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Communication

Evaluating the Efficacy of a Novel Multi-Component Feed Additive for Methane Mitigation and Performance Enhancement in Sheep

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Simple Summary: Livestock farming, particularly with sheep and cattle, releases methane gas, which accelerates climate change by trapping heat more effectively than carbon dioxide. This study examined a new feed mixture, a novel multi-component feed additive, composed of natural substances, such as fatty acids, yeast, plant extracts, and vitamins, to determine its ability to reduce methane emissions from sheep while also improving their growth and health. Twenty sheep were divided evenly into two groups for approximately 10 weeks; one group received the standard feed and the novel feed additive, and the other consumed standard feed only. The sheep fed the novel feed additive produced less methane per unit of feed consumed and increased their feeding frequency, suggesting greater acceptance of the mixture. However, these animals did not exhibit improved weight gain or enhanced health compared to the standard-feed-only group. This suggests that the novel feed additive lowers methane output per unit of feed, offering potential environmental benefits, yet it does not enhance sheep productivity or well-being as anticipated.

Abstract: Enteric methane emissions from ruminants substantially contribute to global greenhouse gas emissions, necessitating effective mitigation strategies that also support animal productivity. This study assessed the efficacy of a multi-component feed additive that combines medium-chain fatty acids (MCFAs), live yeast, plant-based agents, and Vitamin B, in reducing methane emissions, improving feed efficiency, and enhancing growth and immune function in sheep. Twenty crossbred castrated male sheep (52 ± 3.7 kg) were divided into control and treatment groups (n = 10 each), with the treatment group receiving grass pellets supplemented with the multi-component feed additive (20 g/day) for 71 days, including a 30-day acclimatisation period. Feed intake, methane emissions, growth performance, and blood parameters were monitored using BioControl pens, GreenFeed units, and haematological analyses. The treatment group exhibited a 24% increase in daily feed intake ($p < 0.001$) and a 22.2% reduction in methane yield per kg of dry matter ingested ($p < 0.001$), which could be attributed to MCFAs' anti-methanogenic properties and yeast's rumen modulation. However, no significant improvements were observed in daily live weight gain, feed conversion efficiency, or immune parameters, suggesting limited energy utilisation for growth. These findings highlight this novel multi-component feed additive as a promising strategy for methane mitigation in forage-based systems. Further

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dosage optimisation and dietary integration could enhance its application across ruminant species, contributing to sustainable livestock production.

Keywords: enteric methane; NuAdvent+; ruminants; sheep; sustainability; methane mitigation

1. Introduction

Livestock farming contributes to global warming, with ruminants being a major source of methane, a greenhouse gas with a global warming potential (GWP100) 28-times higher than CO_2 [1]. Agriculture accounts for 40–46% of global methane emissions, two-thirds of which arise from enteric fermentation in livestock [2]. As food production rises, enteric emissions are projected to increase by 40% by 2050, making mitigation strategies critical for reducing the environmental footprint of livestock farming. Beyond environmental concerns, methane emissions represent an energy loss of 2–12% of the ruminants' gross energy intake, impacting growth and productivity [3,4].

Dietary interventions have emerged as a promising approach to reducing enteric methane emissions. Feed additives, like medium-chain fatty acids (MCFAs), seaweed, 3-nitrooxypropanol (3-NOP), and probiotics, have shown the potential to alter rumen characteristics to curb methane emissions [5]. MCFAs, particularly lauric and myristic acids, inhibit methanogens and protozoa, reducing methane emissions by up to 50% in different studies [6,7]. However, these strategies may reduce fibre digestibility, particularly in forage-based diets [7]. Yeast supplementation is another viable strategy that reduces methanogen populations while improving nutrient digestibility and energy efficiency [8]. Despite its benefits, the efficacy of yeast is inconsistent across studies, highlighting the need for further optimisation. Hence, this study investigated the efficacy of a novel feed additive (NuAdvent+, Cloud Agri Ltd., Manchester, UK) combining MCFAs, live yeast, a potent blend of plant-based agents (derived from fruits and herbs), and B Vitamins (composed of Vitamin B1, Vitamin B2 (riboflavin), Vitamin B6, and Vitamin B12) on its potential to reduce enteric methane emissions, enhance feed conversion efficiency, and improve immune function and growth performance in sheep, as a model for ruminants.

2. Materials and Methods

2.1. Animals, Feeding and Biophysical Measurements

A total of 24 crossbred (Suffolk × North Country Mule) castrated male sheep with an average initial liveweight (LW) of 52 ± 3.7 kg were allocated into experimental groups pre-trial. Animals were balanced by weight and age and then randomly assigned to treatment or control groups. Animals were fed a ration of grass pellets containing 16% protein and consisting of permanent pasture ryegrass for 71 d (including a 30-d acclimatisation period). Feed was supplied to animals on an ad libitum basis, with available feed replaced daily. Half of the animals had their grass pellets supplemented with NuAdvent+, with the product applied in powder form directly on top of the offered grass pellets. The NuAdvent+ contained, as percentage of dry matter (DM), 3.2% crude protein (CP), 1.02% water-soluble carbohydrates (WSC), and 83.1% ash, and it was supplied at a dosage of 20 g/day. This dose was selected based on manufacturer (Cloudagri®, Manchester, UK) recommendations, as no prior scientific studies were available. Cloudagri® ensures product safety through regular testing of raw materials for undesirable substances, following an annually reviewed plan aligned with risk assessments from the Belgian sector federation and compliant with Belgian and European legislation, as overseen by the Belgian Federal

Agency for the Safety of the Food Chain. The additive was evenly sprinkled over each lamb's daily ration and manually mixed in individual feed bins to ensure uniform distribution, with this process repeated daily to maintain homogeneity.

During the experimental period, animal LW and body condition scores (BCS, scale from 1 to 5; [9]) were measured weekly using a manual weigh-crate mounted on Tru-Test® load bars for LW and BCS measured by a trained assessor. Data were processed through AgriWebb agricultural management software (AgriWebb, Surry Hills, New South Wales, Australia). At the end of the experimental period, blood samples were collected by venepuncture into the jugular vein and extracted into anti-coagulant (EDTA) vacutainers to a maximum of 10 mL of blood drawn per animal. Samples were taken in the morning and then immediately analysed using a VetScan HM5 Haematology Analyser (Zoetis, Leatherhead, UK), with complete blood counts conducted for 20 variables.

2.2. Feed Intake Measurement via BioControl System

Throughout both phases of the trial (phase one: acclimatisation and baseline; phase two: supplementation), animals were housed within BioControl CRFI (Controlling and Recording Feed Intake) (BioControl, CRFI, Rakkestad, Norway) pens measuring 3 m^2 per individual pen. For phase one of the trial, animals were penned in pairs (for animal welfare purposes to avoid isolation) for animals to be trained to access their individual, exclusively allocated BioControl feeder (controlled by animals' specific individual electronic identification tag-activated feed gates). During phase one, four BioControl panels malfunctioned, leaving only 20 automatic feeders for the experimental phase. As a result, we removed 2 lambs from each group and worked with 20 lambs. For phase two of the trial (six weeks), animals were amalgamated into four large groups (two groups of six lambs and two groups of four lambs) for each mob to have access to one of the four GreenFeed Emission Monitoring (GEM) units (C-Lock, Rapid City, SD, USA). All four groups comprised half of the animals from the treatment group and half of the animals from the control group, each of which retained access to a designated individual feeder. Feed intake was measured automatically throughout the trial via the automatic CRFI system. Eating behaviour (i.e., number of visits, time of each visit, total time feeding, amount eaten per visit, eating rate) was also recorded automatically. Feed samples were taken daily and subsequently oven-dried to calculate DM percentage.

2.3. Enteric Methane Emission Measurement via Greenfeed System

During phase two of the trial, daily methane (CH_4) production (g/day) of all animals was monitored by GreenFeed Emission Monitoring (GEM) units present within each of the four large pens. For methane to be measured in the GEM, animals placed their heads within the shrouded opening of the GEM unit, which detects the individual electronic identification tag of each animal and triggers the unit to release a small quantity of concentrated feed pellets [10]. The pellets contained, as a percentage of DM, 19.5% CP, 11.8% WSC, 17.4% acid detergent fibre, 34.7% neutral detergent fibre, and 9.1% ash. Whilst the animal consumed the released pellets, eructated methane was detected and measured. Pellets were released to individual animals at a maximum of eight sampling events per day. During each sampling event, eight grams of pellets were supplied per 'feed drop', with up to five feed drops permitted at each sampling event at an interval of 35 s. Following initial training, this ensured that animals visited GEM units regularly each day for an average of 240 ± 75 s per feeding event. The actual daily intake of pellets from the GreenFeed units by each animal was included in the total DM intake (DMI) calculations; a sample of pellets was oven-dried to determine the DM content, from which the DMI was derived.

2.4. Data Processing and Statistical Analysis

The experimental design was a completely randomised design, with two groups as fixed effects (control and treatment) and 10 replicates each (lamb). Biophysical measurements (LW and BCS) were recorded using AgriWebb and, alongside methane measurements through GreenFeed, feed intake through BioControl, and blood parameters through VetScan; data were subsequently processed to produce a single value per animal (averages calculated when multiple datapoints were measured, e.g., DMI), and then all analyses were performed using R Statistical Software (v4.1.2) [11]. For all the variables tested, differences between control and treatment groups were determined using a t-test. Data are presented as the mean ± SEM values, and a *p*-value of <0.05 was considered to indicate statistical significance, whereas a *p*-value between 0.05 and 0.10 was considered a tendency.

3. Results

3.1. Growth Performance

The mean initial and final LW of the animals was 58.9 kg ($p = 0.18$) and 67.8 kg ($p = 0.14$), respectively, with no difference between groups (Table 1). Therefore, the mean daily LW gain of the animals was 217 g/day, with no difference between groups ($p = 0.49$) (Table 1). Mean overall BCS change was a score of −0.025, with no differences between the treatment and the control groups ($p = 0.14$). However, it should also be noted that BCS is recorded manually by a trained assessor on a scale of 1–5 and is a method with good but imperfect repeatability. Hence, the slight change observed in each group can be considered as negligible.

Table 1. Biophysical and methane production variables of lambs fed a standard feed (Control) and a standard feed plus a multi-component feed additive (Treatment) assessed over a 6-w period.

	Control		**Treatment**		***p*-Value**
Biophysical					
Initial liveweight	57.3	±1.61	60.4	±1.66	0.18
Final liveweight	65.7	±2.12	69.8	±1.64	0.14
DLWG [1] (g/day)	205	±0.02	229	±0.03	0.49
BCS [2] change	0.08	±0.08	−0.125	±0.1	0.14
Feeding behaviour					
Daily intake (kg DM [3])	1.89	±0.04	2.34	±0.03	<0.001
Visits/day	49.5	±1.25	59.1	±1.3	<0.001
Intake/visit (g DM)	34.7	±1.0	35.6	±0.67	0.43
FCR [4]	4.8:1	±1.13	5.7:1	±1.01	0.54
Methane production					
Methane (g) animal/day	41.0	±1.56	43.9	±2.25	0.30
Methane (g) animal/kg liveweight	0.70	±0.02	0.72	±0.04	0.59
Methane (g) animal/kg DLWG	229	±33.0	191	±14.2	0.31
Methane (g) animal/kg DM ingested	24.3	±0.86	18.9	±0.48	<0.001

[1] Daily Live Weight Gain, [2] Body Condition Score, [3] Dry matter, [4] Feed Conversion Ratio: kg DM intake divided into kg of DLWG. Values presented as mean ± SEM; n = 10 animals per group.

3.2. Feed Efficiency and Feeding Behaviour

Mean DMI varied between groups ($p < 0.001$), with lambs supplemented with the multi-component feed additive showing a 24% increase compared to the control group (Table 1). This difference was driven primarily by the higher number of daily visits to the feeder for the treatment group ($p < 0.001$), averaging approximately 10 more visits per day.

However, the average DMI per visit did not differ between groups ($p = 0.43$), with both groups consuming similar amounts per feeding event. Despite the increased daily DMI, FCR did not differ between groups ($p = 0.54$), which, overall, averaged 5.3:1 (Table 1).

3.3. Blood Parameters

Seventeen of the parameters were not different between groups (Table 2). The red cell distribution width (RDW) tended ($p = 0.08$) to differ between groups (24.4 fl for control and 24.9 fl for treatment). Similarly, platelet distribution width tended ($p = 0.10$) to differ between groups (PDW was 4.41 fl for control and 5.46 fl for treatment). However, the only parameter with a statistical difference ($p = 0.02$) was the mean platelet volume (MPV), 5.08 fl for control and 5.66 fl for treatment groups. Notably, the main parameter of interest for this analysis was neutrophil count (and percentage), which did not show any difference between groups ($p = 0.33$), averaging 26.4 10^9/L.

Table 2. Blood parameters of lambs fed a standard feed (Control) and a standard feed plus a multi-component feed additive (Treatment) assessed after a 6-w feed trial.

Parameter [1]	Control		Treatment		*p*-Value
WBC 10^9/L	15.3	±1.20	17.3	±1.63	0.35
LYM 10^9/L	11.0	±0.965	12.8	±1.10	0.24
MON 10^9/L	0.08	±0.006	0.09	±0.008	0.29
NEU 10^9/L	4.26	±0.463	4.41	±0.641	0.84
LYM %	71.6	±2.21	74.5	±1.94	0.34
MON %	0.5	±0.00	0.5	±0.00	NA [2]
NEU %	27.9	±2.21	25.0	±1.94	0.33
RBC 10^{12}/L	13.3	±0.318	13.3	±0.312	0.92
HGB g/dL	13.4	±0.313	13.1	±0.284	0.58
HCT %	34.7	±0.667	35.2	±0.362	0.58
MCV fl	26.4	±0.499	26.6	±0.521	0.79
MCH pg	10.1	±0.228	9.89	±0.202	0.48
MCHC g/dL	38.5	±0.478	37.4	±0.503	0.12
RDWs fl	24.4	±0.200	24.9	±0.222	0.08
RDWc %	27.1	±0.408	27.5	±0.539	0.55
PLT × 10^9/L	314	±33.6	262	±28.9	0.25
PCT %	0.16	±0.02	0.15	±0.02	0.69
MPV fl	5.08	±0.147	5.66	±0.175	0.02
PDWs fl	4.41	±0.423	5.46	±0.421	0.10
PDWc %	22.3	±1.19	24.7	±1.14	0.15

[1] WBC: white Blood Cell; LYM: Lymphocytes; MON: Monocytes; NEU: Neutrophils; RBC: Red Blood Cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDWs: Red Cell Distribution Width (Standard Deviation); RDWc: Red Cell Distribution Width (Coefficient of Variation); PLT: Platelet count; PLC: Procalcitonin; PCT: Platelet Distribution Width (Standard Deviation); MPV: Mean Platelet Volume; PDWs: Platelet Distribution Width (Standard Deviation); PDwC: Platelet Distribution Width (Coefficient of Variation); [2] NA: does not apply. Values presented as mean ± SEM; n = 10 animals per group.

3.4. Methane Production

Daily methane produced per lamb per day did not differ ($p = 0.30$) between groups, averaging 42.5 g/d, and neither did the methane produced per kg of LW, averaging 71 g/kg LW. Methane produced per kg of daily LW gained did not differ ($p = 0.31$) despite notable numerical differences (Table 1), averaging 210 g/kg daily LW gain. However, a

difference was observed ($p < 0.001$) between groups for methane produced per kg of DMI, with the treatment group producing 22.2% less methane than the control group per kg DMI (Table 1, Figure 1). This equated to an average reduction of 5.4 g of methane per kg DMI for the treatment group. The variation in average methane produced per kg DMI per animal was visually scrutinised for temporal trends, indicative of a cumulative treatment effect; however, no trend (either positive or negative) was detected (Figure 2).

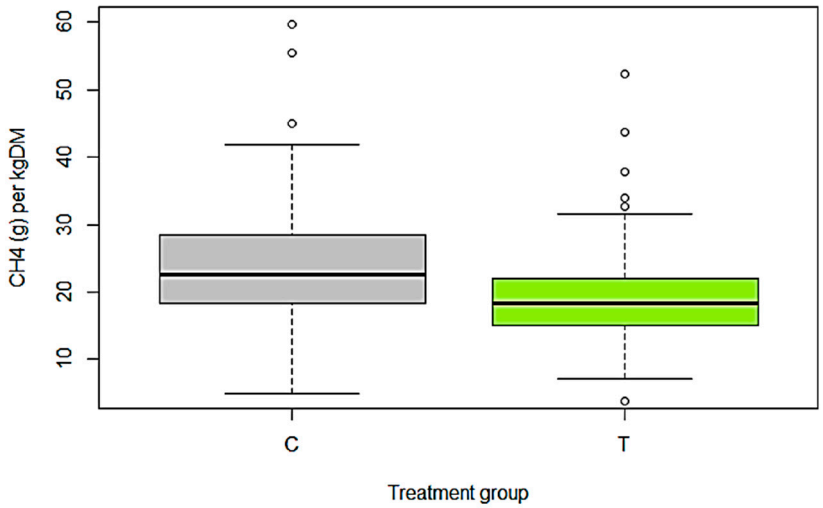

Figure 1. Conditional boxplot showing the quantity of methane (g) produced per kg of dry matter ingested. C: control (grey); T: treatment group (green) (lambs supplemented with NuAdvent+ at 20 g per day).

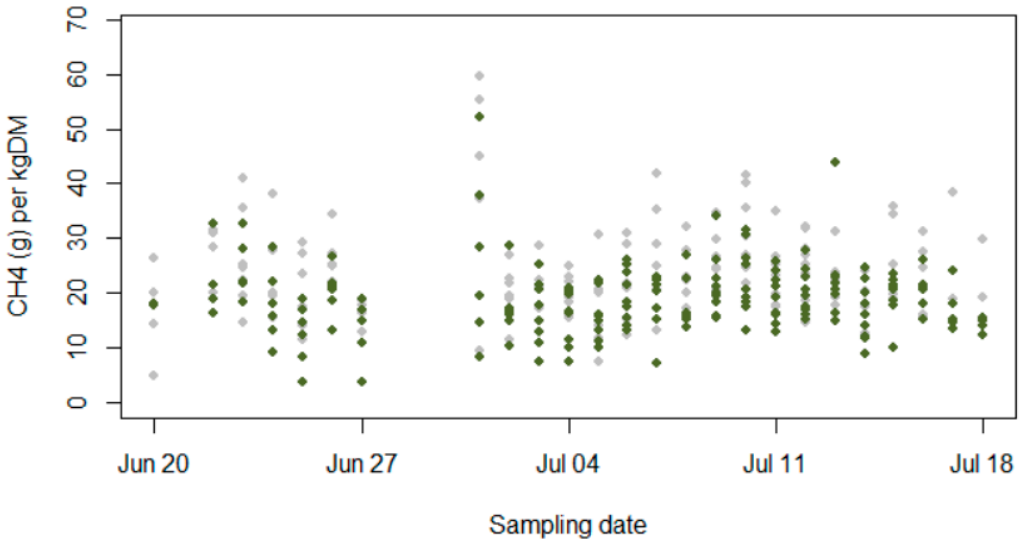

Figure 2. Temporal variation in average methane (g) produced per animal, per kg of dry matter ingested. Grey dots: control; green dots: treatment group (lambs supplemented with NuAdvent+ at 20 g per day).

4. Discussion

This study explored the effects of NuAdvent+, a novel feed additive combining MCFAs, live yeast, plant-based agents (derived from fruits and herbs), and B Vitamins, on sheep as a ruminant model. The investigation focussed on its potential to influence feeding behaviour, reduce enteric methane emissions, and improve growth performance

and immune function. As a newly formulated additive, NuAdvent+ lacks extensive prior research, unlike more established additives such as 3-NOP, which have been widely studied in cattle. This discussion contextualises the findings within the broader scope of dietary interventions for ruminant sustainability.

Feed additives are recognised for their ability to enhance palatability, digestibility, and nutrient absorption, often leading to increased feed intake and improved growth outcomes [12]. The inclusion of NuAdvent+ notably increased daily feed intake in the treatment group, driven by more frequent feeding visits rather than a larger intake per visit. This suggests that the additive, possibly due to its aromatic plant-based components or palatability-enhancing properties, encouraged greater engagement with the feed. Live yeast, a key component of NuAdvent+, may have contributed to this effect, as yeast supplementation has been shown to stimulate appetite and promote the activity of fibre-degrading enzymes like xylanase and Carboxymethyl cellulase [13–15]. However, despite the higher feed intake, this did not translate into improved growth performance or FCR. The lack of significant differences in DLWG and FCR between groups indicates that the increased consumption may not have been efficiently converted into body mass, possibly due to limitations in nutrient utilisation or energy allocation. This finding contrasts with some studies on probiotics and essential oils, which report enhanced growth alongside increased intake [16]. The presence of MCFAs, whilst beneficial for methane reduction, might have subtly impaired fibre digestibility, a known trade-off in forage-based diets [17], potentially offsetting the yeast's positive effects on rumen fermentation. Future research could explore adjusting the MCFAs concentration or pairing NuAdvent+ with diets optimised for fibre digestion to better balance intake and efficiency.

A primary objective of this study was to assess NuAdvent+'s capacity to mitigate enteric methane emissions, a critical factor in reducing the environmental impact of livestock farming. The significant reduction in methane yield per kilogram of DMI in the treatment group highlights the additive's anti-methanogenic potential. The observed reduction in methane yield per kilogram of DMI in the treatment group may be attributed to the synergistic effects of the additive's constituents. The formulation included MCFAs, live yeast, plant-derived extracts, and Vitamin B complex, which are known to inhibit methanogenesis by disrupting microbial populations and altering ruminal hydrogen fluxes, thereby reducing substrate availability for methane production [6,18]. It has already been identified that live yeast supplementation stabilises rumen pH, enhances fibre degradation, and improves microbial efficiency [19,20]. The inclusion of phytogenic compounds from the plant extracts may also contribute to methane reduction by altering rumen fermentation pathways [21]. Similarly, B Vitamins can potentially enhance nutrient and energy utilisation in the animal [22]. These findings align with the established properties of MCFAs, which inhibit rumen methanogens, redirect hydrogen away from methane production, and reduce the availability of fermentable carbohydrates for methanogenesis [6,17]. The live yeast component likely complemented these effects by stabilising rumen pH and fostering a microbial environment less conducive to methanogen proliferation [8]. Notably, whilst methane per unit of DMI decreased, total daily methane output and methane per unit of LW or LW gain showed no significant change. This suggests that the mitigation effect is tied to feed intake dynamics rather than an absolute reduction in methane production, reflecting the higher DMI in the treatment group. The absence of a temporal trend in methane reduction over the trial period further implies that NuAdvent+'s impact is immediate and consistent rather than cumulative, which could be advantageous for practical implementation but limits long-term adaptation insights. Compared to additives like 3-NOP, which can achieve greater methane suppression in cattle [23], NuAdvent+ offers a moderate yet promising reduction, particularly suited to forage-based systems. While the precise contribution of each component cannot be deconvoluted

in this present study, the multi-targeted design of the additive likely underpins its observed anti-methanogenic effect. However, the potential trade-off with fibre digestibility, as noted with MCFAs [7], warrants further investigation to ensure that methane mitigation does not compromise overall rumen function.

Contrary to expectations, NuAdvent+ did not enhance growth performance or BCS, despite its nutrient-rich composition and the inclusion of Vitamin B, which is linked to improved energy metabolism. The lack of difference in DLWG and FCR suggests that the additional energy from increased feed intake may have been diverted to maintenance or methane-related metabolic processes rather than growth. This aligns with the energy loss associated with enteric fermentation, estimated at 2–12% of gross energy intake [3], which NuAdvent+ only partially mitigated. Regarding immune function, blood parameters showed minimal differences between groups, with only MPV reaching statistical significance. The slight increase in MPV in the treatment group could indicate subtle shifts in platelet activity, potentially linked to the anti-inflammatory properties of plant-based agents or Vitamin B, but this was not corroborated by changes in neutrophil counts (or percentage) or other immune parameters. These findings suggest that NuAdvent+'s impact on immune function is negligible under the conditions tested, possibly due to the healthy baseline status of the sheep or the short duration of the trial. Longer-term studies or trials under immune-challenged conditions might better elucidate its immunomodulatory potential.

NuAdvent+ demonstrated promise as a methane mitigation strategy, offering a practical dietary intervention for reducing the environmental footprint of ruminant production. Its multi-component design leverages the synergistic effects of MCFAs, yeast, and plant-based agents, distinguishing it from single-action additives. However, its inability to improve growth performance or feed efficiency highlights the need for optimisation. Adjusting the dosage, currently set at 20 g/day, could enhance efficacy, as could tailoring its use to specific ruminant species, production stages (e.g., growing vs. finishing), or dietary contexts (e.g., high-concentrate vs. forage-based diets). The moderate methane reduction, whilst significant per unit of DMI, suggests that combining NuAdvent+ with other strategies, such as genetic selection or alternative additives, might yield greater environmental benefits. Additionally, exploring its long-term effects on rumen microbiota and animal health could address sustainability concerns beyond methane emissions. Given this study's focus on sheep, extrapolating these findings to other ruminants, such as cattle, requires caution due to species-specific differences in rumen dynamics and methane output.

5. Conclusions

In conclusion, NuAdvent+ offers a viable approach to reduce methane yield in sheep, with notable effects on feeding behaviour. Whilst it is not effective for enhancing growth or immune outcomes in this context, its potential as part of a broader sustainability strategy remains promising, needing further investigation. Further refinement and broader application will be key to maximising its impact across ruminant production systems.

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Institutional Review Board Statement: Prior to commencement, the research protocol of this study was reviewed and approved by Rothamsted Research's Animal Welfare Ethical Review Body (AWERB) in accordance with the Animal Scientific Procedures Act (1986).

Data Availability Statement: Data can be obtained from the Rothamsted Research repository at: https://doi.org/10.23637/b6tiuymu.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

3-NOP	3-nitrooxypropanol
BCS	Body condition score
DLWG	Daily liveweight gain
DM	Dry matter
FCR	Feed conversion ratio
GEM	GreenFeed Emission Monitoring
MCFAs	Medium-chain fatty acids
MPV	Mean platelet volume
PDW	Platelet distribution width
RDW	Red cell distribution width

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