of the low and high emitters were 21.7 ± 0.6 and 26 ± 0.8 g/kg DMI, respectively, prior to the rumen cannulation. The four cows averaged 616 ± 30 kg body weight, 25 ± 1 kg DMI, and 39 ± 2 kg milk yield. Total rumen contents were exchanged between the two methane categories by evacuation and thorough washing (including omasum). Representative rumen content (solids+liquids) samples were collected twice in weeks -1, +1, +3 and +7 from the day of rumen content exchange. Ruminal pH and concentrations of short-chain fatty acids (SCFA) and ammonia were assessed and subjected to repeated measures analysis of variance (SAS). Extractions of biomass total DNA were sequenced and used for a genome-centric metagenome analysis. Specifically, a hybrid metagenome sequencing and de novo assembly strategy was applied using both long-read (ONT) and short-read DNA sequencing methods (Illumina). Ruminal pH and concentrations of SCFA were not different before and after the exchange of rumen content, but the concentration of ammonia was decreased at week +7 as compared to week -1 for the low emitters. Methane yield for the low and high emitters was 12 ± 0.8 and 25 ± 0.2 g/kg DMI, respectively, assessed at week +8 after rumen content exchange. Principle component analysis (PCA) of 16s rRNA gene amplicon data was used for comparisons of overall microbial community composition in all samples. The PCA plot showed that after rumen content exchange the low emitters changed gradually back to their original microbial profiles, whereas the high emitters did not change back. Further shotgun metagenome analysis indicated changes in the relative temporal abundances of *Bifidobacterium merycicum* and *Prevotella*. In conclusion, methane category remained unchanged and host specificity of rumen microbial community in the low emitters reconstituted after rumen transfaunation, while the high emitters almost completely inherited the rumen microbial community from the donor animals without turning to low methane yielding cows.

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Plasma-Based Nitrogen-Fixation Can Reduce Ammonia and Methane Emissions from Livestock Manure

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The application of livestock manure to agricultural land is a sensible approach to nutrient recycling, as a substantial proportion of the nutrients consumed by the animals can be returned to the soil. However, the storage and application of livestock manure are associated with a high loss of nutrients, as well as the emission of local pollutants and potent greenhouse gases. The emission of ammonia from livestock manure constitutes both a loss of valuable nitrogen that must be replaced by fossil-based nitrogen fertilisers to maintain productivity and indirectly exacerbates nitrous oxide emissions (when the ammonia eventually rains down on unproductive land (Van der Hoek et al., 2007)). Stored manure will also emit the potent greenhouse gas methane, which is an important contributor to the greenhouse gas footprint of livestock production. N2 Applied has developed a novel technology that targets the emission of ammonia and methane from livestock manure, and that simultaneously shifts the fixation of nitrogen from the fossil-based Haber-Bosch process to an electricity-based plasma process (Graves et al, 2018). The technology operates solely on electricity which it uses to ionise a flow of air, splitting the nitrogen and oxygen molecules therein, and generating a reactive nitrogen gas. The gas is then absorbed directly in the liquid phase of the livestock manure. Consequently, the manure is enriched with nitrate nitrogen, and its pH is slightly reduced. This stabilises the ammonium in the manure, prevents the loss of ammonia and stops formation of methane. Storage trials conducted by Research Institutes of Sweden show a near elimination of methane emissions over a 3-month storage period following the plasma treatment. The ammonia emissions during storage were reduced by 98 %. After field application, trials by ADAS in the UK have shown that ammonia emissions were reduced by 84 % in the 2020 trial and 91 % in the 2021 trial. No significant differences were found in ammonia emissions from the plasma treated livestock manure and calcium ammonium nitrate. Combined, the effects on nitrogen content and gaseous emissions improves the nitrogen use efficiency of livestock manure and reduces the overall carbon footprint of livestock production. N2 Applied has thus expanded their operation to include six full-scale pilot installations in Northern Europe that will be accompanied by further agronomic and environmental assessments.

The Effect of Life-Stage on Methane Production in Sheep

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Accurate selection of sheep with reduced methane (CH₄) emissions requires a knowledge of an animal's CH₄ output throughout the entire production cycle. The objectives of the study were to investigate the effect of life-stage on CH₄ emissions. Methane emissions from a total of 60 female animals were measured across their lifetime as lambs (<12 months), nulliparous hoggets (12 to 24 months) and ewes (primiparous or greater; 24 to 29 months) using 12 portable accumulation chambers (PAC). Ewes were further classified as pregnant and dry (non-pregnant and non-lactating). Animals were removed from feed for at least one hour prior to CH₄ measurement, weighed and randomly assigned to each chamber. Methane (ppm) concentration was measured at 3 time points (0, 25 and 50 minutes from entry of the animal into the first chamber) using an Eagle 2 monitor. Measurements on the same animals were repeated 14 days later. The effect of life-stage on CH₄ emissions was modelled using a mixed model with life-stage, age, breed and live-weight of the animal, diet type, the chamber of measurement and the time off feed prior to measurement included as fixed effects, with contemporary group (date-PAC run) included as a random effect. The average CH₄ output, expressed in grams /day, measured at each life-stage was 4.5 g/day (lambs), 17.7 g/day (hoggets), 11.3 g/day (pregnant ewes) and 32.6 g/day (dry ewes). Methane emissions differed between each life stage (P<0.01) except between lambs and pregnant ewes (P>0.01). For every additional kilogram in live-weight, CH₄ emissions increased by 0.20 g/day. The repeatability of CH₄ emissions across the animals' lifetime was 0.24. Results from this study show that CH₄ emissions differ across stage of production of the animal emphasizing the relationship between production performance and CH₄ output.

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Evaluation of Oxidizing Inhibitors on Enteric Methane Emissions and Other Production Variables, Using the Rumen Simulation Technique

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Agriculture accounts for 23% of all anthropogenic greenhouse gas (GHG) emissions globally. Enterically-produced methane from ruminants is the single largest contributor of agricultural GHG emissions. International emissions reduction targets have been introduced to limit global warming to well below 2°C compared to pre-industrial levels, as set out in the Paris agreement. Feed additives are one of the most promising methane (CH₄) mitigation strategies. The CH₄ inhibitors reported here are based on the control of rumen oxidation-reduction potential (ORP) using mild oxidising agents, or a combination of mild oxidising agents with halides, such as potassium iodide (KI). The objective of this study was to investigate the effects of these treatments on CH₄ emissions, organic matter digestibility (OMD), and fermentation parameters, using the rumen simulation technique (RUSITEC). Treatments consisted of: Control (no treatment); HALA (0.5X urea hydrogen peroxide (UHP) + KI); HALB (0.5X UHP); HALC (0.25X, UHP + KI) and HALD (0.25X UHP). Concentrations were based on the amount of peroxide in each treatment and expressed in terms of fold-concentrated. The *in vitro* diet consisted of 50:50 grass silage:concentrate on a dry matter basis. After 14 days acclimatisation, daily CH₄ emissions, digestibility, and volatile fatty acid (VFA) concentrations, were measured over a 7-day period. Data was analysed using the Mixed procedure in SAS. All treatments reduced methane in terms of CH₄% (by 32% to 49%), CH₄ volume (L/d; by 46% to 77%), and CH₄mmol/g OMD (by 47% to 76%), relative to the control (P<.0001). HALA and HALB supplementation resulted in high CH₄ suppression (76% and 67% in terms of CH₄ mmol/g OMD, respectively) however they both led to a 6% decrease in organic matter digestibility (P<.0001). HALC supplementation resulted in the highest CH₄ reduction (52% in terms of CH₄mmol/g OMD) without affecting digestibility. Preliminary analysis of VFAs suggests a concentration dependent change in fermentation patterns as a result of treatment. *In vivo* studies are necessary to validate the CH₄ mitigating potential of these CH₄ inhibitors.

Identification of Potential Methane Mitigating Compounds in Ensiled Brown Seaweed *Saccharina latissima*

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Seaweeds are under current investigation for possible methane mitigation properties in cattle. The red tropical *Asparagopsis taxiformis* can induce dramatic reductions in enteric methane emission from cattle due to the presence of halomethanes, such as bromoform, dibromomethane, and dibromochloromethane. However, these compounds have carcinogenic and ozone depleting properties. When the Nordic brown seaweed *Saccharina latissima* was subjected to three out of four different methods of ensiling for 28 days (done by Danish Technological Institute, Denmark; details on methods cannot be provided due to confidentiality), methane production was lowered by up to 80% in an *in vitro* system simulating rumen fermentation and without affecting feed organic matter degradability. Attempts to replicate these findings with two other *S. latissima* batches, harvested and ensiled in the same ways, were unsuccessful. The reason for this is currently being investigated. Our aim is therefore to identify the bioactive compounds in the first batch of ensiled *S. latissima* responsible for the dramatic reduction in methane formation. The ensiled samples were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) for detection and quantification of halomethanes. Halomethanes were not detected in any of the *S. latissima* samples. Further, we used Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS) for metabolic profiling. Metabolomics data was pre-processed using MS-DIAL (version 4.60) and analyzed using Principle Component Analysis (PCA) in Latentix (version 2.13, LatentiX Aps). The results from the PCA showed clear grouping according to the method of ensiling. The first Principle Component (PC1) explained the effect of ensiling pH on the metabolic profiles. PC2 explained the variation in metabolic profiles between control and the treatments with methane mitigating effects. Using PC2, several potential compounds have been tentatively identified. This study shows that different ensiling methods can manipulate the methane mitigating potential of *S. latissima*, which could not be ascribed to halomethanes.

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Effect of Intensive and Integrated Grazing Systems, in Seasons, on Methane Mitigation in Nellore

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The intensification of grazing systems and their integration with crops and forest components result in improvement of animal performance and bring beneficial effects to the environment by reducing enteric methane emissions when expressed per unit of product and/or area. Each production system has its potential to produce carcass weight per area, for example, intensification of grazing systems improves growth and muscle development of Nellore cattle and intensification of pasture cattle production systems produces more meat per hectare. The experimental design consisted of four animals (Nellore bulls - 18-24 mo) in each grazing system of which two animals were monitored for enteric methane production using the SF₆ technique. Animals were weighted every 28 days without fastening and the average daily gain (ADG) was calculated by regression between animal weight during the experimental period. Grazing systems evaluated included: degraded pasture - I and J, dryland pasture with moderate stocking rate (*Urochloa decumbens*) - G and H, dryland pasture with high stocking rate (*Megathyrsus maximus*) - E and F, livestock-forest system with moderate stocking rate (*Urochloa decumbens* and wooded with native forest species) - K and L, irrigated pasture with high stocking rate (*Megathyrsus maximus*) - C and D. Statistical analysis was performed using PROC MIXED of SAS 9.4, treatments were considered different when $P \leq 0.05$ by LSD FISHER test. In spring, there was a lower enteric methane emission per unit area (CH₄/ha) in the K and L systems (67.5 g.day⁻¹.ha⁻¹), whereas, the highest emission was in winter in the C and D systems (182.1 g.day⁻¹.ha⁻¹). The methane emission per ADG, defined as methane emitted per gain of 1 kg of body weight, was lower during the spring (CH₄/ADG) within the irrigated and more intensified systems C and D (0.4 g.day⁻¹) and higher during winter, within the integrated systems K and L (1.0 g.day⁻¹). Thus, methane emissions, when expressed per unit of area, product or weight gain were lower in intensified systems.

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Greenhouse Gases Balance of Pasture-Based Dairy Production Systems in Brazilian Atlantic Forest Biome

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Brazilian cattle production is mostly carried out in pastures and the need to reduce the sector's greenhouse gas (GHG) emissions and its overall environmental footprint has become a priority. The adoption of well-suited breeds and intensification of pasture-based livestock production systems are an alternative to optimize the sector's land usage, through the reduction of deforestation and mitigation of environmental impacts. However, further research on tropical systems is necessary. The objective of this research was to evaluate the effect of Holstein and Jersey-Holstein crossbred cows in different levels of pastures intensification (continuous grazing system with low stocking rate—CLS; irrigated rotational grazing system with high stocking rate—RHS), and the interaction between these two factors on the GHG mitigation. Twenty-four Holstein and 24 Jersey-Holstein crossbred dairy cows were used to evaluate the effect of two grazing systems on milk production and composition, soil GHG emissions, methane emission, soil carbon accumulation (0-100 cm). These variables were used to calculate the carbon balance, GHG emission intensity and land-saving effect. The carbon balance of all systems and genotypes presented a deficit in carbon; there was no difference for genotypes, but RHS was more deficient than CLS (-4.99 to CLS and -28.72 to RHS ton CO_{2e}/ha.year). Likewise, the GHG emission intensity was similar between genotypes and higher for RHS (-0.480 to RHS and -0.299 to CLS kg CO_{2e}/kg FCPCmilk). Although both GHG removals (0.14 to CLS higher than 0.02 to RHS kg CO_{2e}/kg FCPCmilk) and GHG emissions (-0.49 to RHS higher than -0.44 to CLS kg CO_{2e}/kg FCPCmilk) contributed to results in the GHG emission intensity of milk production, the greatest influence was due to the lower removals of GHG from RHS. In contrast, the grazing intensification resulted in higher milk production and land-saving effect of 2.7 ha for each ha of intensified pasture.

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Enteric Methane Emissions from Djallonke Sheep Fed Diets with Increasing Level of Metabolizable Energy

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Livestock is the second largest contributor of methane emissions, which is a potent greenhouse gas. The majority of methane from livestock is in the form of enteric methane, mostly from ruminants. Although there is a wealth of data on cattle enteric emissions, there is paucity of data for small ruminants, even less for species from tropical countries. To help make the data available, we conducted a trial to assess the effect of feed energy concentration on enteric methane emissions from Djallonke sheep. The GreenFeed technology for small ruminants (C-Lock Inc.) was used to directly measure emissions from a local breed of sheep. The experiment was conducted at Farako-Bâ research station, in western Burkina Faso. Sixteen adult males were used in a 4 × 4 Latin square design with four iso-nitrogenous dietary treatments differing from each other in energy level: maintenance (Diet 1), 1.25 × maintenance (Diet 2), 1.5 × maintenance (Diet 3), and 1.75 × maintenance (Diet 4). Animals were housed in metabolic cages and before starting the experiment, they were adapted to the GreenFeed unit and metabolic cages. In each of the four periods, the animals were given the diets for a week and data collection during the following week. During data collection period, methane, carbon dioxide, hydrogen and oxygen gases were measured 16 times during a 48-hour feeding cycle. The results showed a linear increase in methane emissions as a function of dry matter intake. There was also a linear decrease of methane emitted per unit of energy consumed. The methane conversion factor (methane/gross energy intake; Y_m) was estimated to be 9.27%, which is greater than New Zealand average of 6.7%. The results of the study provide valuable data to estimate enteric methane emissions from Djallonke sheep and help in calculating national inventory of emissions in West Africa.

Key words: Enteric methane, Maintenance Requirement, Metabolizable Energy and Djallonke sheep

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Use of *Tithonia diversifolia* as an Alternative Forage for Brazilian Livestock

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Ruminant production is an important source of food, employment, and income for the Brazilian population. Most farmers use pastures as a feed source for these animals' production. However, few of them have the infrastructure, technical knowledge, and income to obtain the necessary inputs for the maintenance of the forages commonly used in Brazil. Consequently, degradation and reduction of the nutritional value of pastures and an increase in greenhouse gas emissions is an important result. *Tithonia diversifolia* is a shrub with high biomass production, low external input dependence and it can be an alternative for this niche of farmers. The objective of this study was to analyze the effects of cutting intensity and frequency of *T. diversifolia* on biomass production, nutritional value, and methane production. Ninety-six plants were used, distributed in randomized blocks in a 2 x 4 factorial with 4 replications (composed of 3 plants): 2 cutting intensities (30 and 40 cm post cutting height) and 4 cutting frequencies (21, 28, 35, and 42 days). These plants were established in lines with 3 meters space between lines and 1 meter between plants. A standardization cut was made at the respecting cutting intensities and the biomass was collected after reaching the days of each frequency previously described. The contents of dry matter, ash, crude protein, ether extract, fiber fractions, lignin, and methane production (by the *in vitro* gas production technique) were analyzed in the collected biomass. Between the two tested intensities, there was a statistical difference only for lignin ($P < 0.05$). Among the frequencies analyzed, a linear increase was observed for biomass production, dry matter, fibrous fractions, and lignin ($P < 0.05$). While for crude protein, ash, and ether extract a linear reduction was observed ($P < 0.05$). There was no statistical difference in methane production between the intensities and frequencies tested ($P > 0.05$). Due to similar values of biomass production and chemical composition among the tested intensities, it can be assumed that *T. diversifolia* can also be managed at 30 cm height, a similar cutting intensity to some traditional forages in Brazil. A cutting frequency of 35 days was the best fit when comparing biomass production and the chemical composition of biomass.

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Impact of a Retrofit In-House Slurry Acidification on Ammonia and Methane Emissions from Fattening Pig Barn

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Acidification of slurry is known to reduce ammonia emissions. In some European countries, for example Denmark, this method is already in use. At present, the system is only integrated in the construction of new barns with flat slurry channels. The aim of this study is to investigate whether acidification technology can also be retrofitted in existing barns with slurry storage under the floor. Furthermore, it is to be investigated whether ammonia and methane emissions are reduced with this method. Therefore, a trial compartment with 32 fattening pigs was retrofitted with the new acidification technology. The pH value of the slurry stored under the floor was reduced to 5.5. For this purpose, part of the slurry was mixed with sulfuric acid in an external process tank and then pumped back into the slurry channels. In the identical reference compartment (partially slatted floor, 4 pens with 8 animals each), the fattening pigs were housed simultaneously. The air volume flow was recorded with measuring fans in the exhaust air ducts. The ammonia and methane concentration in the inlet and outlet air were measured using photoacoustic infrared spectroscopy. In this way, the emission mass flow of ammonia and methane from the respective compartment could be quantified during the entire fattening period. Three fattening periods were carried out. The ammonia and methane emissions rose with increasing animal weight. Compared to the reference compartment without acidification, ammonia emissions were reduced by about 40% through slurry acidification. Furthermore, a reduction of methane emissions by about 67% was observed. The pH value in the slurry channels had to be below pH 5.5 to ensure a considerable reduction in emissions. In-house slurry acidification can thus contribute to environmental and climate protection. In addition, it is possible to still use the slurry channels as storages.

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Livestock Actions in National Climate Commitments: Role of Cattle Health

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Nationally Determined Contributions (NDCs) are the backbone of achieving the goals of the Paris Agreement. While most NDCs submitted include concrete information on adaptation and mitigation commitments, the targets on livestock's contribution and specifically the role of animal health for mitigation of and adaptation to climate change have remained limited. Climate change affects animal health directly through behavioral and physiological changes, and indirectly through the availability of key inputs such as water and feed, as well as the ecology and spread of pathogens. These direct and indirect effects can have significant negative impacts on animal and herd productivity, leading to reduced production efficiency and resilience. Preventing and/or controlling diseases can reduce greenhouse gas emissions (GHGs) or GHG emissions intensity and increase profit of farmers through reduction in milk losses, optimum culling rate, improved reproductive efficiency, improved feed conversion efficiency and reduced feed- and other variable costs. Even though the process of achieving a systematic inclusion of animal health in NDCs and GHG inventories may vary from country to country, there are common challenges in mechanisms of inclusion and gaps in capacity and knowledge on data requirements and quantification of emissions. The synthesis in this paper shows that the management decisions and the types of animal health interventions determine the parameters needed to capture the impact of improving cattle health on GHG emissions. These parameters can be incorporated into Tier 2-based tools directly or predicted through modelling. By engaging in projects aiming to improve production efficiency through improvements in animal health, countries can determine the mitigation and adaptation options relevant for their livestock sector, and also have the opportunity to capture the outcomes of these efforts within their NDC reporting by applying the appropriate measurement, reporting and verification process.

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An Assessment of Industry Preparedness for Methane-lowering Feed Additives Tract

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The objective of this study was to develop an introductory understanding of the interest and preparedness of different factors in the feed additive pipeline (additive-manufacturers, feed or supplement manufacturers and livestock managers). Questionnaires were shared as a MS Word file and as an on-line form (Google forms). Eight additive manufacturers; 14 feed/supplement manufacturers and 26 livestock managers participated. For the feed additive manufacturers, developed (87.5%) and developing (37.5%) countries; and feedlot (87.5%) and dairy (62.5%) industries were considered a high to a very high priority market. Lack of products research was the main constraint for commercialization (62.5%). Feed additives manufacturers thought probiotics (64%), antibiotics (50%) and essential oils (50%) to effectively reduce methane production, while only 28%, 14% and 7% of respondents were aware of 3-NOP, asparagopsis and nitrate, respectively. Products to reduce GHG emissions was considered currently a priority to 14% of feed manufacturer respondents, but it was expected to increase to 43% within 5 years. Production cost (57%) and consumer demand (50%) was considered the main factors for impediment and incentive for moving to low-methane products. Livestock managers already use feed additives (60% probiotics and 50% antibiotics), with animal performance (81%) the main reason for using it. Currently GHG management is not a priority (81%) for livestock managers, but respondents (62%) expect to move to a high priority within 10 years. In addition, animal performance and feed efficiency were the main motivations (92%) to use feed additives to reduce GHG emissions. Although very limited in scope, the data indicate a feed additive pipeline that is poorly prepared to achieve a substantial reduction in global livestock emissions in the next five years. This is on account of limited target markets for additive manufacturers, a poor level of understanding on emerging additives, unrealistic expectation of co-benefits, and lack of clarity regarding potential off-farm financial rewards.

Profitability and Climate Risk Mitigation of Low Carbon Innovations for Livestock Farms in Mexico, Honduras, and Costa Rica

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Governments in Latin America have a strong interest in reducing GHG emissions by transforming the livestock value chain through various low carbon interventions. As a result, several countries have registered the NAMA Livestock as a strategic option to contribute to their Nationally Determined Contributions (NDCs), employing policy changes, deployment of financial mechanisms, and capacity-building programs. However, farmers' adoption of low-carbon innovations needs to be profitable and contribute to climate risk mitigation. In this paper, we evaluate the profitability of a custom-made intervention package for an average farm in Mexico, Honduras, and Costa Rica. A simulation model is used to forecast intertemporal biological and economic key indicators. In addition, montecarlo simulation is used to capture the climate, yield, and market uncertainties. Preliminary results from Honduras show that the low-carbon intervention package of innovation improves farmers' capacity to cover the basic basket from 0.6 to 3.46 times. Furthermore, silvopastoral options reduce the impact of dry spells and heat stress, leading to more resilient cash flows to meet the farmer's food security needs. Finally, the estimated financial burden shows the need to have concessional loans, especially for small farmers implementing low-carbon innovations. Results are intended to complement extension programs in the region for the low carbon transition.

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Live Weight Gain and Methane Production of Grazing Heifers on Tall Fescue and Perennial Ryegrass

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The increased frequency of periods of summer drought jeopardizes the production of high-quality forages with an increased interest in tall fescue as an alternative for perennial ryegrass in North-West European dairy production. However, the voluntary intake and digestibility of grazed tall fescue is supposed to be lower compared to perennial ryegrass. This inhibits the adoption of this species in practice. New varieties of tall fescue have been bred to improve digestibility. Nevertheless, there is a paucity of results from intake and animal performance trials with these new species. We hypothesized that when grazing on these new bred varieties of tall fescue the live weight gain is not negatively affected and methane production is higher when grazing compared to perennial ryegrass. To investigate this, we grazed two groups of heifers during the autumn of 2021 on a pasture with strips of perennial ryegrass and tall fescue in a cross-over design. We monitored live weight gain and methane production. The results indicated a higher methane production on tall fescue (258 g/d) in comparison to perennial ryegrass (246 g/d), but the methane production/kg live weight gain was lower when grazing on tall fescue. These findings add information to the results from a previous trial performed in the summer of 2021 that monitored live weight gain and also dry matter intake and showed a higher live weight gain when the heifers grazed on tall fescue. These preliminary results support the potential value of tall fescue for grazing cattle. To stimulate a broader implementation on dairy farms in North-West Europe more results on intake and animal performance are needed with both fresh and ensiled grass of these new bred varieties of tall fescue.

Effect of Including Kernza Straw in Forage-Based Heifer Diet on Methane Production, Yield and Intensity

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Kernza Intermediate wheatgrass is a cool-season perennial grass utilized as a dual-purpose crop (grain and forage). As a co-product of grain, Kernza straw is a low-opportunity-cost feed for ruminants. Pregnant dairy heifers older than one year of age require lower energy and high fiber diets. Thus, straw is commonly included in their diets to reduce energy density but also dry matter intake (DMI) to help avoid over-conditioning (when housed in free stall barn). The impact of including Kernza straw in heifer diets on performance and enteric methane has not been quantified. The goal of this study was to quantify methane production (g/day), yield (g/DMI) and intensity (g/average daily body weight gain, ADG) by heifers fed increasing levels of Kernza straw. The experiment was performed as a completely randomized design using 24 Holstein heifers with similar bodyweights ($\bar{x} = 507 \pm 14$ kg) fed one of three diets for 12 weeks. Diets were formulated to include 0, 20 and 40% Kernza straw with the remaining portion made up of a 40:60 mixture of corn silage and alfalfa haylage (DM basis). In all cases, the experimental unit was the individual heifer, rather than the pen where the 8 heifers per treatment were housed. Dry matter intake was measured daily, and bodyweight was measured in two consecutive days in week 1 and 12. Enteric methane was measured using a GreenFeed unit (C-Lock, Rapid City, SD) placed in the back alley of the pen between week 5 and 7. For heifers fed diets with 0, 20 and 40% Kernza straw, DMI was 11.8, 10.4, 10.2 kg/d ($P < 0.01$) ADG was 1.3, 1.1, 0.9 kg/d ($P < 0.01$), methane production was 286, 266, 258 g/d ($P = 0.12$), methane yield was 24.4, 25.6, 25.4 g/kg DMI ($P = 0.65$), and methane intensity was 217, 250, 320, g/kg ADG ($P = 0.01$), respectively. Although inclusion of Kernza straw in the diet significantly reduced DMI and ADG, and increased methane intensity, heifer growth performance met industry standards with 40% Kernza straw in the diet.

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Effects of *Aspergillus oryzae* Prebiotic on Enteric Methane Emissions in Steers Fed a Silage-Based Diet

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Twenty-four Angus crossbred steers (10 ± 1 mo, 230.7 ± 22.8 kg of BW), were used to evaluate the effects of the inclusion of an *Aspergillus oryzae* prebiotic in a backgrounding corn silage-based diet on enteric methane emissions and nutrient digestibility. Treatments were: 1) *Aspergillus oryzae* prebiotic inclusion 0.032% DM (AOP) and 2) No inclusion (CTL). The experiment was conducted as a switchback design with 2 periods. Each period consisted of 21 d of adaptation, followed by 5 d of enteric methane sampling plus 5 d of apparent total tract digestibility determination. A 7-d washout interval was used between periods. Steers were housed in 4 pens equipped with two GrowSafe feed bunks each to measure individual dry matter intake (DMI). Treatments did not affect DMI ($P \geq 0.10$). Methane emissions were unaffected by treatments ($P \geq 0.10$) when expressed as grams per day and as grams per kg of DM digested, but AOP tended to increase it when expressed as grams per kg of DMI ($P = 0.09$; 39.1 vs. 32.8 g/kg DMI, for AOP vs. CTL, respectively). Additionally, inclusion of the prebiotic tended to increase DM digestibility ($P = 0.08$), and increased OM, NDF, ADF and CP digestibility ($P \leq 0.05$; 68.7 vs. 67.1, 44.0 vs. 41.5, 44.3 vs. 41.5, 57.8 vs. 53.4% of intake, for AOP vs. CTL, respectively). Considering that the positive impact of AOP on nutrient digestibility was paralleled by marginal changes in methane production, further research should evaluate if feeding AOP helps reducing emissions intensity in grams of methane per kilogram of animal product.

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Can Crossbreeding Reduce the Carbon Footprint of Weaner Calf Production in Beef Cattle?

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An effective way to reduce the carbon footprint from beef production is to reduce the animal numbers and increase the production per animal. Beef cattle producers in developed countries, with temperate climates and intensive systems, can use additive genetic variance with some limited use of heterosis to improve production efficiency. In contrast, producers in developing, tropical, and sub-tropical countries under extensive systems, have to capture production through the effects of heterosis and ensure the conservation of indigenous genetic resources. The effective use of crossbreeding may have specific advantages in the case of beef cattle. A crossbreeding experiment was carried out at the Vaalharts Research Station in the Northern Province of South Africa (hot and arid area), where indigenous Afrikaner, Bonsmara (a composite breed) and Nguni cows are mated with Afrikaner, Bonsmara, Nguni, Angus and Simmentaler bulls in all possible combinations, rendering three purebred and twelve crossbred genotypes. Weaning weights of the calves produced were adjusted by analysis of covariance to a constant Large Stock Unit (conceptually similar to the Animal Unit that is used in the USA). A Large Stock Unit has been shown to produce 94 kg of enteric methane per year (Tier II calculation) in the South Africa commercial beef sector. The mean Large Stock Unit for cows in the dataset was 1.2335. Thus, the methane efficiency was estimated by dividing the least squares means for adjusted weaning weight by 116 (94×1.2335). The methane efficiency of purebred calves averaged 0.98. The average heterosis was 3%. Crossbred calves produced by Angus and Afrikaner sires had the highest methane efficiency (up to 16% heterosis), while the Bonsmara was most efficient among the purebred calves. These results indicate that methane efficiency can be improved through the use of crossbreeding.

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Reducing Methane Production of Stored Feces by *Asparagopsis taxiformis* from Swedish Dairy Cows

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The objective was to reduce methane production from stored cow's feces by the addition of *Asparagopsis taxiformis* to the feces of dairy cows previously fed with or without supplementation of *Asparagopsis taxiformis* in their diets. Pooled fecal samples were collected from a production trial (at 2 different periods) from 2 different group of cows supplemented with or without *Asparagopsis taxiformis* at a level of 0.5% organic matter intake. A 2 × 2 factorial design was set in the laboratory in which cows that were supplemented with *Asparagopsis taxiformis* (2 cows) in their diet were further added *Asparagopsis taxiformis* in their feces (0.5% organic matter). The collected fecal samples from the other 2 cows that were not supplemented with *Asparagopsis taxiformis* in their diet were added with *Asparagopsis taxiformis* as well. This made a total of 4 treatments with 2 replicates each. The same procedure was repeated at period 2 with different cows. Four hundred grams of pooled fresh fecal samples were incubated in 1-liter serum bottles for 9 weeks at 39°C. Methane and total gas production was measured at day 1, 4, 7 and then every second week until the end of the incubation. Bottles were gently shaken 3 times during the week. Methane production was numerically lower ($P = 0.61$) in dairy cows feces that were previously supplemented with *Asparagopsis taxiformis* in their diet. Adding *Asparagopsis taxiformis* to the feces of dairy cows significantly reduced ($P < 0.01$) methane production in the feces by almost 50% compared to the cows feces that was not added with *Asparagopsis taxiformis* in the feces. The current study concluded that methane production was numerically lower from cows feces supplemented with *Asparagopsis taxiformis* in their diet and that methane production could be further reduced in the feces of Swedish dairy cows by the addition of *Asparagopsis taxiformis* to the feces.

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Greenhouse Gas Emissions of Urban and Peri-Urban Livestock Production in Ouagadougou, Burkina Faso

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Livestock production systems in the Global South are often low-yielding and, thus, perceived as disproportionately high emitters of greenhouse gases. Intensification of production is seen as key to reducing their global environmental impact and, in the city of Ouagadougou, Burkina Faso, is driven by the increasing demand of urban consumers for animal products. Urbanization thus led to the emergence of urban and peri-urban livestock production systems with diverse degrees of intensification and specialization. Our study aims to understand and assess the relationship between emission intensity and the production strategies chosen by urban and peri-urban dairy producers in Ouagadougou. Feeding practices, weight gain and milk production of dairy cows were monitored on 18 farms in 10 visits between October 2014 and January 2016. Following the 2006 IPCC guidelines, livestock-related emissions per liter of milk were computed. Local Zebus were mostly kept for meat production and thus barely produced any milk (on average 1,70 liter/day; n = 153). Sahelian Zebus produced on average 8,20 liter/day (n = 55) and exotic crossbreds 9,01 liter/day. Digestibility of Sahelian Zebus' diet was however higher (64.2%) than of exotic crossbreds (62.9%), hinting at better use of resources and potentially a lower emission intensity. Our study contributes to the documentation of the global environmental impact of low-yielding yet intensifying livestock production systems in the Global South. It also highlights the relative importance of intensification strategies focused on breeding or feeding used by livestock producers on the emission intensity of urban and peri-urban livestock production.

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Early Weaned Calves Fed High Energy Did Not Reduce Enteric Methane Emissions during Rearing and Finishing

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Early-age rumen interventions have been proposed as an alternative to modify rumen functionality and reduce enteric methane (CH₄) production thereafter in life. This hypothesis was tested in two long-term experiments under production conditions. Nursing Angus calves grazed, with their dams, a tall wheatgrass dominated pasture until assigned to 2 treatments. In Exp. 1, extra-early (EEW) vs. traditional weaning (TW) (53±10 vs. 205±12 days of age, n=16) were compared. EEW calves were fed alfalfa hay plus commercial feed for 30 days, then pasture hay:cracked maize grain:sunflower meal (30:40:30 dry matter (DM) basis; 2.7 Mcal ME/kg DM, 17% CP) for 95 days. In Exp. 2, calves were weaned at early (EW) vs. TW time (142±28 vs. 213±30 days of age, n=20). EW calves were fed whole-corn silage:maize grain:soy expeller (23:47:30 DM basis; 2.7 Mcal ME/kgDM, 18% CP) during 62 days. In both experiments, after weaning treatment application, calves strip-grazed altogether (3.6 head/ha) alfalfa and Mediterranean fescue. In Exp. 2, calves were divided into 2 feeding treatments (n=10), control (Ctrl) vs. supplemented (Suppl) with 1% live weight/day of ground maize grain. Enteric CH₄ was measured with SF₆ during 5 days while grazing in both experiments. In Exp1, EEW and TW steers had similar CH₄ yield while grazing in spring, summer, or finishing on feedlots (average: 26.6 vs. 25.6 g CH₄/kg DM intake (DMI), P>0.05). In Exp. 2, at day 85 of supplementation no interaction was observed between weaning and supplementing treatments on CH₄ emissions. Methane emission yield was similar (P=0.95) for EW (17.1 gCH₄/kgDMI) and TW steers (18.0 gCH₄/kgDMI); and was lower (P=0.014) for Suppl (14.5 gCH₄/kgDMI) than Ctrl steers (20.7 gCH₄/kgDMI). These set of experiments suggest no effect of early ruminal intervention and no synergetic effect with mid-age supplementation to mitigate CH₄ emission yield subsequently under production conditions.

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Optimising Soil pH and Phosphorus Can Reduce N₂O Emissions Grassland Soils

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The effect of soil pH and soil test phosphorus (STP) on crop yields is well understood. The effect of soil fertility on emissions of greenhouse gases such as nitrous oxide (N₂O) from fertilization is less understood. A number of studies have linked soil pH and STP with soil microbial activity and function with potential effects on nitrification and denitrification in soils. Often these studies are limited to short term field and laboratory incubations and the longer term effects of improved soil fertility on N₂O emissions are less understood. The objective of these studies was to investigate the effect of long term liming and P fertilization on N₂O emissions from N fertilization on temperate mineral grassland soils. This research used the long term liming trial (established in 2011) and long term P fertilization trial (established in 1995) simulated grazed grassland trials. Two experiments were carried out. A soil pH field experiment with 4 soil pH levels receiving 300 kg N ha⁻¹ year⁻¹ as CAN in 8 splits. Frequent N₂O measurements were made using the static chamber method. A laboratory soil P experiment. Soil samples at 2 pH levels were incubated at 70% water filled pore space and 15°C and amended with KNO₃ and glucose. Cumulative N₂O emissions from both experiments were analyzed using ANOVA. There was a significant effect ($P < 0.05$) of soil pH, cumulative N₂O emissions decreased with increasing pH. The emission factor decreased from 2% (pH 5) to 1.2% (pH 6.9). There were significantly higher N₂O emissions from low compared to high soil P. Optimization of soil pH and soil test phosphorus can reduce N₂O emissions from fertilizer. The effect of soil fertility on greenhouse gas emissions needs to be investigated further across a wider range of soils and cropping systems to optimize soil C, N and P cycles.

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Switching to Ammonium Based Fertiliser Can Reduce N₂O Emissions from Wet Grassland Soils

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Nitrogen fertilizer application to wet grassland soils is a major source of nitrous oxide (N₂O) emissions and accounts for 10% of Irish national agricultural emissions. In Ireland, approx. 50% of Nitrogen fertilizer is applied as straight nitrogen fertilizer and the remaining applied as N-P-K compound fertilizer. Straight nitrate based fertilizer has a country specific emission factor (EF) of 1.49% and urea based fertilizers have an EF of 0.25%. The compound fertilizer EF is 1% but the effect of nitrate to ammonium ratios on the EF is unknown. The research objective was to evaluate the effect of compound fertilizer type on the N₂O EF. A field experiment was conducted under simulated wet denitrifying conditions with a range of commercial compound fertilizers applied at 80 kg N ha⁻¹ in June and July to a cut grassland sward. Emissions were measured using static chambers over a 3 month period. The effect of fertilizer type on cumulative N₂O emissions was analyzed using general linear mixed model and means compared using Tukey HSD test. There was a significant effect ($P < 0.05$) of fertilizer type on cumulative N₂O emissions. Low nitrate based compound fertilizers reduced cumulative emissions by 31 to 44%. This research is being expanded to evaluate the effect of compound fertilizer formulation on N₂O and ammonia emissions across multiple sites and years to assess this potential mitigation measure for wet grassland soils and to refine the EF for inclusion in the national inventory.

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Improving Carbon Footprinting of Welsh/UK Lamb Production

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The climate change impacts of agricultural systems, particularly those associated with livestock production, are receiving increasing attention worldwide. Lamb production systems in the UK are complex, involving frequent animal movement and stratification whereby lowland, upland and hill pastures are exploited to maximise production. Accurate quantification of product footprints is therefore difficult and an understanding of emissions variability across different altitudes is underdeveloped. The study objectives were therefore to: (i) measure emissions of enteric methane (CH₄) and nitrous oxide (N₂O) from urine and dung from UK lamb production at different altitudes; (ii) develop a carbon footprinting tool based on life cycle assessment (LCA) to represent the complexity of sheep systems through disaggregation of important model parameters by altitude e.g., emission factors, feed characteristics and animal input data. Methodologies included use of a GreenFeed system to quantify enteric CH₄ from grazing sheep in each pasture. A static chamber system was used to measure N₂O emissions from sheep urine/dung in lowland and upland pastures. Field experiments, including pasture quality measurements, have been undertaken and results are being processed for use in the model. The measured data, alongside data from wider literature, will be used to create improved emission factors for upland systems, allowing development of time and altitude disaggregated carbon footprints. A sensitivity analysis will also be performed to recommend priorities for improvement within lamb production footprints. Initial data suggests that the emission factors for N₂O derived from urine in upland pastures are lower than those from the lowlands. However, ruminant CH₄ emissions in the uplands may be lower in absolute terms but higher per functional unit due to poorer pasture quality. Overall footprints may appear lower than previously published literature due to the lower N₂O emissions observed at higher altitudes. Finally, the new carbon footprint model will be used to explore farm management scenarios and mitigation options in the context of net zero greenhouse gas emissions, with the intention of supporting policy decision making for environmentally sustainable sheep production in upland areas.

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Breeding Forage Cultivars with Improved Nutritive Value

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Cattle production is one of the main agricultural products in the southeastern (SE) USA and warm-season perennial grasses and cool-season legumes are the main feed source for livestock. The ability of ruminants to convert forage biomass into animal-based products is remarkable, but this process must become more efficient. Nutritive value (NV) indicates the nutrient content in feeds and it is an important factor to elaborate diets. Improving NV in forage species could be achieved by multiple approaches, such as breeding, and fertilizer management. The Forage Breeding and Genetics Lab at the University of Florida focuses on improving yield and nutritive value in two forage species: bermudagrass (*Cynodon spp.*) and alfalfa (*Medicago sativa* L.).

Experiment 1. A bermudagrass collection composed of 283 genotypes were assessed for crude protein (CP), phosphorous concentration (P), *in vitro* digestible organic matter (IVDOM), and neutral detergent fiber (NDF) using wet chemistry. The whole germplasm was evaluated in two harvests; while 15 genotypes were evaluated across 11 harvests during two years in Citra, Florida. Significant genetic variability was found in this population for NV, indicating potential improvements for these traits through breeding. Specifically, P and IVDOM presented large variation, while NDF had lower variation. Breeding line 240, PI-316510, and PI-3166536 presented superior NV than Tifton 85 (best control cultivar).

Experiment 2. A trial composed of three cultivars (Florida 77, Florida 99 and B805) and two breeding lines (AP15, AP17;) were seeded in four replicated plots at a seeding rate of 20 and 27 kg.ha⁻¹. Dry matter yield (DMY, kg.ha⁻¹) was analyzed for two harvests for all cultivars, and *in situ* ruminal DM disappearance was measured for AP15 and B805 after incubating samples for 0, 3, 6, 9, 12, 24, 48, 72, and 96 h in triplicates in rumen of two non-lactating Holstein dairy cows. AP15 (1908 kg.ha⁻¹) and AP17 (2131 kg.ha⁻¹) produced the highest DMY, and B805 produced the lowest DMY (1362 kg.ha⁻¹). B805 had lowest lignin concentration, greater net energy of lactation, and greater milk per ton of forage compared to other varieties. Neutral detergent fiber degradability (NDFD) was greater for B805, FL77, and FL99 when seeded at 20 kg.ha⁻¹; however, NDFD was greater for B805 compared to other cultivars at 27 kg.ha⁻¹. Both the rate and extent of DM degradation was greater for B805 compared with AP15. In conclusion, planting density had no effects on nutritive value; however, B805 had greater nutritive value, and *in situ* ruminal DM degradability compared to other cultivars, while it had the lowest DMY.

Experiment 3. Incorporating legumes into grass swards provides numerous benefits to forage-livestock systems. Bermudagrass-alfalfa mixtures are of increasing interest to producers in the SE USA. A defoliation study was conducted to define management practices to increase the likelihood of successful alfalfa-bermudagrass mixtures and determine NV in mixtures. Samples from mixtures representing different botanical composition between bermudagrass and alfalfa were assessed using wet chemistry for CP, IVDOM, NDF, and ADF, as well as through near-infrared reflectance spectroscopy (NIRS). A NIRS calibration was developed for alfalfa/bermudagrass samples to predict NV, and high accuracies were obtained for all traits: CP (0.97 ± 0.05), IVDOM (0.95 ± 0.02), NDF (0.99 ± 0.01), and ADF (0.98 ± 0.02). This information will allow faster evaluation of bermudagrass/alfalfa mixtures for NV.

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Using Partial Life Cycle Assessment to Enhance National Climate Actions to Reduce Methane from Livestock Systems

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More than 100 countries have joined the initiative of the United States of America and European Union on Global Methane Pledge to cut 30% of methane emissions by 2030 from 2020 levels. For many regions, enteric fermentation and manure handling are the leading anthropogenic sources of methane emissions from livestock. Technical capacity building and policy support for signatories to the Pledge will be an essential part of increasing ambitions for livestock systems to enhance national climate actions. Life cycle assessment is a useful approach to identify where methane emission 'hotspots' occur, what the sources of these emissions are and therefore what measures can be introduced to reduce them. The objective of our research is to use the Global Livestock Environmental Assessment Model (GLEAM) – a partial life cycle assessment tool – to strengthen inventories of greenhouse gas emissions from diverse livestock systems, and to identify specific mitigation measures that are appropriate and effective. Briefly, we partnered with national institutions to collect and compile nationally available livestock activity data from the peer-reviewed literature and national statistics. We conducted multi-stakeholder workshops with data providers to better parameterize and validate the model for use for subnational (i.e., farm-level) and national mitigation scenario assessments. Five key results are expected from this research: 1) Improved livestock activity databases that better represent livestock production system typologies at subnational levels; 2) Identification of data gaps and future areas for data collection and improvement; 3) A baseline of methane and other greenhouse gas emissions from business-as-usual scenario; 4) Scenario analyses of methane and other greenhouse gas mitigation reduction potential; and, 5) Estimation of progress made towards sectoral development goals and national climate actions where they exist. This assessment will support increased ambitions in the Nationally Determined Contributions and provide evidence-based information to integrate livestock into national climate planning and policies.

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***In Vivo* Study of Combining *Asparagopsis taxiformis* and Phloroglucinol to Reduce Methane Production and Improve Rumen Fermentation Efficiency in Goats**

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Most CH₄ mitigation strategies based on feed additives result in excess ruminal H₂ being expelled and consequently not accompanied by improved rumen fermentation efficiency that could benefit animal productivity. The present study aimed to confirm previous *in vitro* results and evaluated the combined treatment of *Asparagopsis taxiformis* (AT), a potent methanogenesis inhibitor, and phloroglucinol, an organic compound that can be degraded by rumen bacteria using H₂ as electron donor, yielding acetate as the terminal product. Eight Murciano-Granadina adult goats were used in a replicated 4x4 Latin square design. Four treatments were considered: i) control; ii) AT at 0.5% DM; iii) phloroglucinol at 20 g/kg DM diet (PHL) and iv) combination of AT and phloroglucinol (AT-PH). Each experimental period consisted of 10 days of adaptation to the treatments, followed by 3 days for CH₄ and H₂ measurements in open-circuit respiration chambers and 1 day for rumen fluid collection at 3h post-feeding for VFA analysis and rumen microbial community characterization. Results showed that PH treatment did not affect CH₄ production, while AT and AT-PH significantly (P<0.001) reduced CH₄ production by 30-40 %. The AT treatment increased H₂ expelled, which was reduced by 74 % when AT was combined with PHL (P<0.001). Total VFA and acetate concentrations were significantly increased by AT-PHL compared to AT. Moreover, PHL supplementation decreased the concentrations of archaea, protozoa and anaerobic fungi. This study demonstrated that phloroglucinol could be used as means to capture ruminal H₂ produced in excess in a methanogenesis inhibition scenario and redirect it towards acetate production, which could potentially lead to improvements in animal productivity.

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Safety Assessment Method of Novel Methane Reducing Feed Additives Fed to Dairy Cattle

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Novel feed additives are currently tested in feeding trials to reduce enteric methane emission from dairy cattle. To perform an initial safety validation of a novel feed additive for the cow and consumer, *in vitro* cell-based assays are used. Biological samples originate from a dose-response feeding trial with four Danish Holstein dairy cattle, where the methane reducing compound was introduced directly into the rumen through a rumen cannula. The effects of serum and whey from untreated (C) and treated (T) cows exposed to a daily dose of the methane reducing compound were tested on bovine mammary epithelial cells (MAC-T) and fetal human intestinal epithelial cells (FHs 74 Int; FHI). The effect on cell viability was examined with a resazurin metabolism assay (AlamarBlue) after 72h of cultivation of cells in a media containing whey or serum samples. Whey samples were tested in concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5 and 10%, while serum samples were tested in concentrations of 1, 5 and 10% in basal medium. The results from these assays are expressed as a relative viability value to basal medium. The preliminary results with serum show that MAC-T and FHI cells had similar viability, whether the serum derived from treated or untreated cows, although a numerically slightly lower viability was observed for treated cows (MAC-T: C=1.63 and T=1.52, P=0.23; FHI: C=2.77 and T=2.65, P=0.17). Viability in general increased linearly with increasing concentration of serum. With whey, the highest MAC-T viability was found with 1% whey in the media, and there was a significantly higher cell viability with whey from treated cows (C=1.11, T=1.20, P<0.01). Future assays will show if the response of whey is different on FHI cells. The addition of targeted and untargeted metabolomics studies for bioactive compounds in the biological matrices will support the understanding of the observed effects.

The Use of Proxies for Genomic Selection for Low Methane in the New Zealand National Sheep Flock

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New Zealand is the first country to roll out a national breeding scheme for the selection of low methane emitting sheep. To date over 10,000 sheep have been measured with hundreds of thousands of genomic breeding values for methane emissions delivered to the industry. We will describe the implementation of genomic prediction for lowered methane emissions in the New Zealand national sheep flock. We describe the practical implementation and roll out of a national breeding scheme and define the estimated physical and economic impact. Using a combination of research from selection lines, progeny test flocks and commercial producer flocks, we have developed several proxies to estimate methane emissions in sheep. These include portable accumulation chamber measures, milk fatty acid profiling, rumen size, feed intake, rumen microbial profiling and genotyping. Here, we look at the utility of these proxies for the selection of low emitting sheep for breeding and the practical inclusion of combining multiple predictors within a breeding scheme. Finally, we will discuss the potential for across country evaluations and how the findings could be transferred to other national sheep breeding schemes and to other ruminant livestock production systems.

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Carbon Balance for a Pasture-Based Dairy Cattle in Chile: Case Study

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The aim of this study was to determine carbon balance for a pasture-based dairy cattle system in Chile. We selected a representative dairy farm from southern Chile, where a field farm survey was carried out to collect information on an annual year basis, 2020 (herd size, land use, productive parameters, and farm and cattle management). Model for carbon emissions were carried out in Excel[®]. Data of land use was resolved by using digital cartography. For forest land, carbon removals were calculated using an ecophysiological approach with field measurements and emissions were estimated using information collected in the farm survey. For pasture it was assumed that emissions = removals. GHG emissions (CH₄, N₂O and CO₂) and removals were calculated using the 2006 IPCC Guidelines for National GHG Inventories. Annual carbon balance was calculated using the following equation: carbon balance (CO₂-eq) = emissions – removals. Total carbon emissions were 1969 t CO₂-eq, while removals of CO₂ reached -713 t CO₂-eq, which represented 36% of total emissions, with a carbon balance of 1256 t CO₂-eq /year. Rumen fermentation (60%) and excreted (urine and dung) deposited into pasture (17%) were the main emissions sources, followed by N fertilization (synthetic and organic N fertilizer; 7%), manure management (6%), indirect emissions (5%) and other sources. Based on GHG emissions from animals, dairy cattle and growing animals (heifers and calves) represented 52% and 40% of total animal emissions. In conclusion, despite the important contribution of forest withing the dairy farm, emissions are greater that removals, however, forest can upset 36% of the carbon emitted, representing an important C removal and a mitigation strategy from pasture-based cattle systems in Southern Chile.

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Mitigation of Greenhouse Gas Emissions from Suckler Cow Beef Production Systems

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Various mitigation scenarios, involving cow reproductive performance (calf mortality rate and number of calves born per cow per year), young bull beef production efficiency (i.e. age at slaughter and carcass weight), and supplementation of an inhibitor promising for enteric methane (CH₄) reduction (3-nitrooxypropanol; 3-NOP) was investigated using the whole-farm model HolosNorBeef in both typical herds of British and Continental breeds and commercial herds of Aberdeen Angus, Hereford, and Charolais. The scenarios were based on the observed variation among the worst and best performing 1/3 of herds in the Norwegian Beef Herd Recording System. HolosNorBeef is an empirical model based on the HolosNor model and the methodology of the Intergovernmental Panel on Climate Change with modifications to Norwegian conditions. HolosNorBeef considers direct emissions of CH₄, nitrous oxide (N₂O) and carbon dioxide (CO₂) from on-farm livestock production and indirect N₂O and CO₂ emissions associated with inputs used on the farm. The corresponding soil carbon (C) emissions are estimated using the implemented Introductory Carbon Balance Model (ICBM). In typical herds, improving cow reproductive performance reduced emission intensities 3% across breeds. Continental breeds showed greater potential of reduction of emission intensities from improving young bull beef production efficiency. A best-case scenario combining cow reproductive performance and young bull beef production efficiency reduced total emissions by 11.7% across breeds. When including the effect of the inhibitor assuming a 33% reduction of enteric CH₄ emissions during the housing period (Sept 15-May 31) in the best-case scenario, emission intensities could be further reduced by 8.3%. In commercial herds, the effect of mitigation options showed variability across individual farms, which warrants tailored mitigation strategies rather than a 'one measure fits all' approach.

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Forage-Finished Beef Steers and Enteric Methane in Uruguay: Reducing Emissions by Managing Forage Fiber Content

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In Uruguay, methane (CH₄) emitted from livestock sector contributes with 46.1 % of the total national emissions, where more than 70% of the beef finishing cattle are fed with forage varying the quantity or quality offered. As CH₄ emissions are affected by quantity and quality of food intake, there is a need to quantify these changes. This work aimed to quantify the effect of different NDF content of forage diets on CH₄ emissions from beef steers during the finishing stage. A total of 36 Angus steers (\bar{x} = 437 kg live weight, LW), were blocked by LW and randomly assigned to one of two treatments. In addition, each block was grouped in three lots (n = 12) and accommodated in three different pens, where both treatments were applied using automatic individual feeding systems for 97 days. The treatments were: low-quality diet with high NDF (54.9%, H_NDF) and high-quality diet with lower NDF (47.8%, L_NDF). Dry matter intake (DMI), LW, and average daily gain (ADG) were determined. At the end of the feeding period, CH₄ emissions were measured using the SF₆ tracer gas technique during five consecutive days. Statistical analysis was performed on 30 animals due to a sampling effectiveness of 86%. The results show that DMI and ADG were significantly higher (P < 0.05) in L_NDF animals (9.9 vs 8.2 kg DMI/day and 0.68 vs 0.32 kg/day ADG, respectively). The total daily CH₄ emission per animal was higher (P = 0.0476) in L_NDF than H_NDF (215 vs 194 g CH₄, respectively); however, the emission intensity expressed per unit of DMI was 8% lower (P = 0.0363; 21.7 vs 23.7 g CH₄/kg DMI) and almost 1.9 times lower per unit of LW gain (P < 0.0001; 327 vs 633 g CH₄/kg LW) when compared to H_NDF. These results confirm that high quality forage diets with low fiber content used at finishing stages are an alternative to reduce enteric emissions intensity while improving productivity variables.

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Low Carbon Beef: A Case Study in a Sandy Soil from Brazilian Cerrado

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Global demand for food is increasing, which pressures for greenhouse gas emissions reduction or neutralization to mitigate the climate change. In this context, Brazil has developed technologies to produce beef with lower carbon emissions. This study aimed to evaluate a low carbon strategy for intensifying beef cattle raising in areas of consolidated use in sandy soils of the Brazilian Cerrado, West of Bahia's State, through enteric emissions, liveweight gain (LWG) and changes of stocks of soil organic carbon (SOC) from 0 to 40 cm layer. SOC calculations were performed in 2019 and 2021 using the DNDC model and Urquiaga et al. (2016) protocol, while enteric methane emissions used the ALU model. From July 2019 to June 2020, 10 months Nellore cattle were evaluated in two treatments: pasture using low carbon beef protocol (LCB, Embrapa), characterized by soil-plant-animal management practices; pasture using farm conventional management (FCM). Native Cerrado (CER) was used as a control for SOC. Enteric emissions for LCB and FCM treatments were 1.1 tCO₂ eq and 1.4 tCO₂ eq head/year, respectively. The LWG was 1.4 and 0.4 t/ha/year for LCB and FCM, respectively. The initial SOC by DNDC model was 13.5 tC ha⁻¹ for CER; 22.4 tC ha⁻¹ for LCB and 18.2 tC ha⁻¹ for FCM and final SOC was 14.4 tC ha⁻¹ for CER; 18.6 tC ha⁻¹ for LCB and 16.0 tC ha⁻¹ for FCM. The initial SOC by Urquiaga et al. (2016) protocol was 26.2 tC ha⁻¹ for CER; 29.2 tC ha⁻¹ for LCB and 26.8 tC ha⁻¹ for FCM and final SOC was 23.9 tC ha⁻¹ for CER; 23.5 tC ha⁻¹ for LCB and 21.8 tC ha⁻¹ for FCM. In conclusion, LCB was more efficient than FCM, since it was possible to increase beef production with lower per capita emissions and greater soil carbon accumulation.

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Effects of Cashew Nut Extract on *In Vitro* Ruminal Fermentation, Methane Production and Ruminal Microbial Communities

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The objective of this study was to evaluate the effects of cashew nut shell extract (CNSE) and monensin on ruminal *in vitro* fermentation, methane production, and ruminal microbial communities. Treatments were: control (CON, without additives); 2.5 μ M of monensin (MON); 100 ppm of CNSE (CNSE100); and 200 ppm of CNSE (CNSE200), each incubated with 52 mL buffered ruminal fluid and 500 mg of total mixed ration for 24 h using serum vials. The experiment was done as a complete randomized block design with 3 runs. Run was used as blocking factor. Each treatment had 5 replicates, in which 2 were used to determine nutrient digestibility and 3 were used to determine pH, ammonia nitrogen (NH₃-N), volatile fatty acids, lactate, total gas and methane production and microbial community composition. Treatment responses were statistically analyzed with the MIXED procedure of SAS. Orthogonal contrasts were used to test the effects of (1) additive (CON vs. MON, CNSE100, and CNSE200); (2) CNSE- (MON vs. CNSE100 and CNSE200); and (3) DOSE-(CNSE100 vs. CNSE200). Significance was declared at $P \leq 0.05$ and tendencies were considered when $0.05 < P \leq 0.10$. We observed that pH ($P = 0.02$), acetate ($P = 0.04$) and acetate:propionate ratio ($P < 0.01$) were lower for CNSE100 when compared to CNSE200, propionate was greater ($P < 0.01$) for CNSE100 compared to CNSE200, the total gas of CON was greater ($P = 0.02$) compared to all treatments and the total methane production was lower ($P = 0.04$) in both CNSE treatments compared to MON. Also, compared to MON, DM digestibility of CNSE treatments was greater ($P = 0.05$). Compared to MON, CNSE treatments tended to decrease ($P = 0.06$) DL-lactate concentration. No effects ($P > 0.05$) were observed for NH₃-N, and NDF digestibility. Overall, the inclusion of CNSE decreased methane production of *in vitro* ruminal fermentation, making CNSE a possible methane mitigation additive for dairy cattle diets.

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Effects of Dietary Fiber Source on Enteric Methane Emission from Growing Pigs and Gestating Sows

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Three experimental diets were formulated to assess the impact of soluble and insoluble dietary fiber (DF) sources on enteric methane emission from gestating sows and growing pigs: a control diet (CON) based on wheat and barley; a diet with high content of soluble DF based on sugar beet pulp (SBP); and a diet with high content of insoluble DF based on wheat bran (WB). Two experiments were carried out as a repeated 3 x 3 Latin square design with six cannulated growing pigs and six cannulated gestating sows. After 7 days adaptation period, animals were placed in metabolic cages for 7 days to determine digestibility and ileal and fecal flow of the nutrients. During the experiment, animals were placed into respiration chambers for 48 hours for measurement of gas exchange. The proportion of non-starch polysaccharides (NSP) was higher in ileal digesta when sows were fed WB and SBP diets compared to the CON diet, with the highest values obtained in sows fed WB diet ($P < 0.05$). For growing pigs, NSP proportion in ileal digesta was not affected by diets. Feeding WB resulted in an increased content of NSP excreted in feces, compared to CON and SBP diet ($P < 0.05$). In growing pigs, enteric methane production amounted to 1.58 and 1.91 L/kg DM intake when fed CON and WB diet, respectively. Feeding pigs the SBP diet numerically increased methane production (2.99 L/kg DM intake). Sows fed CON and SBP diets had the lowest and highest daily methane production, respectively, while the daily methane emission from sows fed WB was intermediate ($P < 0.05$). In conclusion, enteric methane production was affected by the source of DF and animal age, with the highest values obtained when fed a diet with high content of soluble fiber to gestating sows.

The Farm-Gate Methane Footprint of Diverse Beef Cattle Genotypes in South Africa

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The aim of this study was to quantify the farm-gate methane footprint of some of the major and diverse South African beef cattle breeds. A simulation study was done to simulate the methane production (expressed as methane intensity) of a weaner calf production system for nine diverse beef cattle genotypes. The genotypes were chosen on their relative numbers and the availability of data. The breeds involved were Afrikaner, Nguni, Bonsmara, Angus, Hereford, Brahman, Brangus, Charolais and Simmentaler. A farm size of 1 200 ha with a carrying capacity of 6 ha/Large Stock Unit was simulated, which could carry 200 Large Stock Units. Frame size specific equations were used to estimate cow Large Stock Units for the different breeds. In South Africa the enteric methane emission of a Large Stock Unit is estimated to be 94 kg methane/year (Tier II estimate). The methane intensity (kg methane/kg live weight) was calculated by dividing the annual methane emissions from the farm per year (200 Large Stock Units x 94 kg methane = 18 800 kg methane) by the total kg live weight that leaves the farm. The total live weight available for disposal annually was calculated by adding the total kg of surplus calves and total kg of culled cows. It was concluded that the Afrikaner (0.60), Bonsmara (0.59), Angus (0.59), Brahman (0.61) and Brangus (0.62) breeds have low methane intensities and is environmentally friendly. The Nguni (0.68) and Hereford (0.64) have medium methane intensities, while the Charolais (0.85) and Simmentaler (0.74) have high methane intensities and may be regarded as environmentally unfriendly. It must be noted that the financial aspects of the different breeds were not considered. This information can be used to develop baseline emissions for the beef cattle sector and for the promotion sustainable production with low environmental impacts.

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Effect of Methane Category and Lactation Stage on Immune Response and Rumen Microbiome

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The objective was to delineate relationships of immunocompetence and rumen microbiome with enteric methane yield (MY, g/kg dry matter intake, DMI) in early- and late-lactating dairy cows. We expected (1) greater correlations between immune response and MY in early than late lactation, (2) lower immune responses in low than high MY cows, and (3) reflection of methane category and lactation stage in rumen microbial community structure. DMI, body weight (BW), energy-corrected milk (ECM) yield and MY in respiration chambers were quantified in early- (n=20, 31 days in milk, DIM) and late-lactating (n=14, 390 DIM), multiparous Holstein cows. We studied the *in vitro* immune response to lipopolysaccharide (LPS) using whole blood and to phytohaemagglutinin (PHA) and concanavalin A (ConA) using peripheral blood mononuclear cells (PBMC). 16S rRNA in rumen liquids (oesophageal tubing) were sequenced for microbial populations identification and relative abundance. Data were subjected to analyses of variance and correlation, principal component analyses and linear discriminant analysis Effect Size (LEfSe). Cows weighed 545-917 kg, had 9-24 kg DMI/day, 16-51 kg ECM/day, 363-751 g methane/day, 70-620 pg/ml tumor necrosis factor α (TNF α) in supernatants, and PBMC proliferation indices (PI) of 1.9-5.5 (ConA) and 1.4-3.8 (PHA). Immune responses (PI-ConA, PI-PHA) were not correlated ($P > 0.1$) to MY in early- and late-lactating cows, except for a positive correlation ($P = 0.053$) between TNF α response and MY in early lactation. Lower immune responses (PI-ConA $P=0.03$, PI-PHA $P=0.052$, TNF α -LPS $P = 0.026$) were observed in low compared to high MY cows in early lactation (n = 10 vs. n = 10). Early-lactating, low MY cows were also characterized by a lower ($P = 0.01$) feed conversion efficiency (ECM/DMI). Rumen microbial community structure differed ($P < 0.05$) between lactation stages and between low and high MY cows. Methanobacteria and Christensenellaceae were enriched in early lactation. Abundance of Methanospaera and Acetitomaculum was higher in low MY cows, whereas enrichment of Muribaculaceae was observed in high MY cows. Additional energy through higher fermentation activity (higher MY, distinct microbiome) improves both immunocompetence and feed conversion efficiency during energetically demanding early lactation.

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Correlations Between Eating Time and Methane Emissions in Dairy Cattle and Sheep

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Reducing methane emissions from dairy cattle and sheep is of environmental importance. With the growing popularity of wearable sensor devices, the time spent eating (eating time) per eating event (ETE) could potentially be relatively easy and cheap to obtain on dairy cattle and sheep farms. Four hundred and ten Holstein cows were fed compressed cubes consisting (on a dry matter basis) of 74% alfalfa hay, 25% crushed barley, and 1% minerals, and methane emissions were estimated using a modified sulphur hexafluoride technique over 5 days. Additionally, methane emissions of 445 Australian Maternal Composite ewes were measured at post-weaning, hogget and adult ages with the use of portable accumulation chambers. Ewes were fed *ad libitum* in automated feed intake facilities with cereal straw-based pellets that had a composition of 40% cereal straw, 20% cereal grain (barley or wheat), 15% legume grains (beans, lupins or lentils), 15% oat hulls, 7% almond hulls, 1% lime, 1.5% bentonite, 0.5% gypsum. Methane emissions, defined as daily methane production, methane yield, and residual methane production were calculated for both populations. Phenotypic correlations between ETE and methane emissions traits were calculated in R. ETE was negatively correlated (between -0.08 and -0.32) with methane emission traits in both populations, suggesting that those animals that spend more time eating per eating event emit less methane. Further studies are required to confirm these associations.

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Effect of Nutritional Supplementation on Methane Emissions of Grazing Beef Cattle in Colombian Humid Caribbean

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The Colombian Caribbean region contributes 38% of total beef production with 2.1 million head of cattle grazing 6.7 million hectares of tropical grasses such as *Bothriochloa pertusa*, *Dichantium aristatum*, *Megathyrsus maximus* among others. Supplement feeding may be a strategy to enhance animal performance. The aim of this study was to evaluate the effects of energetic-protein supplementation (ENS) with agro-industrial by products on dry matter intake (DMI), daily weight gain (DWG), total methane emissions (CH₄, g/d), CH₄ yield (CH₄/DMI, g/kg), CH₄ intensity (CH₄/DWG, g/kg), and gross energy losses as CH₄ (Y_M) of ten beef F1 Romosianuano x Cebu steers (420 ± 12 kg) grazing *Megathyrsus maximus* cv. Sabanera with 1.8 kg of ENS. Changes by effects of ENS were evaluated by comparing experimental periods using a completely randomized design. Three experimental periods were evaluated, each one had 30 days of ENS. Over the last three days of each period, CH₄ emissions were measured and determined by integrating measurements from a hand-held laser detector at four times per day at 05:00h, 09:00h, 13:00h and 17:00h, with five minutes of measurements for each animal. DMI and intake of gross energy were different (P<0.05). ENS increased above-average 7.1 kg of DMI and 7.6 Mcal.d⁻¹. Furthermore, ENS decreased (P<0.05) by 33.4%, CH₄ g. animal. d⁻¹, with a daily CH₄ production of 106.69 ± 14.32 g.animal.d⁻¹ with ENS and 160.23 ± 22.45 g.animal.d⁻¹ without ENS. Y_M was reduced by 38.3% compared to non-supplementation. CH₄ yield and CH₄ intensity were different (P<0.05). Calculated emission factor according to IPCC (2016) was 29.3±3.93 kg of CH₄/head/year with ENS compared to 44±6.17 CH₄/head/year without supplementation. This study shows that ENS can improve beef cattle production in grazing, leading to decreases in enteric methane emissions and drive sustainable livestock production.

Enteric Methane from Dairy Cattle Grazing on Traditional Pastoral and Silvopastoral System of Cundinamarca Farms

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Cundinamarca, second largest milk producer in Colombia, with 4,800,000 liters day⁻¹ and 70,000 families depending on a sustainable production. This study aimed to evaluate if integrating animal+pasture+ silvopastoral systems (SPS) would help neutralize the impact of enteric methane (CH₄) emission and would have positive effect on production and quality milk. The research was carried out in Tibaitata research Centre in Cundinamarca. Ten Holstein lactating cows were randomly assigned to two grazing treatments: *Cenchrus clandestinus*, traditional pastoral system (TPS) and silvopastoral system (SPS) integrated by woods-tree (*Eucalyptus sp*, *Alnus acuminata* and *Acacia melanoxylon*) between two lines of a forage-shrub (*Sambucus peruviana*). Each treatment was divided in three grazing paddocks of 0.3 ha. The experimental design was randomized complete block with sub-samplers. The blocks defined by measurement period and the sub-samples by cows assigned to treatments. The study lasted fifteen days with animals grazing five days in each of the three paddocks; over last three days of each period were measured emissions CH₄ and recorded production and quality of milk in terms of milk fat (F), protein (P) and total solids (TS), and fat corrected milk (FCM) was calculated. Enteric CH₄ was determined by integrating reads from hand-held laser detector on three times per day at 08:00h, 12:00h and 16:00h. Results showed that animals in SPS had significantly ($P < 0.05$) higher milk quality and yield (3.8% F, 11.9% TS and 18.02 FCM) compared to (2.9% F, 11.1% TS and 12.7 FCM) TPS. Intake of gross energy and digestibility energy were different ($P < 0.05$). SPS increased above-average 8.8 and 9.1 Mcal.d⁻¹, respectively. Furthermore, SPS decreased ($P < 0.05$) 37.1% CH₄/ kgFCM, and numerical reduced by 21.7% CH₄ g.animal.d⁻¹ and 27.7% the Ym compared to TPS. This showed that grazing dairy cows in a SPS can improve milk yield, lead to decrease in methane emissions and drive sustainable production.

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Effect of Sward Age and Proportion of Plantain on N₂O Emissions from Cattle Urine Patches

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Introduction of plantain (*Plantago lanceolata* L.) into grazed pasture swards has recently been suggested as a strategy to reduce nitrous oxide (N₂O) emissions and nitrate (NO₃⁻) leaching in New Zealand agricultural systems. An experiment was undertaken to assess the effect of sward age and proportion of plantain on N₂O emissions from urine collected from cattle fed a ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture diet. Urine (682 kg N ha⁻¹) was applied to plots with varying proportions of plantain mixed with ryegrass/white clover (i.e. 0%, 30%, 45% and 100% Plantain). One set of plots was established in November 2017 (Old plantain) and another in November 2019 (New plantain). Gas measurements were conducted from February to May 2020 in both trials using a standardized static chamber technique and N₂O concentrations were determined by gas chromatography. The average cumulative N₂O emissions over 99 days were 3.2 kg N ha⁻¹ [(average emission factor (EF₃)= 0.45%)] and 1.2 kg N ha⁻¹ (average EF₃= 0.15%) for the old and new plantain trials, respectively ($P < 0.10$). The proportion of plantain in the swards did not affect N₂O emissions in the old trial ($P > 0.10$). In the new trial a linear decrease on N₂O emissions was found from 0% plantain (100% ryegrass/white clover) to 45% plantain swards ($P < 0.10$, $r^2 = 0.93$), but no significant effect was found at 100% plantain sward. Our results suggest that plantain swards in the first year of establishment have a greater potential to reduce N₂O emissions in grazing systems, and that increasing proportion of plantain mixed with ryegrass (up to 45% plantain) leads to a significant decrease in N₂O emissions. In contrast, a 2-year established plantain sward does not seem to be an effective strategy to reduce N₂O emissions from cattle urine patches.

Influence of Nitrogen Fertilizers on Nitrous Oxide Emissions in Tropical Pastures

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The objective of this study was to quantify nitrous oxide emissions from grasslands soil fertilized with nitrogen fertilizers sources. Treatments were: no nitrogen fertilization (control), urea, ammonium nitrate and ammonium sulfate fertilization in marandu palisade grass (*Urochloa brizantha*), split into three applications in the forage growing season, totaling 150 kg nitrogen ha⁻¹. The design used was completely randomized. The static closed chamber methodology was used to quantify nitrous oxide production and gas chromatography to measure nitrous oxide emissions. The evaluations were subdivided into three periods, starting immediately after each fertilization, totaling 127 days. In the first period, the emissions from the urea treatment were close to the emissions from the control treatment ($P = 0.007$), averaging of 0.99 mg N-N₂O m⁻² and 23.77 mg N-N₂O m⁻², respectively. In the second period, emissions were lower in the treatment without nitrogen fertilization (0.704 mg N-N₂O m⁻²), but did not differ between treatments that received nitrogen fertilization, with average emissions of 10.80 mg N-N₂O m⁻² ($P < 0.001$). In the third period, emissions were lesser in the treatment without nitrogen fertilization (0.108 mg N-N₂O m⁻²) treatments urea and ammonium sulfate, 8.34 mg N-N₂O m⁻² and 4.80 mg N-N₂O m⁻², respectively, and ammonium nitrate treatment presented the greatest emissions (8.92 mg N-N₂O m⁻²; $P < 0.001$). Total emissions during the experimental period were lower in the treatment without nitrogen fertilization, but they did not differ between treatments with nitrogen fertilization ($P < 0.001$). The total N₂O emissions measured were 1.80 mg N-N₂O m⁻² for control treatment, 45.52 mg N-N₂O m⁻² for urea, 48.46 mg N-N₂O m⁻² for ammonium nitrate and 45.79 mg N-N₂O m⁻² for ammonium sulfate. During the studied period, there was not nitrous oxide consumption. In this study, it was found that fertilization stimulated nitrous oxide emissions. However, there is no difference in total nitrous oxide emissions between nitrogen fertilizers.

The Identification of Rumen Microbial Biomarkers Associated with Residual Methane Emissions in Cattle

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The identification of rumen microbial biomarkers associated with the methanogenic potential of ruminants will aid the development of genetic selection programmes as a methane mitigation strategy for livestock. Recently, residual methane emissions (RME), has been identified as the optimal phenotype for assessing the methanogenic potential of ruminants, due to the traits independence from animal productivity but positive correlation with daily methane emissions (DME). However, there is dearth of data available on the contribution of the rumen bacterial and archaeal microbial communities to inter animal divergence in RME. Therefore, the objective of this study was to investigate the composition of the rumen microbiota in a population of finishing beef cattle previously ranked as high and low on the basis of RME. Rumen fluid samples were successfully obtained from 260 animals, having previously undergone detailed measurements of methane output (GreenFeed System), using a trans-oesophageal sampling device (FLORA rumen scoop). Rumen microbial DNA was extracted with 16S rRNA amplicon libraries generated and sequenced on the Illumina MiSeq to determine microbial biomarkers associated with RME. Sequences were classified using DADA2 with taxonomy assigned to sequences variants using the RefSeq + RDP database. An increased relative abundance ($P < 0.05$) of lactic acid producing bacterial genera (*Intestinibaculum*, *Olsnella*) and *Selenomas* was observed in low RME animals. Within the rumen methanogen community, an increased abundance ($P < 0.05$) of the methylotrophic genera *Methanosphaera* as well as the *Methanobrevibacter* RO clade was observed in low RME animals. In addition, the relative abundances of both *Intestinibaculum* and *Olsnella*, as well as *Methanosphaera* and the *Methanobrevibacter* RO clade, were negatively correlated ($P < 0.05$) with both RME and DME. Findings from this study highlight the abundance of both lactic acid producing bacteria and methylotrophic methanogens as potential rumen microbial biomarkers to target as part of the genetic selection of low RME cattle.

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Rumen Microbial Biomarkers Improve Prediction Equations for Enteric Methane Emissions in Cattle

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The objective of this study was to assess the utility of applying daily feed intake, bodyweight, rumen fermentation and rumen microbiota measures as underlying predictors of daily methane emissions (DME) in a population of intensively finished beef cattle. Rumen fluid samples were successfully obtained from 260 animals, as part of feed efficiency performance test during which detailed measurements of feed intake, growth and enteric emissions (GreenFeed System) were conducted over a 90 day test period. Rumen microbial DNA was extracted with 16S rRNA amplicon libraries generated and sequenced on the Illumina MiSeq targeting bacteria and archaea microbial populations. Amplicon sequence data was analyzed in DADA2 and taxonomy assigned to sequences using the RefSeq + RDP database. The contribution to DME of dry matter intake (DMI), daily carbon dioxide emissions (DCE), animal body weight, indices of rumen fermentation and the abundance of bacteria and archaea shown to correlate with DME was assessed using a stepwise regression in SAS. For the variables assessed, DCE (adjusted $R^2 = 0.43$) and animal body weight (adjusted $R^2 = 0.05$) accounted for 48.3% of the variation in DME. The ruminal abundance of three bacterial genera (*Mogibacterium*, *Selenomonas* and *Intestinibaculum*) and the *Methanobrevibacter* SGMT clade accounted for a further 20.4% of the variation in DME. These findings show, that in a population of intensively finished beef cattle offered a consistent diet in this study, the optimal combination of traits to predict DME were DCE, animal bodyweight and the abundance of key rumen microbes associated with methanogenesis, as when combined these variables explained 70% of the variation in DME.

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Organo-Modified Nano-montmorillonite by Quaternary Ammonium Cationic Surfactant Affected the Aflatoxin B1 Toxicity and Ruminal Nutrient Degradability and Methanogenesis *In Vitro*

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The objectives of the current study were to develop the organically modified nano montmorillonite (OMNM) by chemical and mechanical modifications and to use it as a novel feed additive for ruminant diets. Egyptian natural montmorillonite was modified using a quaternary ammonium cationic surfactant (cetyltrimethylammonium bromide) and ground to obtain the nanoscale powder particle size form. The dose-response effects of the OMNM supplemented to a basal diet (50% forage: 50% concentrate) contaminated or not with Aflatoxin B1 (AFB1) (20 ppb) were evaluated *in vitro* using the semiautomatic gas production (GP) system. The experimental treatments were control (basal diet without any supplementations), natural montmorillonite (NM) supplemented at 5 g/kg DM, and OMNM diets supplemented at low (0.5 g/kg DM) and high doses (1 g/kg DM). The physicochemical characteristics of the developed OMNM were enhanced compared to NM clay. The Zeta potential of NM clay was negative (-23) and became more negative (-28) after the organo modifications. The OMNM had a higher cation exchange capacity than its natural form. Diets supplemented with or without AFB1 produced higher ($P < 0.01$) GP than the control diet supplemented with AFB1, while no differences were observed between the NM and control diets. The OMNM supplementation successfully reduced ($P < 0.01$) methane production (CH_4) compared to the control and NM diets, and the contrast test showed linear ($P < 0.01$) CH_4 reduction by the increasing level of the OMNM clay. Supplementation of AFB1 adversely ($P = 0.01$) affected the fiber degradability, while both NM and OMNM supplementations positively affected ($P < 0.05$) the fiber degradability compared to the control diet supplemented with AFB1. No differences were detected on ruminal protozoal counts either by clay supplementation, while AFB1 tended to reduce ($P = 0.08$) the protozoal populations. Diets contaminated with AFB1 tended to have lower ($P < 0.01$) total short-chain fatty acids (SCFAs), propionate, and butyrate concentrations, while shifts towards high ($P = 0.006$) acetate were observed. These results highlighted the *antimethanogenic* and the positive effects of OMNM on reducing the adverse effects of diets contaminated with AFB1.

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***Moringa oleifera*, an Unconventional Food as Strategy of GEE Mitigation in Livestock Production**

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The unconventional foods in animal production aims to contribute to systems sustainability through food alternatives incorporation whilst keeping or improving diet quality and animal performance. The objective of this study was to evaluate the influence of *Moringa oleifera* inclusion in sugarcane (*Saccharum officinarum*) silage (SCS) regarding silage nutritional quality and ruminal methane (CH₄) production through in vitro gas production assay. After cutting, sugarcane was chopped and added with 25 (SM25), 50 (SM50) and 75% (SM75) of chopped Moringa, dry matter basis. The chopped material was ensiled in 3 experimental silos (3 of 2 kg plastic buckets per treatment) added with 1% of micro processed lime. Silages were opened after 30 days when the samples were collected for chemical composition and in vitro gas production analysis. Data were submitted to analysis of variance and means compared by Dunnet's test ($P < 0,05$). Silages with Moringa reduced dry matter content from 416 to 386 and 355 g/kg DM (SE = 3.8; $P < 0.001$) respectively, for SCS vs SM50 and SM75; crude protein increased from 28 for SCS to 56, 76 and 89 g/kg DM (SE= 3, $P < 0.001$) for SM25, SM50 and SM75, respectively. The neutral detergent fiber, acid detergent fiber and lignin for SM50 and SM75 also increased in relation to SCS, whilst gas production potential was not influenced by Moringa inclusion ($P > 0.05$). The CH₄ production was not affected by Moringa (110 ± 6.0 ml/g OMD, $P > 0.005$). Organic matter digestibility was reduced ($P < 0.001$) with 75% of Moringa inclusion (509 and 459 (SE= 10.8) respectively for SCS and SM75. The addition up to 50% of Moringa in SCS was shown to improve silage quality without impairing nutrient digestibility, so it could be considered as an alternative for a high-quality roughage source able to achieve efficiently of increasing supply of crude protein in diets at a low cost of production.

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Inhibition of Methanogenesis by Exploring Hydrogen Destination

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In the face of global climate crisis, ruminal methane emissions pose severe environmental pressure and therefore need resolving. Non-antibiotic methods tested here to inhibit methanogenesis are: 1) enzymatic inhibition of methanogenic pathways by bromochloromethane (BCM); and 2) provision of alternative hydrogen sinks that are competitive against methanogenesis such as nitrate and nitrite reduction to ammonia and sulfate reduction to hydrogen sulfide. While these molecules participating in either method may effectively inhibit methanogenesis, method 1 most likely suffers from the accumulation of hydrogen and decreases dry matter intake and digestibility whereas method 2 may not attain complete inhibition. Therefore, the best solution is to combine both methods so that hydrogen spared from the blocked methanogenic pathways has alternative destinations. The hypotheses for the two experiments conducted so far are whether BCM directly inhibits methanogenesis and whether the provision of alternative hydrogen sinks can competitively inhibit methanogenesis. We conducted *in vitro* fermentation studies with the following independent variables: 3 dietary energy levels (forage-to-concentrate = 70:30, 50:50, and 30:70); 3 levels of each additive (BCM, 3-nitro-1-propionic acid or 3NPA, nitrate, and sulfate); and 3 time points (6, 12, and 24 hours) to examine the additive effects of these variables on methanogenesis and fermentation profiles (short-chain fatty acids, lactic acid, and hydrogen concentrations) as dependent variables. The data from *in vitro* uses of BCM and nitrate individually have shown up to 98% and 90% efficacy in reducing methanogenesis, respectively. The highest level of 3NPA in the experiment decreased methane production across all three diets above, though not as effectively as BCM and nitrate. All levels of sulfate supplementation did not significantly, but tended to, decrease methane production. The anti-methanogenic effects of these compounds are shown to be additive. However, the changes in other fermentation profiles must be examined for practical implications.

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Prediction of Enteric Methane Emission in Southeast Asia Beef Cattle Using an International Database

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Beef cattle in Southeast Asia (SE-Asia) are raised under diverse climatic conditions and feeding systems and have different enteric methane (CH₄) emission characteristics compared with those in the industrialized countries. The objectives of this study were to: i) develop prediction models for CH₄ emission (g d⁻¹ animal⁻¹), yield [g kg⁻¹ dry matter intake; DMI⁻¹], intensity [g kg⁻¹ average daily gain⁻¹], and CH₄ conversion factor (*Ym*) using an international dataset of individual animal records from SE-Asia, ii) evaluate the impact of different dietary forage inclusion rates representing the diverse feeding systems in SE-Asia, and iii) cross-validate prediction models from this study with published data. A total of 398 individual beef cattle observations from SE-Asia were used for the analysis. Linear models developed by incrementally adding covariates revealed that a CH₄ emission model using only DMI fitted to all data had a root mean square prediction error (RMSPE) of 16.9%. Subsets containing all-data with forage proportion in the diet of 100% (all-forage), 50 to 85% (high-forage) and <50% (low-forage) had RMSPE of 16.5, 14.7, and 17.4%, respectively. Linear multiple equation based on DMI and dietary NDF concentration (DMI + NDF_C, RMSPE = 15.2%; all-data) improved prediction accuracy over that of DMI alone. Measurements to obtain additional dietary and animal variables were not justified. Methane yield and CH₄ emission intensity could not be reliably modelled with the current database. Methane conversion factor (*Ym* = 7.0) recommended by IPCC (2019) for high-forage diets may be used for predicting CH₄ emission for SE-Asia beef cattle when dietary forage content is 50 to 85% (RMSPE = 15.1%), but not the *Ym* recommended for all- and low-forage diets (*Ym* = 6.3; RMSPE = 25.6%). Study recommended CH₄ emission prediction equations for the different dietary forage levels based on DMI+NDF and DMI alone were:

- For all-forage diets: [39.25 (11.58) + 20.22 (1.92) × DMI - 0.41 (0.11) × NDF]; and [13.98 (9.89) + 22.79 (1.89) × DMI].
- For high-forage diets: [34.32 (6.78) + 19.81 (1.14) × DMI - 0.43 (0.09) × NDF]; and [14.27 (6.00) + 19.75 (1.27) × DMI].
- For low-forage diets: [36.03 (4.91) + 18.77 (0.86) × DMI - 0.34 (0.05) × NDF]; and [20.15 (4.42) + 19.59 (0.90) × DMI].

The present study reaffirmed that region-specific models are needed to reliably predict beef cattle enteric CH₄ emission at national or regional levels, particularly for low- and middle-income countries.

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Rumen Fermentation and Microbial Adaptation to Three Red Seaweeds Using the Rumen Simulation Technique

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Various red seaweeds have shown to inhibit enteric methane production; however, adaptation of the microbial community to their presence is not well understood. The objective of this study was to examine the effect of three red seaweeds (*Asparagopsis taxiformis*, *Mazzaella japonica*, *Palmaria palmata*) on *in vitro* fermentation, methane production, and microbial adaptation using the rumen simulation technique (RUSITEC). The experiment was conducted as a completely randomized design with four treatments, duplicated in two identical RUSITEC apparatus equipped with eight fermenter vessels each. The four treatments included the control (barley straw and barley silage) and the three red seaweeds added to the control diet at 2% diet DM. The experimental period was divided into four phases including a baseline phase (d 0-7; no seaweed included), adaptation phase (d 8-11; seaweed included in treatment vessels), intermediate phase (d 12-16) and a stable phase (d 17-21). The degradability of organic matter ($P = 0.04$) and neutral detergent fiber ($P = 0.05$) was decreased by *Asparagopsis* during the adaptation phase but returned to control levels in the stable phase. *Asparagopsis* was the only seaweed to suppress methane production ($P < 0.001$), with the suppressive effect increasing ($P < 0.001$) across phases. *Asparagopsis* supplementation resulted in a decrease ($P < 0.001$) in acetate, propionate and total VFA production, with an increase in butyrate, caproate, and valerate production. Amplicon sequencing showed a clear impact of *Asparagopsis* on bacterial and archaeal communities, with little effect observed with the other two seaweeds. The introduction of *Asparagopsis* caused an immediate decline in archaeal diversity, which persisted for the duration of the experiment. In contrast, bacterial community diversity declined during the adaptation to *Asparagopsis*, before recovering to baseline levels. In conclusion, our results suggest that the bacterial community requires an adaptation period to the introduction of *Asparagopsis* to the diet.

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Effects of Three Macroalgae from the Northern hemisphere on Enteric Methane Emission from Dairy Cows

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During fermentation of the feed in the forestomachs of ruminant animals, methane is formed as a by-product. However, methane is a potent greenhouse gas, and thus of concern in relation to climate change. Several macroalgae from the Northern hemisphere have shown some potential *in vitro* to reduce methane emission from ruminants. This study aimed to investigate the effects of three Nordic macroalgae on enteric methane emission and feed intake when fed to dairy cows. The experiment was conducted as a 4 × 4 Latin square design using four lactating rumen, duodenal, and ileal cannulated Danish Holstein dairy cows. The cows were fed a standard lactation diet without (CON) or with addition on DM-basis of either 4% ensiled *Saccharina latissima* (LAT), 4% *Ascophyllum nodosum* (NOD), or 2% *Sargassum muticum* (MUT) for periods of 21 days. All algae products were dried and ground prior to mixing into the experimental diets. Gas exchange was measured the last 4 d in each period, using four individual transparent respiration chambers based on open-circuit indirect calorimetry. Furthermore, dry matter intake (DMI) and milk yield were recorded. The inclusion of the three Nordic macroalgae in the diets of dairy cows did not reduce methane emission expressed as L per d or as L per kg DMI (P-value 0.14 and 0.84, respectively). Additionally, the inclusion of macroalgae did not affect the DMI or milk production (P-value 0.34 and 0.51, respectively). Thus, it can be concluded that the supplementation of *Ascophyllum nodosum*, *Sargassum muticum*, or ensiled *Saccharina latissima* in the feed ration of dairy cows had no impact on neither methane emission nor cow productivity.

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Enteric Methane Emission of Dairy Cows Supplemented “Compound X” in a Dose-Response Study

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The emission of enteric methane from dairy cows constitutes a large part of the contribution from agriculture to global warming. The new feed additive “Compound X”, at present under patenting, has been shown to reduce methane emission derived from rumen fermentation in *in vitro* studies and in one *in vivo* pilot study with dairy cows. This study aimed to investigate the dose-response effects of “Compound X” on enteric methane emission, milk yield, and feed intake of dairy cows. The experiment was conducted as a 4 × 4 Latin square design using four lactating rumen, duodenal, and ileal cannulated Danish Holstein dairy cows. Four different levels (CON, LOW, MEDIUM, and HIGH) of “Compound X” were supplemented intra-uminally twice daily to the cows over a period of 14 days. Gas exchange was measured the last 4 d in each period, using four individual transparent respiration chambers based on open-circuit indirect calorimetry. Additionally, milk yield and dry matter intake (DMI) were recorded. Data were analyzed using the mixed procedure of R. Methane emission (L/d) was significantly lower for MEDIUM and HIGH compared to CON. MEDIUM decreased emission by 47%, while HIGH reduced emission by 79%. However, DMI and milk yield were also significantly lower for HIGH compared with CON. The two highest dosages, MEDIUM and HIGH, significantly lowered methane yield (L/kg DMI) by 35% and 79%, respectively. Methane intensity (L/kg ECM) was also significantly lower for MEDIUM and HIGH compared to the two remaining levels. MEDIUM reduced the intensity by 48% and HIGH reduced the intensity by 73% compared to CON. Thus, it can be concluded that intra-uminal supplementation with MEDIUM or HIGH levels of “Compound X” had a large reducing effect on methane emission. However, HIGH also significantly lowered DMI and milk yield.

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Carbon Balance of Dairy Systems in Argentina

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The aim of this study was to evaluate the carbon footprint (CF) and carbon balance (CB) in real dairy systems in the province of Santa Fe, Argentina. Nine contrasting dairy farms were selected through a simple random sampling of surveys conducted by INTA: total mixed ration (TMR) (n=1), pasture-based with partially mixed ration (PMR) (n=4) and pasture-based with separately delivered feed (SF) (n=4). PMR and SF systems included systems with high animal stocking rate (SR; ≥ 1.5 cows/ha) and high diet concentrate amount (CA; ≥ 6 kg/cow/day), high SR and low CA, low SR and high CA and low SR and low CA. Estimation of GHG emissions (E/ha; tnCO₂eq/ha) and CF were carried out by IPCC Tier 2 (2019). Soil organic carbon sequestration (SOC) was estimated by Ricard and Viglizzo (2020) methodology. The CF and the CB (GHG- SOC) per kg of fat and protein corrected milk (FPCM) were calculated for each farm up to the farm gate, considering an average milk allocation factor of 90%. The E/ha and CF were 9.3 tnCO₂eq/ha and 0.75 kgCO₂eq/kgFPCM, respectively. SOC and CB were 0.17 and 0.58 kgCO₂eq/kgFPCM, respectively. Systems with low SR had lower E/ha. SF systems presented similar average CF (0.75 kgCO₂eq/kgFPCM) and lower CB (0.56 kgCO₂eq/kgFPCM) respect to PMR (0.76 and 0.59 kgCO₂eq/kgFPCM, respectively) and TMR (0.73 and 0.63 kgCO₂eq/kgFPCM, respectively). SF-LSLC had higher emissions from crop and pasture residues, but had the highest SOC. In SF and PMR with low SR, SOC represented 30.3 and 27.2% of the CF in average, in SF and PMR with high SR, 23.1 and 20.0%, respectively, and in the TMR, 14%. Results suggests that in Argentina pasture-based dairy systems is the potential to reduce milk CF through the application of practices that do not necessarily imply the intensification of the systems.

Feeding By Protected Fat on Methane Emission of Holstein Cows in Summer

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The aim of this study was to evaluate the effect of supplementation with a protected fat (PF) source on CH₄ emission and in Holstein cows under heat stress conditions. Thirty mid-lactation Holstein cows were used in a 12-week trial during the summer 2020-2021 INTA Rafaela, Argentina in dry lot pens. They were distributed in 15 blocks by number of lactations (2.0 ± 1.1), lactation days (182 ± 80) and milk production (29.4 ± 5.7 kg/day) at the beginning of the trial and randomly assigned within each block to the following treatments (diets): PF = supplementation with PF (4.9 and 0.7 kg/day of concentrate and PF, respectively) or WPF= without PF (isoenergetic replacement of fat by corn grain). The rest of the diet consisted of a totally mixed ration *ad libitum* (26.0% corn silage, 33.2% alfalfa silage, 8.5% ground corn, 18.1% soybean meal, 5.2% soybean expeller and 9.0% alfalfa hay). For the enteric CH₄ evaluation, 16 cows (8/treatment) were selected and the CH₄ emission was estimated using SF₆ tracer technique (Johnson et al., 1994) for 5 days, in the 7th week. Simultaneously, was measured the dry matter intake (DMI), milk production (MY) and composition, and energy-corrected milk production (ECM) (Tyrrel and Reid, 1965). No significant differences ($p > 0.05$) were observed in enteric CH₄ emission (421 and 415 gCH₄/d for CGP and SGP, respectively). Neither when the emission was expressed per unit of ECM (15.9 vs. 16.5 gCO₂/kg ECM) for PF and WPF, respectively) or per unit of DMI (19.4 vs. 18.7 gCH₄/kg DMI, for PF and WPF, respectively). In the productive response study, we obtain an improvement in MY corrected for solids and fat production in the PF group, with no significant effect on enteric methane emission.

Managing Enteric Emissions from Grazing Systems Using Leucaena

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Across northern Australia commercial Leucaena (*L. leucocephala glabrata*) has been planted to support productive and profitable operations producing "grass-fed" beef. Beef production systems should be evaluated in terms of yearly livestock productivity and herd methane emissions to demonstrate the effectiveness of Leucaena on decreasing enteric emission intensity and how this contributes to abatement opportunities.

A two-year field trial was conducted on Belmont research station QLD (LAT 23.2134° S; LONG 150.390258° E.) to determine the impact of Leucaena grazing systems on herd scale methane emissions, productivity and rumen function. *Bos indicus* cross steers [n = 60, initial mean (\pm sem) LW 296 \pm 5.2 kg] were continually managed on Leucaena with inter rows containing Rhodes (*C. gayana*) and Green panic (*P. maximum*), or pasture only dominated by Rhodes grass. Herd scale emissions were determined using open path lasers on four occasions and liveweight measured at regular intervals over 600 d. Herd scale data indicated that steers grazing Leucaena had lower daily emissions (g CH₄/head) by up to 18% on two occasions than those grazing grass only. Mean daily gains were 0.59 and 0.42 kg/d for Leucaena and Rhodes grass fed steers respectively which extrapolated to 335 g CH₄/kg carcass weight (CW) compared with 596 g CH₄/kg CW. Rumen microbial analysis showed Leucaena diets increased the relative abundance of methylotrophic methanogens while decreasing the proportion of other functional groups of methanogens. In Leucaena fed cattle there was a shift in the bacterial populations and fermentation to more reduced end products which may contribute along with leucaena tannins to decreased methane emissions. The addition of Leucaena to beef production systems has the potential to increase productivity and gross margin, whilst reducing emissions intensity. Provided net farm emissions are maintained or reduced, Leucaena appears conducive to sustainable intensification of beef production in grazing systems.

Intensity of Emissions Per Unit of Product for Bovine Livestock in Colombia

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The aim of this study was to estimate the intensity of greenhouse gas emissions per unit of product, for 6 bovine age groups in Colombia in 10 livestock regions; the analysis of the intensity of emissions per unit of product contributes to a better understanding of the environmental efficiency of production systems. Greenhouse gas emissions were estimated under multi-model equations, the methodological level TIER 2 was obtained for enteric CH₄ and manure management; Direct and indirect N₂O emissions of categories from manure management were calculated using the advanced TIER 1 methodology. The product units used were 1kg of meat and milk protein, 1kg of milk corrected for fat and protein, and 1kg of live weight gain. The intensity of emissions per unit of product was calculated as the relation between the total emission of each age group in CO₂eq and the unit of product. As a result, the mean intensity in Colombia was 124.59 ± 72.28 kg CO₂eq per kg protein year⁻¹, beef cows presented the highest intensities (204.83 ± 32.47 kg CO₂eq per kg protein year⁻¹) followed by fattening cattle (149.35 ± 20.80 kg CO₂eq per kg protein year⁻¹). Milk productions systems, the high and low production dairy cows showed an average of 1.47 ± 0.44 kg CO₂eq per kg milk corrected for fat and protein year⁻¹, the high production dairy cows had a lower emission of 38.34% compared to the national average. The intensity of the emission per kg of live weight gain estimated in the categories of pre-weaned calves, replacement heifers and fattening cattle presented an average intensity of 10.86 ± 4.97 kg CO₂eq per kg of live weight, with average values of 5.02 kg CO₂eq per kg for pre-weaned calves and 15.14 kg CO₂eq per kg live weight gain for replacement heifers. The variations in emission intensities by animal category reflect the heterogeneity in the country, in terms of livestock agroecosystems and contrasting productive systems, ranging from the plains of the Orinoco region and the Tropical Forests to the high Andean areas in the Cundiboyacense region. passing through medium and highly productive regions such as Magdalena and the South Central region, located in the inter-Andean valleys of Colombia; This work has been the basis for stakeholders mitigation actions for Colombia currently.

Integration of Livestock-related Targets into National Policies and Climate Action

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The global climate ambition is off track to reach the Paris Agreement goal to keep global temperature rise well below 2°C limits and preferably below 1.5°C as by 2030 compared to the pre-industrial level. Countries, therefore, are encouraged to revisit and enhance their commitments to reduce greenhouse gas emissions across all sectors in 2022. More than 103 countries recently joined the Global Methane Pledge to cut 30% of methane emissions by 2030 from 2020 levels. Livestock alone contributes 32% percent of total anthropogenic methane emissions. Around one-third of all national climate commitments, or nationally determined contributions, include mitigation measures or targets in the livestock sector, in particular to reduce methane. Achieving these commitments, however, will depend on the extent to which livestock and climate change commitments are embedded into national policies, strategies, action plans and laws. These actionable policies are a prerequisite to achieving the Paris agreement in agri-food systems. This study assesses the degree of policy coherence and integration of climate change and livestock commitments within national policies and action plans, as well as identifies opportunities to raise ambition in climate action by integrating more livestock targets. The study relies on a policy analysis framework, which evaluates different policies and nationally determined contributions using policy assessment criteria for alignment. Furthermore, stakeholder consultations were conducted to validate the methodology and outcomes in four countries: Costa Rica, Nicaragua, and Rwanda. The results summarize the alignment between nationally determined contributions and national policies, baseline GHG emissions from livestock systems based on the Global Livestock Environmental Assessment Model (GLEAM), outcomes from stakeholder consultations, technical and policy gaps to be closed for the implementation of climate action and policy recommendations to support the achievement of the SDG 13 on Climate action and the Paris Agreement.

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Let Graze or Feed Indoors: Mitigating Methane through Pasture Grazing Compared to Silage Feeding Indoors

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The objective of this study was (1) to collate a treatment mean database of enteric methane production from lactating dairy cows on pasture or fed grass silage indoors, and (2) to compare the two feeding regimes. We hypothesized that cows on pasture are characterized by lower methane yield (MY, g/kg dry matter intake, DMI) and methane emission intensity (MI, g/kg energy-corrected milk yield, ECM) than cows fed grass silage indoors. A comprehensive search of ISI Web of Science produced 52 experiments from which 159 treatment means (pasture n=62, indoors n=97) were subjected to analysis of variance using R. Additionally, a database subset (pasture n=21, silage n=24) was examined with cows that had similar ECM ranging from 20 to 25 kg/day. Dietary forage proportion (on DM basis) in pasture and silage fed cows was $82 \pm 15\%$ and $60 \pm 12\%$, respectively. Daily DMI, ECM and methane production ranged from 9.3 to 25 kg, 2.0 to 46 kg, and 203 to 614 g, respectively. DMI, ECM and methane production were lower ($P < 0.01$) in pasture than silage fed cows. MI did not differ ($P = 0.13$) between feeding regimes and MY was 7% lower ($P < 0.01$) in cows on pasture. For the database subset, DMI and ECM did not differ between dietary treatments, but methane production was 21% lower ($P < 0.01$) on pasture compared to feeding grass silage indoors. Accordingly, cows on pasture compared to indoors had a 14 and 21% lower ($P < 0.01$) MY and MI, respectively. Nutrient concentrations were not available for all observations but indicate that dietary NDF intake did not differ between groups (about 7 kg/d, $P \geq 0.20$, database and database subset). Thus, the lower MY and MI from pasture fed cows cannot be explained by lower fiber intakes. Methane production (g/d) and DMI (kg/d) were positively correlated, both in the complete database (correlation coefficient, $CC > 0.6$, $P < 0.01$) and in the database subset. In the latter, methane production (g/d) from pasture grazing cows increased with 10 g/kg DMI ($CC = 0.5$, $P < 0.05$), and with 25 g/kg DMI ($CC = 0.8$, $P < 0.05$) in silage fed cows. Reduction in methane emission on pasture compared to grass silage fed indoors is remarkable considering that the proportion of concentrates in the diet fed indoors was twice as high (20% vs. 40%), which normally results in lower emissions.

Using Spot-Sample Breath Measurements of Methane Emissions from Dairy Cattle for Genetic Evaluations

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Many strategies exist to reduce enteric methane emissions from dairy cattle, of which the most widely recognized methods include feeding strategies. A developing method is animal breeding, which results in a cumulative, permanent, and cost-effective solution for farmers. Our aim was to estimate genetic parameters from spot-sample measurements by two non-invasive breath recording devices: sniffers (ppm) and the GreenFeed (g/cow/day). The devices were installed on 25 farms, where four farms recorded with both systems simultaneously although at a different location in the barn. The data were corrected for hour of the day, whereafter averages per week were taken. In total, 31,861 weekly averages from 1,770 cows were recorded for sniffers, and 4,444 weekly averages from 737 cows for GreenFeed. The heritability and a genetic correlation between measuring methods were estimated from mixed models using a restricted maximum likelihood approach. The models included fixed effects for: herd*year*week, parity, and 3rd order Legendre polynomials for days in milk, and random effects for the genetic cow effect and the repeated measurements for each cow. The estimated heritabilities were similar to the heritability of milk yield and show that differences between cows are partly due to genetic factors, and therefore selecting for lower emitting cows is possible ($h^2 = 0.29$ for sniffer, and 0.39 for GreenFeed). The genetic correlation between enteric methane emissions recorded by sniffers and GreenFeed was 0.62 ± 0.13 , which suggests that the two devices ranked cows similarly from low to high emitting and could be combined in genetic evaluations. In conclusion, breath recorded methane emission shows potential to be included in breeding programs. Before enteric methane can be included in a breeding goal, genetic correlations with other breeding goal traits have to be estimated, so a reduction in methane emissions will not negatively influence production, feed efficiency, conformation, fertility, or health.

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Comparison of The Laser Methane Mini and GreenFeed System to Measure Enteric Methane in Dairy Cows

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The GreenFeed system (C-Lock Inc., USA) is nowadays the most frequently used technique to measure enteric methane in dairy cows. This system requires voluntary visits from cows and risks to miss data from a number of cows that do not visit the device. Therefore, sensor devices such as the handheld Laser Methane Detector (LaserMethane Mini (LMM), Crowcon Detection Instruments Ltd., UK) could be used as an alternative to measure methane emissions. GreenFeed and LMM both measure methane concentrations, but they are used in different ways. Therefore, it is unclear how well their measurement results correspond, especially since the LMM was originally developed for methane leak detection. In this study a comparison between GreenFeed and LMM measurements was made to find out whether results are comparable. During a period of four weeks, ten cows housed in a freestall barn were measured daily in the morning using the LMM, while throughout the day the cows had access to the GreenFeed. For LMM measurements, cows were fixed in a chute outside the barn to prevent influence of surrounding cows on the measurement. The area was shielded using grass bales to reduce the influence of wind and weather. Emissions were measured by aiming the LMD in the nostril of the cow for at least four minutes while maintaining the same distance (1.7 m) and sensor height throughout measurements. The results showed that it was not easy to gather qualitative data using the LMM as cows were often moving their head, thus disturbing the measurement by making it very difficult to keep aiming at the same spot.

LMM measurement data were analyzed by calculating the mean peak height of all CH₄ concentration measurements (ppm.m, P_{MEAN}), and averaged per cow. GreenFeed (GF) emission data were used as provided by C-Lock, and raw GF data of the respective successful cow visit data were calibrated and consecutively processed using the same algorithm as LMM data. When comparing GF emission data with LMD P_{MEAN} using a linear regression, no relation was found ($R^2 = 0.00$). Similarly, a regression of GF P_{MEAN} and LMM P_{MEAN} yielded an R^2 of 0.08. Lastly, a regression of GF emission data and GF P_{MEAN} yielded an R^2 of 0.13. We concluded that the results of both measuring devices are not easily comparable, and that the processing method of LMM raw data did not yield comparable results when used on raw GF data and compared with emission data calculated from the same raw GF data.

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Models to Predict Ammonia and Nitrous Oxide Emission Factors for Land-Applied Manure and Urine

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The DATAMAN and MELS projects collated data on greenhouse gas and ammonia (NH₃) emissions from manure management (housing, storage and field) from across the world. The objective of our study was to analyse the 'field' component of the DATAMAN database (www.dataman.co.nz) to develop models to predict emission factors for both NH₃ and nitrous oxide (N₂O). For NH₃, we focused on cattle and swine slurry applied to land using a range of slurry application methods (broadcast, trailing hose, trailing shoe, and open slot injection), while for N₂O emission factors, we focused on cattle and sheep urine deposited onto soils. Modelling and estimation of parameters were undertaken with multi-variable linear mixed effects models fitted to the cube root transformed data. The NH₃ predictive models for cattle and swine slurry showed slurry dry matter content and the slurry application method having a statistically significant influence on NH₃, explaining 14% and 17%, respectively, of the variability. The N₂O predictive model developed for cattle urine showed the emission factor increased as both soil water content and soil pH increased, explaining 21% of the N₂O emission factor variability. All other significant predictive models, including those for the sheep urine N₂O emission factor, were based on single variables. Given the known influence of a range of variables on these emissions, the single-variable models are limited in their application. The multi-variable predictive models help to improve our understanding of relatively complex processes underlying greenhouse gas emissions and aid the development of models for national inventories and farm-scale tools.

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Does Basal Diet Composition Impact the Methane Mitigation Potential of 3-Nitrooxypropanol in Dairy Cattle?

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The objective was to determine whether the methane mitigation potential of 3-nitrooxypropanol (**3-NOP**) in dairy cows was affected by basal diet composition. Eight rumen-fistulated, multiparous Holstein-Friesian dairy cows were assigned to a double 4 × 4 Latin square design. The four treatments were (on DM basis) a grass silage-based diet (**GS**; 67% grass silage and 33% concentrate) and a corn silage-based diet (**CS**; 13% grass silage, 54% corn silage, and 33% concentrate) supplemented with a placebo (**NOP0**) or 80 mg 3-NOP/kg DM (**NOP80**). Each experimental period consisted of 14 d adaptation in a free-stall barn followed by 4 d of measurements in climate respiration chambers. On the last 2 d of adaptation, rumen fluid was collected 1 h before, and 1, 2, 3, 4, and 6 h after morning feeding. Rumen pH was recorded at 1-min intervals using indwelling pH loggers during the respiration chamber period. NOP80 decreased ($P = 0.038$) DM intake (**DMI**) with 1.0 kg/d, both with GS and CS. NOP80 did not affect milk yield and composition ($P > 0.100$). Ruminal pH increased ($P = 0.036$) and ruminal acetate to propionate ratio decreased ($P = 0.012$) with NOP80 both with GS and CS. Methane yield (g/kg DMI) decreased ($P = 0.002$) with NOP80 for both GS and CS, but the decrease in methane yield was numerically smaller for GS (-12.6%) compared with CS (-24.2%). A similar pattern was observed for methane intensity (g/kg energy corrected milk), which decreased ($P < 0.001$) with NOP80 for both GS and CS, but the decrease was numerically smaller for GS (-14.6%) compared with CS (-24.7%). In conclusion, 3-NOP effectively decreases methane emissions in dairy cows but potentially to a greater extent with a corn silage-based diet compared with a grass silage-based diet.

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Methane Emission from Dairy Cows Receiving 3-Nitrooxypropanol for One Complete Year

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The objective of this study was to determine the effect of 3-nitrooxypropanol on methane emission and production characteristics from dairy cows receiving 3-nitrooxypropanol in their diet for one complete year, including the dry period. Sixty-four late-lactation Holstein-Friesian dairy cows (33% primiparous) were blocked in pairs, based on expected calving date, parity, and milk yield. The experiment started with a covariate period of 3 weeks in which all cows received the same basal diet and baseline measurements were performed. Directly after, cows within a block were randomly allocated to 1 of 2 dietary treatments: a diet including on average 59 mg 3-nitrooxypropanol/kg DM (**3-NOP**) and a diet including a placebo (**CON**). The diet consisted of grass silage, corn silage, and concentrate. The ratio between grass silage and corn silage was constant throughout the experiment (70:30; DM basis). The forage to concentrate ratio depended on lactation stage (i.e., the dry period and early, mid, and late lactation) and milk yield. Diets were provided as a total mixed ration in feed bins (Hokofarm B.V.), which automatically recorded feed intake. The GreenFeed system (C-Lock Inc.) was used to measure methane emissions. Overall, 3-NOP did not affect daily milk yield (26.4 and 27.5 kg/d for CON and 3-NOP, respectively) and total DM intake (21.0 and 20.8 kg/d for CON and 3-NOP, respectively), but resulted in a higher energy-corrected milk yield (32.3 and 34.2 kg/d for CON and 3-NOP, respectively). Methane yield (g/kg DM intake) over the year was reduced by 19%, being 20.1 and 16.3 g/kg DM intake for CON and 3-NOP, respectively. Preliminary analysis show that the mitigation potential of 3-NOP depended on the quality of the grass silage (i.e., NDF digestibility in %), which appeared positively related to the mitigation potential of 3-NOP ($R^2 = 0.59$).

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Enteric Methane Emissions from Holstein Friesian Heifers

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Enteric methane emissions from dairy cattle are considerably contributing to the greenhouse gas emissions from the agricultural sector. Beside mature dairy cattle, young stock is a source of enteric methane emissions that cannot be ignored. According to the IPCC guidelines, a TIER 2 method is used to estimate the enteric methane emissions of growing cattle in Flanders, although data during the rearing period is limited. An observational study was performed using the GreenFeed system (C-lock inc.) in which enteric methane emissions of 169 Holstein heifers of different ages were monitored. The animals were housed in a free-range stable in small groups, varying from 8 to 14 animals. Within a group, all animals were around the same age, with the youngest being 6 months and the oldest being 25 months. Every measuring period consisted of 28 days. At the beginning and end of each period, all animals were weighted in order to calculate a mean live weight gain over the measuring period. Once a day, animals were fed a roughage mixture consisting of grass silage, maize silage and straw. The inclusion of straw increased with increasing age. A concentrate was provided via the GreenFeed system to allow methane emission measurement. An average CH₄ production was calculated over the measuring period of 4 weeks. The enteric methane production varied from 135 to 315g CH₄ per day. Beside the measured enteric methane production, the enteric methane emissions were also calculated, according to the IPCC guidelines (TIER 2 method) as provided in the greenhouse gas inventory. The calculated methane production was significantly ($p > 0.01$) lower compared to the methane production measured until the age of 15 months. Thereafter, the calculated methane production did not differ. These results provide information for further improvement for the estimation of the production of greenhouse gas emissions in Flanders.

Increasing Inclusion Rates of Green Algae Residues on Ruminal *In Vitro* Fermentation Profile and Methane Production

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Increasing algae cultivation for fuel production or human consumption will result in greater byproduct availability for livestock. Certain algae residues have potential as dietary ingredients in ruminant diets due to their great concentration of crude protein (20% approx.) and their demonstrated methane abatement capabilities. However, research is limited regarding the use of post-extraction residue (PEAR) of green algae. The objective of this experiment was to evaluate the effects of increasing inclusion rates of green algae (*Chlorella* sp.) residues on ruminal *in vitro* fermentation profile and methane production in a corn silage-based diet. Incubations were conducted on three separate days using corn silage and gin trash as substrate (approximately 70:30). Treatments were 0, 1, 5, and 10% of PEAR inclusion in the substrate DM. Ruminal fluid was collected from two Angus-crossbred steers fed ad libitum corn silage and gin trash. Final pH, concentration of volatile fatty acids (VFA) and ammonia nitrogen (NH₃-N), *in vitro* organic matter digestibility (IVOMD), total gas production, and concentration of methane (CH₄) were determined after 24 h of fermentation. Variables were evaluated using the Proc mixed of SAS and orthogonal polynomial contrast. Concentrations of crude protein, fatty acids, and organic matter of PEAR were 19, 1, and 49%, respectively. Final pH and concentration of VFA and NH₃-N were not different ($P > 0.05$) between PEAR inclusions. The proportion of VFA and acetate:propionate ratio were not affected ($P > 0.05$) by PEAR inclusion. A linear increase ($P < 0.01$) in IVOMD and a cubic effect ($P < 0.01$) in total gas production was observed with increasing inclusion of PEAR; however, CH₄ production was not affected ($P > 0.05$) by PEAR inclusion. In summary, the inclusion of PEAR did not substantially modify the ruminal *in vitro* fermentation profile, nor did it affect CH₄ production in a corn-silage based diet.

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Non-protein Nitrogen Supplementation on *In Vitro* Fermentation and Methane Production in a Corn Silage-based Diet

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Including non-protein nitrogen (NPN) sources is an alternative to improving rumen fermentation and animal performance and reducing methane emissions in low-protein diets. The aim of the current study was to evaluate the effect of different NPN sources on *in vitro* fermentation and methane production in a corn silage-based diet. Treatments were control (without NPN), urea, and five proportions of biuret and nitrate mixtures (100:0, 75:25, 50:50, 25:75, and 0:100). Incubations were conducted in three separate days during 24 h using corn silage and gin trash as substrate. Each treatment, except control, was formulated to be isonitrogenous using 1% urea inclusion as a reference. Ruminal fluid was collected from two Angus crossbred steers fed ad libitum corn silage and gin trash, plus 100 g of a urea-biuret-nitrate mixture at least 35 d before collection. Concentration of volatile fatty acids (VFA) and ammonia nitrogen (NH₃-N) were determined at 12 and 24 h. Final pH, *in vitro* dry and organic matter degradability, gas production, and concentration of methane (CH₄) were determined at 24 h. Supplementation of NPN increased ($P < 0.05$) the concentration of total VFA and acetate at 12 h, but not 24 h. Nitrate supplementation increased ($P < 0.05$) the molar proportion of acetate and reduced ($P < 0.05$) the molar proportion of butyrate at 12 and 24 h. NPN supplementation increased ($P < 0.01$) the concentration of NH₃-N at 12 and 24 h. Dry and organic matter degradability and final pH were not affected ($P > 0.05$) by treatment. The fractional rate of gas production was increased ($P < 0.05$) with NPN supplementation. Increasing nitrate proportion linearly reduced ($P < 0.05$) CH₄ production. In conclusion, supplementation of NPN improved fermentation at 12 h, while inclusion of nitrate decreased CH₄ production under *in vitro* conditions in a corn silage-based diet.

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Feed-focused Strategies to Mitigate Greenhouse Gas Emissions in Livestock Systems in Developing Countries

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Reducing methane emissions from livestock is beneficial both for reducing livestock's impact on global warming and for improving livestock productivity through saving dietary energy, as enteric methane (CH₄) emission is responsible for a loss of up to 20% of metabolizable energy. Given higher depends on low quality roughages, livestock in developing countries are important contributors of greenhouse gas emissions. Strategies developed so far to reduce methane emission have not recognized the objective realities of smallholder production systems in developing countries and therefore are either not affordable to smallholders or smallholders will not have the incentive to implement them. In the absence of strong regulatory mechanism that can impose environmental standards, smallholder farmers need incentives that would promote more carbon neutral livestock production systems. The feed subsector, being the most important subsector determining the number of emissions from livestock, provides an opportunity for reducing the GHG emissions from livestock production in developing countries. Smallholder producers in developing countries, however, need affordable and technically feasible technologies that can be applied to reduce GHG emissions while enhancing livestock productivity. Fortunately, most feed-focused strategies for reducing GHG emissions from livestock also simultaneously improve productivity and feed consumption efficiency. In this review, we identified innovations with relevance to smallholder producers. Based on relevance and applicability, the feed-based innovations aimed at reducing GHG emission can generally be categorized into technologies that improve feed quality and animal nutritional status, feed additives, secondary plant metabolites, climate smart feed systems and manure management. Most of these innovations are still under development and need further research before they can be made available for smallholder farmers. A few others, such as carbon negative silvopastoral and pasture-based production systems, are already part and parcel of traditional feed systems in many developing countries and can be enhanced for additional reduction in GHG emissions among smallholder livestock production systems.

Key words: Greenhouse gas emissions, Livestock, Feed, Methane (CH₄), Smallholder, Developing

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Effect of Water–Soluble Carbohydrates Content in *Lolium perenne* on Enteric Methane Emissions: Meta–Analysis

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Our objective was to study the relationship between enteric methane (CH₄) production (g/d) and yield (g/kg dry matter [DM] intake) as a function of water–soluble carbohydrates (WSC) concentration (g/kg DM) in perennial ryegrass (*Lolium perenne*; RG). Data were obtained from *in vivo* experiments (sheep and cattle) published in peer–reviewed journals (27 experiments from 19 studies). The WSC range in RG was 41.4–330 g/kg DM. The CH₄ production range was 196–462 g/d for cattle and 12.2–31.9 g/d for sheep, with a CH₄ yield range of 16.1–28.4 g/kg DM intake. The mixed–effects regression model for CH₄ production included as variables WSC concentration, the random effect of the experiment nested in the studies, and the interaction between WSC concentration and the experiment, and DM intake (kg/d) as a co–variable. The model for CH₄ yield included WSC concentration, the random effect of the experiment nested in the studies, and the interaction between WSC and experiment. Both CH₄ production and yield were negatively associated to WSC concentration ($P \leq 0.019$). For every g/kg DM increase in WSC concentration, CH₄ production decreased by 0.169 g/d, and CH₄ yield by 0.0311 g/kg DM intake. The negative association between CH₄ emissions and WSC concentration may be related to the fact WSC are fermented to a lower acetate to propionate ratio compared to fiber, with propionate acting as an electron sink that competes with CH₄ formation. Importantly, there was no interaction ($P \geq 0.285$) between WSC concentration and the experiment effect in both models, suggesting that the WSC effect on CH₄ might be independent from ruminant species, or CH₄ measurement method. In conclusion, the increase in WSC concentration in RG slightly reduced enteric CH₄ production and yield.

Keywords: Perennial ryegrass, greenhouse gases, sheep, cattle

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Greenhouse Gas Profiles and Emissions from New Zealand Sheep and Beef Farms

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A dataset containing production efficiencies and greenhouse gas (GHG) emissions from 170 New Zealand sheep and beef farms was used to examine the relationships between farm management, biophysical resources, and GHG emissions. Feed inventories and GHG emissions were obtained using the farm-scale model FARMAX; emissions were calculated from FARMAX outputs using Agricultural GHG Inventory methodology. Farms were grouped using a quantitative approach based on physical constraints and management attributes. Mean annual biological GHG emissions from the modelled farms were 3,662 kg CO₂ equivalents (CO₂-e) per effective hectare (i.e., unit of area in grazing + crops; herein ha) and ranged from 157 to 7,096 kg CO₂-e/ha. As stocking rate and animal product (wool + net carcass weight) per ha increased, GHG emissions increased. However, there was considerable variability; farms with GHG emissions of approximately 4,000 kg CO₂-e/ha had an almost three-fold difference in animal production (range 129 to 360 kg/ha). Methane accounted for 80% of total emissions (CH₄ + N₂O). Of the variables selected, feed conversion efficiency (FCE; kg DM intake per kg animal product) and total farm area were the only variables that showed a negative correlation with GHG emissions per ha. A subset of farms (n = 23) with data for the 1994 and 2016 seasons was used to assess the effect of time on GHG emissions. During this period, FCE was enhanced from 34.0 to 29.7 (the smaller number reflects a more efficient conversion) and GHG emissions were reduced from 3,182 to 3,001 kg CO₂-e/ha. The latter dataset offers potential to understand the extent of management and/or biophysical constraints to further efficiency gains, whereas the former dataset provides a holistic assessment of the farm-scale drivers of GHG emissions and a comprehensive baseline from which future trends in farm-scale GHG emissions can be established.

Effect of 3-Nitrooxypropanol Alone and in Combination with Essential Oils on Methane Production and Dairy Cow Performance

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The effect of supplementing dairy cattle with 3-nitrooxypropanol (3-NOP, Bovaer 10®) alone or combined with a blend of essential oils (BEO, Crina® Ruminants) on methane emissions and milking performance compared with cows fed a placebo was investigated over 84 d. Sixty multiparous cows (BW: 606±70 kg, milk yield: 40.7±9.8 kg/d, DIM: 80±32) were divided into 3 treatments (Control; 3-NOP: 60mg 3-NOP/kg DM and COMBO: 60mg 3-NOP/kg DM + 1.5 g BEO/d). Individual intake of a diet (15% CP, 31.1% NDF, 1.70 Mcal of NEI/kg, DM basis) was monitored and cows had free access to GreenFeeds (C-Lock Inc., USA) to measure enteric emissions. Sniffers (Guardian NG Edinburgh Instruments Ltd., UK) recorded methane emissions at the exit of the milking parlor. Data were analyzed using a mixed-effects model for repeated measures. Methane emissions recorded using GreenFeeds were 27.4% and 34.2% lower ($P < 0.05$) in 3-NOP (262.0 ± 11.72 g/d) and COMBO cows (238 ± 11.72 g/d) than Control cows (361 ± 11.72 g/d), respectively, indicating potential synergy with the combination. Methane emissions recorded with Sniffers were 15.2% and 19.5% lower ($P < 0.05$) from 3-NOP (422 ± 17.6 l/d) and COMBO cows (401 ± 17.6 l/d) than Control cows (498 ± 17.6 l/d). Inhibiting methane resulted in an increase in H₂ emissions greatest for 3-NOP (5.24 ± 0.44 g/), followed by COMBO (4.85 ± 0.44 g/d) and Control (2.56 ± 0.44 g/d). DMI, Milk yield and quality were unaffected by treatments; however, feed intake increased ($P < 0.05$) in 3-NOP and COMBO cows over time, resulting in a reduction in feed efficiency (milk/intake). In conclusion, both GreenFeeds and Sniffer techniques can be used to assess potential effects of feed additives on methane emissions. Both, 3-NOP and its combination with BEO are effective substances to reduce methane emissions from dairy cattle without affecting milk yield but decreasing feed efficiency over time.

Effect of NPN Source, Dietary Protein Level, and Production Level on Methane Emission in Dairy Cows

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Nitrate is a hydrogen sink and is expected to reduce methane. The aim of this study was to investigate the interaction between NPN source, dietary protein level, and production level (genetic yield index) on methane emission in dairy cows. Forty-eight Danish Holstein dairy cows (24 primiparous; 24 multiparous) were used in a 6×4 incomplete Latin square design. Cows were blocked in 8 groups based on parity and DIM. Six experimental diets were formulated and included 2 NPN-sources (nitrate or urea) \times 3 crude protein (CP) levels (low, medium, and high; 144, 154, 163 g CP/kg DM) in a factorial arrangement. Gas emission was measured by 4 GreenFeed units. Data were analyzed in R studio (version 3.6.3) using the Linear Mixed-Effects Models procedure. Milk yield and energy-corrected milk yield (ECM) decreased as the CP level increased, while milk fat and urea concentration increased. The addition of nitrate significantly reduced DMI, ECM, CH₄ production, CH₄/DMI, CH₄/ECM, and milk protein and lactose concentrations, whereas H₂ production and milk urea concentration were increased. As production level of cows increased, DMI, ECM, and daily milk fat and protein yield increased. Reduction effect of NPN addition on DMI, ECM, and daily CH₄ and CO₂ production was greater on multiparous cows than primiparous cows. No interactions were observed between production level and NPN source and between CP level and NPN source on methane emission. In conclusion, dietary CP level had no effect on methane emission in dairy cows, nitrate addition reduced DMI, ECM, and CH₄ and CO₂ production and increased H₂ production. However, there was no interaction between NPN source, dietary protein level, and production level on methane emission.

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Sulfuric Acid Modified Vermiculite Cover for Reducing Ammonia and Methane Emissions from Animal Slurry Storage

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Animal slurry storage is an important source of NH_3 and CH_4 emission which has raised a high attention regarding its influence on air quality and environment. There is an urgent need to develop an efficient and safe technology for reducing NH_3 and CH_4 emission. A novel method by using H_2SO_4 -modified expanded vermiculite cover (H_2SO_4 -VM) was proposed, and it was supposed to achieve a combined effect of cover barrier and slurry acidification. Firstly, the constructed H_2SO_4 -VM method was compared with the commonly used H_2SO_4 -acidification of slurry (H_2SO_4 -AC) for their overall gas mitigation effect (ME). It's found that H_2SO_4 -VM and H_2SO_4 -AC achieved comparable NH_3 (87% vs 90%) and CH_4 (52% vs 50%) ME during a 78 days storage period, meanwhile the N_2O emission, H_2S emission, and H_2SO_4 consumption in H_2SO_4 -VM were 28%, 93% and 39% lower than those in H_2SO_4 -AC, respectively, indicating the superiority of the H_2SO_4 -VM. Then, the key modifying parameters for achieving higher gas ME of H_2SO_4 -VM were explored. It's found that no wash was allowed before drying of the acid immersing vermiculite, thus helping maintain the acidity of the H_2SO_4 -VM, which facilitated the long lasting of covering effect and also the surface slurry acidification effect. Meanwhile, the H_2SO_4 -VMs modified with different acid concentration (3M, 5M, 7M H^+) were tested for their gas ME. It's found that 3M, 5M, 7M H_2SO_4 -VM could achieve the NH_3 ME by 57%, 64% and 49%, and CH_4 ME by 22%, 23% and 20%, respectively, during a 84 days storage period. The highest NH_3 and CH_4 ME may be caused by the better surface acidification effect together with the best covering effect of 5M treatment. It's suggested that H_2SO_4 -VM can be a possible alternative for reducing NH_3 and CH_4 emissions from animal slurry storage, and field test was urgently need for evaluating the in-situ effect.

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The Macroalgae *Asparagopsis taxiformis* Decreases Dry Matter Intake and Milk Production in Dairy Cows

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Asparagopsis taxiformis (AT) has been identified as a potent methane-mitigating feed additive in ruminants but there are concerns related the stability of the brominated compounds responsible for the anti-methanogenic effect of AT over prolonged storage. Therefore, the purpose of this experiment was to determine the effect of AT stored for over 20 mo on enteric methane emission and lactational performance of dairy cows. Eighteen Holstein cows were placed in a replicated 3 x 3 Latin square design with 3, 28-d periods. Treatments were control (basal diet), 0.50% AT, and 0.75% AT [feed dry matter (DM) basis]. Enteric methane emission was measured using the GreenFeed system. Data were analyzed using the MIXED procedure of SAS with treatment and period in the model, and square and cow within square as random effects. Daily methane emission decreased linearly ($P < 0.001$) with AT dose: 404, 338, and 283 g/d (SEM = 13.7), for control, 0.50, and 0.75% AT, respectively. AT inclusion, however, decreased linearly ($P < 0.001$; from 24.7 to 19.2 kg/d; SEM = 1.03) DM intake, which resulted in only a small decrease in methane yield ($P = 0.07$; 17.1 vs. 15.6 g/kg DM, control and AT treatments, respectively). Energy-corrected milk yield (ECM; 37.0, 36.1, and 31.8 kg/d, respectively; SEM = 1.05) were linearly decreased ($P \leq 0.01$) by AT. Methane emission intensity was also linearly decreased ($P < 0.001$) by AT (11.0, 9.4, and 9.0 g/kg ECM, respectively). Additionally, there was a 3% decrease ($P < 0.002$) in cow body weight for 0.75% AT, compared with the control. In conclusion, *Asparagopsis taxiformis* stored over 20 mo and included at up to 0.75% (feed DM basis) in the diet of dairy cows decreased methane yield by only 9% and decreased DM intake by 22% and milk production by 8%.

Methane, *Asparagopsis taxiformis*

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Screening of Tropical Macroalgae for Enteric Methane Mitigation Effect *In Vitro*

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Methanogenesis and mitigation of enteric methane emissions from ruminants have been the focus of ongoing research to address livestock contribution to climate change. This experiment investigated the effects of 14 macroalgae specimen (8 distinct species) on methanogenesis and gas production *in vitro*. Macroalgae were collected over the span of 6 mo from the Caribbean Species were analyzed for their effect on pH, total gas production and composition. Incubations were carried out for 24 h and replicated. Rumen inoculum was collected from 2 rumen-cannulated lactating Holstein cows fed a standard 52% forage (corn silage and alfalfa haylage) and 48% concentrate feeds diet. Dried and ground total mixed ration fed to the donor cows was used as substrate in the incubations at 0.01% (w/v) and macroalgae were included in the feed mix at 2.0% (dry matter basis). Control (total mixed ration alone) was also included in each incubation in triplicate. Gas production was continuously monitored with an automated gas production system and headspace samples were collected at 24 h and analyzed for methane concentration. Inoculum pH was measured at the beginning and termination of the incubation. Data were analyzed by incubation set with the MIXED procedure of SAS with treatment, incubation, and treatment \times incubation in the model. Compared with Control, final pH was decreased ($P < 0.05$) by *Hypnea* sp. and *Padina* sp. from 6.46 to 6.39 and 6.41, respectively. Total gas production (mL/g of dry matter) was increased ($P \leq 0.05$) by *Gelidelia aserosa*, *Padina* sp. and *Sargassum acinarium* 15 to 21% compared with Control. Methane yield (mL/g of dry matter) was increased ($P \leq 0.05$) by *Bryothamnion triquetrum*, *Dichotomaria marginate*, *Padina* sp., *Cladophora* sp., *Chaetomorpha* sp. and *Sargassum acinarium* 13 to 27% compared with Control. No macroalgae in this set of experiments decreased methane yield.

Enteric methane, Macroalgae, Rumen fermentation

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Factors Affecting *In Vitro* Methane Yield in 78 Grass Silages from Norway

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The effect of grass-clover silage on enteric methane (CH₄) production is largely unknown, although it constitutes a large part of ruminant diets in several parts of the world. We aimed to identify the quality attributes of grass-clover silage associated with variation in *in vitro* CH₄ yield (MY). We hypothesized that MY would be affected by silage nutrient concentrations and silage fermentation products. Round bales (n = 78) of grass and grass-clover silage from 37 farms in Norway were sampled, incubated, and screened for MY. Production of CH₄ was expressed on the basis of incubated organic matter (MY-OM) and *in vivo* digestible OM (MY-dOM). In situ technique was used to quantify the concentration of indigestible neutral detergent fiber (iNDF, aNDFom), and correlation and principal component analysis were used to examine the data. Among all investigated silage composition variables, neutral detergent fiber and water-soluble carbohydrate (WSC) concentrations correlated strongest with MY-OM (r=-0.63 and r= 0.57, respectively, P<0.05). Concentration of iNDF was negatively associated with MY-OM (r= -0.48, P<0.05). *In vivo* organic matter digestibility (OMD) and concentration of ammonia-N (NH₃-N) in silages were also correlated to MY-OM (r= 0.44 and r= -0.32, respectively, P<0.05). Molar proportion of butyrate was the most prominent rumen short chain fatty acid (SCFA) positively associated with MY-OM and MY-dOM (r=0.23 and r= 0.36, respectively, P<0.05), whereas the most prominent SCFA negatively associated with MY-OM and MY-dOM was the molar proportion of propionate (r= -0.23 and r= -0.26, respectively, P<0.05). In summary, concentration of WSC in grass silages increased MY-OM and MY-dOM, while concentration of NDF and iNDF decreased MY-OM and MY-dOM. In conclusion, low MY silages are related to lower OMD and thereby expected to lower animal production, illustrating the contradiction between selecting silages that are low in MY and those that support high levels of animal production.

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Effects of Gliricidia Supplement on Methane Production of Cattle Fed Rice Straw or Napier Grass

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Shrub- and tree-legumes have important roles in providing rumen degradable nitrogen in tropical ruminant production systems, and their tannin content can also reduce ruminal methane production. A study was conducted to ascertain the effects on total feed intake and methane production, of including Gliricidia in cattle diets composed predominantly of rice straw, and then when consuming predominantly Napier grass. Eight Ongole cows were group housed and offered rice straw alone then of rice straw and Gliricidia, followed by another set of consecutive studies when Napier grass was offered followed by Napier grass plus Gliricidia. The basal roughage and the Gliricidia were each offered in C-Lock SmartFeed feed intake recording bins, with cattle being adapted to diet for 14d then measured for a further 14-day experimental period. Methane production was measured by Greenfeed emission monitors with emissions reported over the last 14-days of the entire period.

The basal feed affected the daily methane production (g CH₄/d) and methane yield (as % of GE), with these being greater on rice straw than on Napier grass. Including Gliricidia in the ration increased the energy intake of animals fed rice straw, but not when animals were fed the Napier grass. Similarly, Gliricidia reduced methane production and methane yield of rice straw fed cattle, but not of the nutritionally superior Napier grass. It is concluded that supplementary Gliricidia is most effective in stimulating feed intake and reducing methane yield, when roughage quality is lowest.

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Enteric Methane Emission Intensity of Lactating Cows Fed Soy-Hull

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Soy hull is an important by-product of the Argentinean crop industry. Including it in the diets of ruminants would increase carbon circularity of mixed crops-livestock systems, yet it may increase enteric methane production. The aim of this study was to evaluate if soy hulls could be used as a partial replacement of corn grain in the diet of lactating cows without negatively affecting their feed use efficiency or methane emissions. We hypothesized that the use of a highly degradable fiber (soy hulls) in the diets of dairy cows would have no effect on milk production nor methane emission intensity. Six Holstein lactating cows were fed a 50:50 (dry matter basis) corn-silage:concentrate TMR (18% CP, 12 MJ/kg ME) during two 25-day periods. Three replicated 2x2 Latin squares were used to evaluate 2 concentrates, containing either 45% soy hulls or 45% corn grain, in 2 periods at 60 ± 7 and 84 ± 7 days in milk. Cows were housed in individual pens, and fed and milked twice daily. Feeds were sampled and intake recorded from day 18 to 25 of each period. From day 22 to 25 of each period cows were housed individually in respiration chambers to determine enteric methane production. Milk production from each milking was measured throughout the experiment. Cows given the soy hulls tended to consume 4% more feed (28.1 vs. 27.0 kg/d, $P = 0.068$) and emitted 6% more methane (484 vs. 456 g/d, $P = 0.072$) than cows given corn grain. There was no effect of treatment on milk production (39.6 vs. 39.8 kg/d, $P = 0.682$) or methane emission intensity (12.3 vs. 11.9, $P = 0.278$). This suggests that soy hulls can be used to replace corn grain in the diet of dairy cows with no detrimental effects on milk production or methane intensity.

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The Effect of Feeding a Lactobacillus Mixture to Cows on Methane Yield and Milk Production

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Numerous dietary strategies based on additives or supplements have been proposed to mitigate ruminal methanogenesis but only a few have shown a persistent effect *in vivo* without negative impacts to animal health or productivity. Use of direct-fed microbials is one possible option that could be both sustainable and acceptable to consumers and producers but there is little information on their effect on enteric methane.

Forty lactating, multiparous, Holstein-Friesian cows were assigned one of two dietary treatments. The Control diet consisted of 7.2 kg DM of a grain mix (wheat, barley, canola meal and minerals) and *ad libitum* vetch hay. The Mylo diet consisted of the Control diet plus 10 mL of Mylo® (Terragen Biotech, Coolumb Beach, Queensland, Australia). The grain mix was fed in the dairy during milking and the hay in an automatic feeding system where 14 feed bins were allocated to each treatment group. Methane was measured using the modified sulfur hexafluoride technique. Treatment groups were kept separate at all times. Cows in the Control group ate the same as cows in the Mylo group (25.4 vs 24.8 kg DM/day) and produced the same quantity of milk (29.9 vs 30.3 kg/day). Cows offered the Control diet produced the same methane as cows offered the Mylo diet (799 vs 765 g/day), with the same methane yield (31.6 vs 31.1 g/kg DM) and methane intensity (27.1 vs 25.2 g/kg milk). Adding 10 mL of Mylo to the diet of dairy cows had no effect in this experiment. However, the dose per kg of feed consumed was less than intended due to dry matter intake of cows being greater than expected.

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Dry Matter Intake and Methane Production from Sheep Offered Diets Differing by Sward Type

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Due to its high digestibility and grazing tolerance, perennial ryegrass (PRG) is the most widely used forage in ruminant production systems across temperate regions. However, the inclusion of alternative forages is gaining popularity in order to enhance animal performance as well as reduce reliance on chemical inputs and anthelmintics. The objective of this study was to assess the effect of sward type on dry matter intake (DMI) and methane (CH₄) output in sheep. The experiment was a 5x5 Latin square design. Twenty wether sheep were housed in metabolism crates across five periods. Treatments offered were PRG only or PRG plus: white clover, red clover, chicory or plantain at a ratio of 75%:25%, respectively. Individual DMI was measured daily with CH₄ measurements collected using Portable Accumulation Chambers (PAC) at the end of each measurement period. Average live weight across all treatments was 64.9kg. Sward type significantly affected DMI ranging from 1.55 ± 0.038 (PRG) to 1.76 ± 0.038 (PRG plus Chicory) kg DM/animal/day (P<0.05). Methane production was highest for animals consuming PRG plus chicory 22.29 ± 0.801 g CH₄/day and lowest for animals consuming PRG plus white clover 18.81 ± 0.786 g CH₄/day (P<0.05). Animals offered PRG only (14.31 ± 0.596 g CH₄/ kg DMI) had a CH₄ yield higher than all other sward types (P<0.05). Methane per unit live weight was lowest for animals offered PRG plus white clover 0.83 ± 0.036 g CH₄/ kg^{0.75} (P<0.05). Results suggest that animals offered PRG plus a companion forage had increased DMI and ranked lower for methane yield (g CH₄/ kg DMI) in comparison to PRG only. To conclude, animals offered PRG plus white clover ranked lowest for CH₄ output whether expressed as production (g CH₄/day), yield (g CH₄/kg DMI) or unit live weight (g CH₄/ kg^{0.75}).

***In Vitro* Rumen Microbial Degradation of Bromoform and the Impact on Rumen Fermentation**

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Among the feed additives with most potential to reduce enteric CH₄ emissions, the macroalgae *Asparagopsis* have shown the highest effectiveness. The anti-methanogenic action of *Asparagopsis* relies on the presence of halogenated CH₄ analogues such as bromoform as the key active compound. However, the interaction of bromoform with rumen microbiota is still unknown. This study aimed to investigate the degradation pattern of bromoform from *Asparagopsis taxiformis* (AT) in the rumen and to elucidate whether this degradation process is diet-dependent. An *in vitro* batch culture system was used considering two treatments, Control (CTL) and AT (2 g/100 g DM diet) and using two diets, high-concentrate (HC) and high-forage diet (HF). Incubations lasted for 72 h and samples of headspace and fermentation liquid were taken at 0, 0.5, 1, 3, 6, 8, 12, 16, 24, 48 and 72 h to assess the pattern of degradation of bromoform into dibromomethane and the VFA, CH₄ and H₂ fermentation profile. The addition of AT reduced CH₄ production by 90% in both diets and significantly reduced the acetate-propionate ratio. The concentrations of bromoform throughout the incubation process showed a rapid degradation with 70% degraded in 30 min and nearly 90 % after 3 hours of incubation. All of the bromoform was degraded within 12 hours of incubation. Along with the bromoform degradation, dibromomethane acted as degradation product and its concentration quickly increased during the incubation up to 6 hours. Then a gradual decline in its concentration was observed towards the end of the incubation. Neither bromoform degradation nor dibromomethane synthesis were significantly affected by the type of diet used as substrate (HC or HF), suggesting the fermentation rate is not a driving factor involved in bromoform degradation. Overall, the results show that bromoform is quickly degraded in the rumen regardless of the diet fed to the animal.

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The Between Animal Variance in CH₄, CO₂ and CH₄/CO₂ Concentration Ratio Measured in Farm Conditions

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The between-animal percentage coefficient of variation (%bCV) in enteric methane flux (CH₄) and Carbon Dioxide flux (CO₂) characterizes the differences in high and low emitting animals within a herd. It is an important statistic because %bCV determines the numbers of animals needed in experimental designs, the uncertainty of inventory estimates, and can be used to assess the effectiveness of breeding programs aiming to reduce CH₄. Further, a benchmark %bCV can be used when reviewing the viability of indirect proxy methods, such as the sniffer methods for estimating CH₄ which produce highly variable results. It was hypothesized that in production dairy farms with varied management strategies, the %bCV in CH₄, CO₂, and CH₄/CO₂ ratios are not highly variable. Five GreenFeeds (C-Lock Inc) were used to measure CH₄ and CO₂ from 1994 lactating dairy cattle on 18 dairy farms in varied locations and management strategies in the Netherlands. On each farm, the animals were first habituated to the GreenFeed, then CH₄ and CO₂ was measured for a period of 14 days. The CH₄ and CO₂ visit data was averaged by animal to determine the CH₄, CO₂, and CH₄/CO₂ concentration ratio and then the %bCV was determined. Across all farms, the averaged %bCV for CH₄, CO₂, and CH₄/CO₂ ratio was 17% (9%-27%), 12% (8%-20%), and 11% (5%-18%), respectively. When removing early and late lactation animals from the analysis and excluding grazing animals, the averaged %bCV in CH₄, CO₂ and CH₄/CO₂ ratio was 12% (6%-19%), 10% (5%-15%), and 7% (5%-15%), respectively. The %bCV in CH₄/CO₂ ratio was significantly lower than both CH₄ and CO₂. This study showed that %bCV is relatively consistent and predictable between animals in different farms and locations for mid lactation animals that have similar expected feed intake rates.

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