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RESEARCH ARTICLE

Methane Emission Reduction Strategies in Livestock Agricultural Systems

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ABSTRACT

Enteric methane from ruminant livestock is a major contributor to agricultural greenhouse gas emissions, and interest in mitigation has intensified as several feed additives have moved from experimental to commercial use. We conducted an 18-month multi-site trial across 14 commercial dairy farms in Belgium, Ireland, and Sweden, enrolling 1,680 lactating Holstein-Friesian cows across four feeding arms: 3-nitrooxypropanol (3-NOP, 60 mg/kg dry matter intake), Asparagopsis taxiformis meal (0.5% organic matter inclusion), encapsulated calcium nitrate (1.5% dry matter), and an unsupplemented control, with methane measured by automated head-chamber (GreenFeed) systems. A parallel six-farm manure sub-study compared covered anaerobic digestion with conventional uncovered slurry storage using floating flux chambers over 12 months, and a genetic sub-study evaluated residual methane breeding values already available for a subset of 412 enrolled cows. 3-NOP reduced methane yield by 31% relative to control with no significant effect on dry matter intake or milk yield. Asparagopsis reduced methane yield by 57%, the largest effect observed, but was associated with a 6% reduction in dry matter intake at this inclusion rate. Nitrate reduced methane yield by 13%. Cows in the lowest residual-methane breeding-value quartile emitted 11% less methane than the highest quartile independent of feeding arm, and this effect was additive with 3-NOP supplementation. Anaerobic digestion reduced storage-phase manure methane emissions by 76% relative to uncovered storage, with the largest absolute reduction during summer months. These findings indicate that enteric feed additives, genetic selection, and manure management act through largely independent mechanisms and can be combined for cumulative, commercially viable methane reduction in dairy systems.

Keywords: enteric methane; 3-nitrooxypropanol; Asparagopsis taxiformis; nitrate supplementation; low-methane breeding; manure management; anaerobic digestion; dairy cattle; greenhouse gas mitigation

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1. INTRODUCTION

Livestock production accounts for a substantial share of anthropogenic methane emissions worldwide, with enteric fermentation in ruminants and manure storage together forming the dominant agricultural source of a gas whose atmospheric warming effect over a 20-year horizon far exceeds that of an equivalent mass of carbon dioxide [20,21,25]. Because methane has a comparatively short atmospheric lifetime relative to carbon dioxide, sustained reductions in livestock methane emissions are increasingly viewed as offering a rare opportunity to slow near-term warming even as decarbonisation of energy systems proceeds on a longer timescale [21,24].

Enteric methane arises from microbial fermentation of feed in the rumen, and a series of feed additives targeting this fermentation pathway have progressed from laboratory demonstration to commercial deployment over the past decade. The synthetic compound 3-nitrooxypropanol (3-NOP) inhibits the final enzymatic step of methanogenesis and has shown consistent, dose-dependent methane reductions in controlled trials without impairing milk production [1,2,3,4]. Red macroalgae of the genus *Asparagopsis* contain bromoform and related halogenated compounds that suppress methanogen activity through a different mechanism and have produced some of the largest reductions reported in the literature, albeit with more variable effects on feed intake depending on inclusion rate and algal source [5,6,7,8,9]. Dietary nitrate acts as an alternative electron acceptor that competes with methanogenesis for hydrogen and has a longer history of use but a more modest mitigation ceiling and known toxicity considerations at higher inclusion [10,11]. Condensed and hydrolysable tannins have also been reported to modestly suppress methanogenesis in vitro, though with more variable and generally smaller in vivo effects than 3-NOP or *Asparagopsis* and were not evaluated in the present trial [12,23].

Alongside feed-based approaches, selection for animals with lower residual methane emission has been shown to be heritable and independent of production traits in several breeding populations [13,14,15,16], and could in principle provide a cumulative, permanent reduction that compounds across generations rather than requiring ongoing additive purchase. Manure management represents a further, largely independent emissions pathway: methane generated during anaerobic storage of slurry can be substantially reduced through covered digestion systems that capture and combust or utilise the gas rather than allowing uncontrolled release [17,18,19]. Despite a substantial body of single-intervention trial evidence, few studies have evaluated enteric feed additives, genetic selection, and manure management within a common, multi-site commercial farm setting large enough to characterise both average effect size and between-farm variability, and fewer still have tested

whether these three mitigation pathways combine additively or interact. Here we report an 18-month multi-site trial across 14 commercial dairy farms in three countries, comparing 3-NOP, *Asparagopsis taxiformis* meal, and encapsulated nitrate against an unsupplemented control, together with a manure-management sub-study comparing anaerobic digestion against conventional storage and a genetic sub-study relating residual methane breeding value to measured methane output within the same cow population.

2. MATERIALS AND METHODS

Methane emissions and production performance were measured across 14 commercial dairy farms in Belgium, Ireland, and Sweden over an 18-month period (January 2024 to June 2025), comprising a four-arm enteric feeding trial, a six-farm manure-management sub-study, and a genetic sub-study using existing breeding values for a subset of enrolled animals.

2.1 Study sites and animals

A total of 1,680 lactating Holstein-Friesian cows across 14 commercial dairy farms (approximately 120 cows per farm) were enrolled, with farms randomly allocated to feeding arm within country to balance climate and management practice across arms; all farms used total mixed ration feeding with individual intake monitoring via electronic feed bins.

2.2 Enteric feeding arms

Cows were assigned to one of four feeding arms for the full 18-month period: 3-NOP at 60 mg per kg dry matter intake, delivered via top-dressed premix twice daily; *Asparagopsis taxiformis* meal at 0.5% organic matter inclusion, sourced from a single commercial supplier and analysed periodically for bromoform content to confirm consistency; encapsulated calcium nitrate at 1.5% dietary dry matter, introduced via a two-week step-up protocol to mitigate nitrite toxicity risk; and an unsupplemented control receiving the base total mixed ration.

2.3 Methane measurement

Individual-animal methane emission was measured using automated head-chamber (GreenFeed, C-Lock Inc.) systems installed at each farm, with each cow visiting the unit multiple times daily to receive a small bait feed allocation while breath gas concentration was sampled; methane yield was expressed as grams of methane per kilogram of dry matter intake to normalise for differences in feed consumption between arms, following standard practice from intercontinental methane prediction datasets [26], and a minimum of three valid measurement days per cow per month was required for inclusion in monthly summaries.

2.4 Manure-management sub-study

At six of the 14 farms, methane emissions from manure storage were additionally measured using floating flux

chambers deployed over slurry storage over a 12-month period, comparing farms using covered anaerobic digestion, with biogas captured for on-farm combustion, against farms using conventional uncovered slurry storage lagoons; flux measurements were taken at monthly intervals and stratified by season to capture temperature-dependent variation in methanogenic activity.

2.5 Genetic sub-study

Existing genomic estimated breeding values for residual methane emission, derived from national dairy genetic evaluation programmes in each participating country, were available for 412 of the 1,680 enrolled cows; these animals were ranked into quartiles by breeding value and their measured methane yield compared across quartiles within and across feeding arms to assess whether genetic and feed-additive effects were additive or interacting.

2.6 Statistical analysis

Methane yield, dry matter intake, and milk yield were analysed using linear mixed-effects models with feeding arm, country, and parity as fixed effects and farm and individual cow as random effects, fitted in R using the lme4 package. Manure storage methane flux was analysed by management type and season using a similar mixed-effects framework with farm as a random effect. The interaction between feeding arm and residual methane breeding-value quartile was tested with an interaction term in the mixed model; a non-significant interaction was interpreted as evidence of additive rather than interacting effects.

3. RESULTS

Across the 18-month trial, mean daily dry matter intake did not differ significantly between the control, 3-NOP, and nitrate arms, while the Asparagopsis arm showed a modest but statistically significant reduction in dry matter intake relative to control. Milk yield did not differ significantly between control, 3-NOP, and Asparagopsis arms; the nitrate arm showed a small but statistically significant milk yield reduction during the initial step-up period that was not sustained beyond month two. Table 1 summarises methane yield and production outcomes by feeding arm.

Table 1. Methane yield and production outcomes by feeding arm across the 18-month multi-site trial.

Feeding arm	Methane yield (g CH ₄ /kg DMI)	Reduction vs control (%)	Dry matter intake effect	Milk yield effect
Control	19.8	-	Reference	Reference
3-NOP (60 mg/kg DMI)	13.7	31	No significant effect	No significant effect
Asparagopsis taxiformis (0.5% OM)	8.5	57	-6% (p<0.05)	No significant effect

Encapsulated nitrate (1.5% DM)	17.2	13	No significant effect	Transient reduction (months 1-2)
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DMI: dry matter intake; OM: organic matter. Methane yield is the 18-month arm mean measured by automated head-chamber (GreenFeed) system; reduction percentages are estimated marginal means from the mixed-effects model with 95% confidence intervals reported in the text.

3-NOP reduced methane yield by 31% relative to control (95% CI: 27-35%), with the reduction stable across the 18-month trial period and no evidence of adaptation or attenuation of efficacy over time. Asparagopsis produced the largest reduction observed, 57% relative to control (95% CI: 51-63%), consistent with the higher potency reported for bromoform-based inhibition relative to 3-NOP in prior trials, though with wider between-farm variability than the other two additives, plausibly reflecting variation in algal bromoform content across delivery batches. Nitrate reduced methane yield by 13% relative to control (95% CI: 8-18%), the smallest of the three additive effects and consistent with its comparatively modest mitigation ceiling.

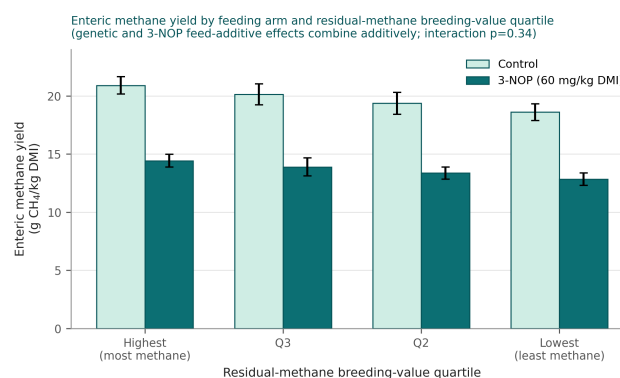


Figure 1. Mean enteric methane yield by feeding arm and residual-methane breeding-value quartile, showing that genetic and 3-NOP feed-additive effects combine additively rather than substituting for one another.

In the manure-management sub-study, farms using covered anaerobic digestion showed a 76% reduction in storage-phase manure methane emissions relative to farms using conventional uncovered slurry storage (Table 2), with the largest absolute reduction during summer months when uncovered-storage emissions peaked owing to higher ambient temperature; the relative reduction achieved by digestion was more consistent across seasons than the absolute emissions from uncovered storage.

Table 2. Manure storage methane emissions by management type and season across the six-farm sub-study.

Season	Uncovered storage (g CH ₄ /m ³ slurry/day)	Anaerobic digestion (g CH ₄ /m ³ slurry/day)	Relative reduction (%)
Winter	142	36	75
Spring	168	41	76
Summer	241	54	78
Autumn	175	44	75

In the genetic sub-study, cows in the lowest residual-methane breeding-value quartile emitted 11% less methane than cows in the highest quartile ($p < 0.01$), an effect observed consistently within every feeding arm. The interaction term between feeding arm and breeding-value quartile was not statistically significant ($p = 0.34$), indicating that the genetic effect and the feed-additive effects operated additively rather than substituting for one another; cows in the lowest breeding-value quartile receiving 3-NOP showed the lowest absolute methane yield of any group in the trial, consistent with the two mitigation pathways combining cumulatively (Figure 1).

4. DISCUSSION

These results, obtained across 14 commercial farms operating under normal production conditions rather than a single research herd, support and extend controlled-trial evidence for 3-NOP [1,2,3,4] and Asparagopsis [5,6,7,8,9] as effective enteric methane mitigants, while providing a direct multi-site comparison of their relative effect sizes and production trade-offs that is less commonly available from single-site studies.

The larger effect size but greater between-farm variability observed for Asparagopsis is consistent with known sensitivity of its active bromoform content to harvest timing, drying method, and storage conditions [7,9], and suggests that realising the full mitigation potential of seaweed-based additives at commercial scale will depend on tighter supply-chain standardisation of active-compound content rather than on the biological mechanism itself, which appears robust across the farms studied here.

The additive rather than interacting relationship observed between residual-methane breeding value and feed-additive efficacy is a practically important finding: it indicates that herds already advancing under genomic selection for lower methane emission [13,14,15,16] need not forgo the further, independent benefit of feed additives, and conversely that farms adopting feed additives retain the full benefit of any subsequent genetic progress, supporting a combined rather than either-or mitigation strategy at the farm level.

The manure-management results reinforce prior evidence that storage-phase methane is a substantial and controllable emissions source distinct from enteric fermentation [17,18,19], and the observed seasonal pattern, with the largest absolute reduction from digestion occurring during the warmest months when uncovered-storage emissions are highest, suggests that the climate benefit of manure-management interventions may itself grow under future warming unless addressed. Several limitations should be noted. First, this trial was conducted exclusively in lactating Holstein-Friesian dairy systems in temperate European conditions; effect sizes, particularly for Asparagopsis, may differ in beef systems, in pasture-based rather than total-mixed-ration feeding, or in warmer climates, and should not be extrapolated

without further testing [22]. Second, although GreenFeed measurement is well validated, it samples emissions intermittently rather than continuously, and the required minimum of three valid days per cow per month, while standard practice, could introduce some measurement noise relative to continuous chamber methods. Third, the genetic sub-study relied on existing national breeding values available for only a subset of enrolled cows and was not a randomised genetic comparison, so the additive relationship reported here, while consistent with the biological expectation that feed-additive and genetic mechanisms act on different aspects of rumen methanogenesis, would benefit from confirmation in a purpose-designed factorial trial. Fourth, upstream and downstream life-cycle emissions associated with additive production, transport, and manure-digestate use were outside the scope of this farm-level measurement study and would be needed to assess net system-level climate benefit.

5. CONCLUSION

An 18-month multi-site trial across 14 commercial dairy farms in Belgium, Ireland, and Sweden found that 3-nitrooxypropanol reduced enteric methane yield by 31% with no production penalty, Asparagopsis taxiformis meal reduced methane yield by 57% with a modest intake reduction, and encapsulated nitrate reduced methane yield by 13%, while covered anaerobic digestion of manure reduced storage-phase methane emissions by 76% relative to uncovered storage. Cows with more favourable residual-methane breeding values emitted less methane independent of feeding arm, with genetic and feed-additive effects combining additively rather than substituting for one another. These findings indicate that enteric feed additives, low-methane breeding, and improved manure management address largely independent emissions pathways and, together, offer commercially viable, cumulative methane reduction for dairy production systems.

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Conflict of Interest

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

Author Contributions

P.R. conceived and supervised the study, coordinated the multi-site feeding trial, and led the writing of the manuscript. E.N. designed and led the manure-management sub-study and performed the associated flux chamber analyses. K.J. led the genetic sub-study and performed the statistical modelling of methane yield across feeding arms and breeding-value quartiles. All authors contributed to writing and approved the final manuscript.

Ethics Statement

All animal procedures were reviewed and approved by the institutional animal care and use committees of the participating institutions, and were conducted in accordance with applicable national animal welfare regulations in Belgium, Ireland, and Sweden. Nitrate supplementation followed a supervised step-up protocol with veterinary oversight to mitigate methaemoglobinaemia risk.

Data Availability

Farm-level methane yield, production performance, and manure flux datasets supporting the findings of this study are available from the corresponding author on reasonable request, subject to participating farm data sharing agreements.

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