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Leverage of Essential Oils on Faeces-Based Methane and Biogas Production in Dairy Cows

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Abstract: Currently, there is an ongoing intensive search for solutions that would effectively reduce greenhouse gas emissions (mainly methane) into the environment. From a practical point of view, it is important to reduce methane emissions from cows in such a way as to simultaneously trim emissions from the digestive system and increase its potential production from feces, which is intended as a substrate used in biogas plants. Such a solution would not only lower animal-based methane emissions but would also enable the production of fuel (in chemical form) with a high yield of methane from biogas, which would boost the economic benefits and reduce the use of fossil fuels. We tested the effect of administering an essential oil blend consisting of 5.5% oils and fats on methane and biogas production from dairy cow feces during fermentation. Three subsequent series (control and experimental) were conducted in dairy cows fed a total mixed ration (TMR) rich in brewer's cereals and beet pulp, with 20% dry matter (DM) of the total diet. Cows from the experimental group received 20 g/cow/day of essential oil blend, namely a commercial additive (CA). The study showed that CA can increase the production of methane and biogas from dairy cow feces. It can be concluded that in the experimental groups, approx. 15.2% and 14.4% on a fresh matter basis and 11.7% and 10.9% on a dry matter basis more methane and biogas were generated compared to the control group, respectively. Therefore, it can be assumed that the use of CA in cow nutrition improved dietary digestibility, which increased the efficiency of the use of feces organic matter for biogas production.

Keywords: biogas; cattle; dairy cows; essential oils; forage diet; methane

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1. Introduction

The continuous increase in demand for dairy products proportionally increases GHG emissions [1–4], primarily methane (CH_4) [3,5,6]. This gas, when converted into CO_2 equivalent (CO_2eq), has a very negative impact on the greenhouse effect, 25–28 times [7–10] greater than carbon dioxide itself. Moreover, in parallel with increased milk production, huge amounts of "waste", like manure, are generated, forcing the search for a sustainable solution to this issue. Manure is a valuable natural fertilizer, but, at the same time, its improper management contributes to harmful GHG emissions and odor-generating ammonia [11–13]. This promotes acidification and the formation of particulate matter, primarily as a result of the volatilization of ammonia and nitrogen oxides, as well as the eutrophication that is largely related to leaching of nitrates and phosphates from surface waters and soil [14–16]. Manure management, including agrotechnical treatments, often leads to considerable costs, but above all, to the consumption of valuable fossil energy sources [14,15,17]. To reduce these costs, relatively cheap solutions can be employed, including compaction and heap cover [18–20], solid fraction separation [18,19,21,22], composting [23–32], and others [19,33,34]. At the moment, however, the most proven solution to the above-mentioned problems, although requiring significant investments, is the application of manure within methane fermentation processes [35–38]. Moreover, retrofitting a biogas plant with a cogeneration unit (CHP) will not only drastically reduce the production of greenhouse gases and other harmful impacts, but will also be a source of renewable energy as a result of methane recovery [39–43].

Although almost 60% (ranging from 30 to 75% depending on the country) of biomass is used in animal production (as feed or bedding [44]), only a few percent of this stream goes to biogas units and, although this share is still growing, this growth is very slow [41,45–47]. There are several reasons for this situation, including low biogas efficiency with a minor degree of organic degradation, mainly due to the high water and fiber content [14,48–50], the need to build large-capacity sealed tanks to store the resulting digestate [39], and lack of a sufficiently large and well-balanced (carbon to nitrogen ratio, organic or lignocellulose content, humidity, etc.) resource of substrates for year-round protection of the biogas plant feedstock [51–54]. The abovementioned aspects contribute to the insignificant profitability of investments in biogas plants. One of the solutions to increase the energy (and, at the same time, financial and ecological) efficiency of manure use is by reducing enteric methane emissions from farm animals and maximizing biogas production from manure. Utilizing organic waste, like feces/manure from ruminant production for energy production, offers a more sustainable option for ruminant production. For example, Denmark is the most biogas-minded country in Europe. Already, a third of manure goes to biogas plants. The policy is to increase this until it is almost fully manure-based. Poland is also highly interested in using manure as a source of substrates for biogas plants. However, studies on the effect of using feed additives to increase biogas production from manure while reducing enteric methane emissions are limited [55,56]. The literature most often indicates that the reduction of enteric methane emissions by feed additives is closely related to the reduction in manure used in biogas production [57].

The hypothesis of the present study assumed that obtaining biogas (especially methane) from the feces of cows fed a diet with a nutrient additive would potentially limit the methanogenesis process at the level of the rumen, but would not affect the subsequent fermentation of the excrement. Such an action is not only of environmental importance (it reduces methane emissions from dairy farming), but, at the same time, generates a positive economic aspect for the farm, namely a higher yield of methane per mass unit. This means higher potential revenues from the use of manure as a substrate for a possible biogas plant, which is the most advantageous way of utilizing this type of waste. Thus, the objective of this study was to investigate the effect of a diet supplemented with a feed additive based on blended essential oils, which potentially reduces enteric methane emission on the methane efficiency of feces from dairy cows, analyzed during "batch culture" laboratory tests.

2. Materials and Methods

The conducted research consisted of two main stages. The first consisted of a series of experiments conducted on a selected group of dairy cows that were given an additive to the feed, which was supposed to increase the methane yield of excreted feces. The second leading stage involved conducting laboratory analyses of the biogas yield of the obtained feces.

2.1. Materials and Methodology of In Vivo Research

The experiment was conducted in vivo on cannulated dairy cows as a continuation of research from another project, where the commercial additive (CA) consisted of 5.5% of oils and fats (including coriander seed oil, eugenol, geranyl acetate, and geraniol), 26% of crude protein, 6% of crude fiber, 18.5% of crude ash, 5.8% of Ca, and 0.2% of Na was used. CA was used to reduce the methanogenesis process, mainly from the rumen.

A total of 4 multiparous rumen-cannulated Holstein–Friesian dairy cows (625 ± 20 kg body weight; 120–150 days in milk) as manure donors were randomized into two groups and treated with conventional and CA-enriched diets (CON vs. CA) in a replicated 2 (groups) × 2 (periods) crossover design. The donor animals were fed with a total mixed ration (TMR) twice a day. However, the experimental diet (CA) was supplemented with CA (20 g/cow/day). The ingredients and chemical composition of the TMR are shown in Table 1.

Table 1. Feeding ingredients and chemical composition of the TMR for cannulated dairy cows.

TMR Ingredients	g/kg Dry Matter	Chemical Composition	g/kg Dry Matter
Corn silage	388	Dry matter g/kg as fed	432
Alfalfa silage	82	Organic matter	906
Meadow grass silage	91	aNDF	367
Beet pulp	103	Crude protein	159
Brewer's grain	95	Ether extract	26.3
Concentrate	119	VEM	943
Rapeseed meal	108		
Mineral vitamin premix	14		
Forage:concentrate ratio	76:24		

During the adaptation and sampling period, donor animals were housed in tie stalls with rubber mats. Individual feeding systems was used and the animals had the unrestricted access to water and salt blocks. During the feces sampling period, relative humidity in the barn was maintained at a level of about 55%, and the average temperature was maintained at 18 °C. Each period lasted 39 days, wherein 30 days were allocated for adaptation and 9 days for sampling (3 days of ruminal fluid collection, 3 days of methane emission measurements, and 3 days of feces collection). Feces amounts were sampled immediately after defecation (to avoid straw and urine contamination), weighed, and collected (20% *w*/*w*) as feces subsamples. Feces subsamples were transferred in a cool (4 °C) atmosphere directly to the laboratory for further testing.

2.2. Methodology of Laboratory Physical Analysis

The collected, cooled feces samples were initially analyzed for dry matter content (dry matter, DM, in accordance with the Polish Standard PN-75 C-04616/01 [58,59]), as per the standard procedure of drying the samples (each in 3 repetitions) at 105 °C for 24 h. The dry organic matter (dry organic matter, DOM) content was then determined and tested in accordance with the Polish Standard PN-Z-15011-3 [59,60], namely burning of dry samples (in 3 repetitions) at a temperature of 525 °C for 3 h (muffle furnace L 40/11/B410 series, manufacturer: Nabertherm, Lilienthal, Germany). The pH was determined following the Polish Standard PN-90 C-04540/01 [59,61]. This type of input data was necessary for determining the starting conditions of the fermentation process in the subsequent periodic tests (e.g., loading the organic load of the chamber) and for calculating the biogas and methane productivity of the substrates.

2.3. Methodology of Laboratory Methane Fermentation Tests

All samples from 6 series (6 series of the 3 main samples in 3 repetitions, i.e., a total of 27 analyses) went to a certified biogas laboratory of the Poznań University of Life Sciences, Poland [62].

The 18 main feces samples were used for the study, which were tested in a special test bench for methane fermentation in so-called "batch culture tests" (periodic analyses). This test setup comprises 3 chamber sections where the individual fermenters (a capacity of

2 dm^3 each) are immersed in a thermal bath of 39 °C to maintain mesophilic conditions (Figure 1).

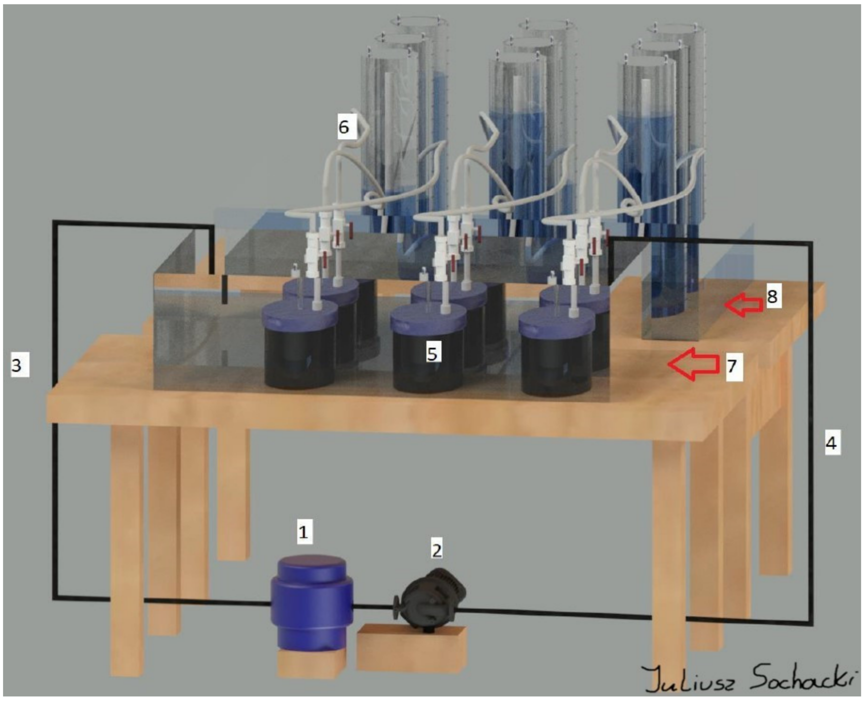

Figure 1. Stand for batch culture test: 1, water heater; 2, water pump; 3 and 4, insulated water system; 5, fermenters (2 dm^3 capacity); 6, biogas lines; 7, water bath; 8, tube system measurement of the produced biogas.

The experiment commenced with purging the fermenters with nitrogen to remove oxygen, hence, immediately creating anaerobic conditions (oxygen is an inhibitor of methanogenic bacteria), followed by placing the tested substrates in fermenters and, depending on the dry matter content, mixing in appropriate proportions with standardized inoculum by the procedures described in international standards DIN 38 414/S8 and VDI 4630 [63,64]. Under the standard procedure, additional fermentation of the inoculate was conducted as means of a control. Moreover, to verify the research results, a fermentation test was executed on the reference substrate, namely microcrystalline cellulose. It gave a biogas yield of 745 m^3/Mg on dry organic matter (DOM), thus, confirming the credibility of the research conducted (the standard assumes the biogas efficiency of this substrate in the range of 740–750 m^3/Mg DOM). All samples were fermented in 3 replicates, and the individual results in the series were the arithmetic mean.

Measurements of the volume and composition of the resulting biogas were conducted daily at 24-hour intervals (Figure 2). The volume was read from the scale on the “stand for batch culture test” tubes (Figure 1, item 8). The measurements were stopped if the daily biogas production for a given measurement was less than 1% of the total obtained biogas volume. Thus, the graph axis (Figure 2) ends at day 41, as this was the longest measurement period of all individual series and repetitions.

It should be noted that the graph presented in Figure 2 shows raw data (substrate with inoculum) and was used to determine the duration of the substrate gasification process under real conditions; it was not applied directly to assess the biogas efficiency of a pure substrate. Our research made it possible to compare individual substrates (the sample series) only after taking into account the inoculum and converting the read volumes in relation to the starting fresh matter (FM). This was dictated by the process procedure (DIN 38 414/S8 standard), in which the starting weight of the sample is determined based on dry matter (DM) content.

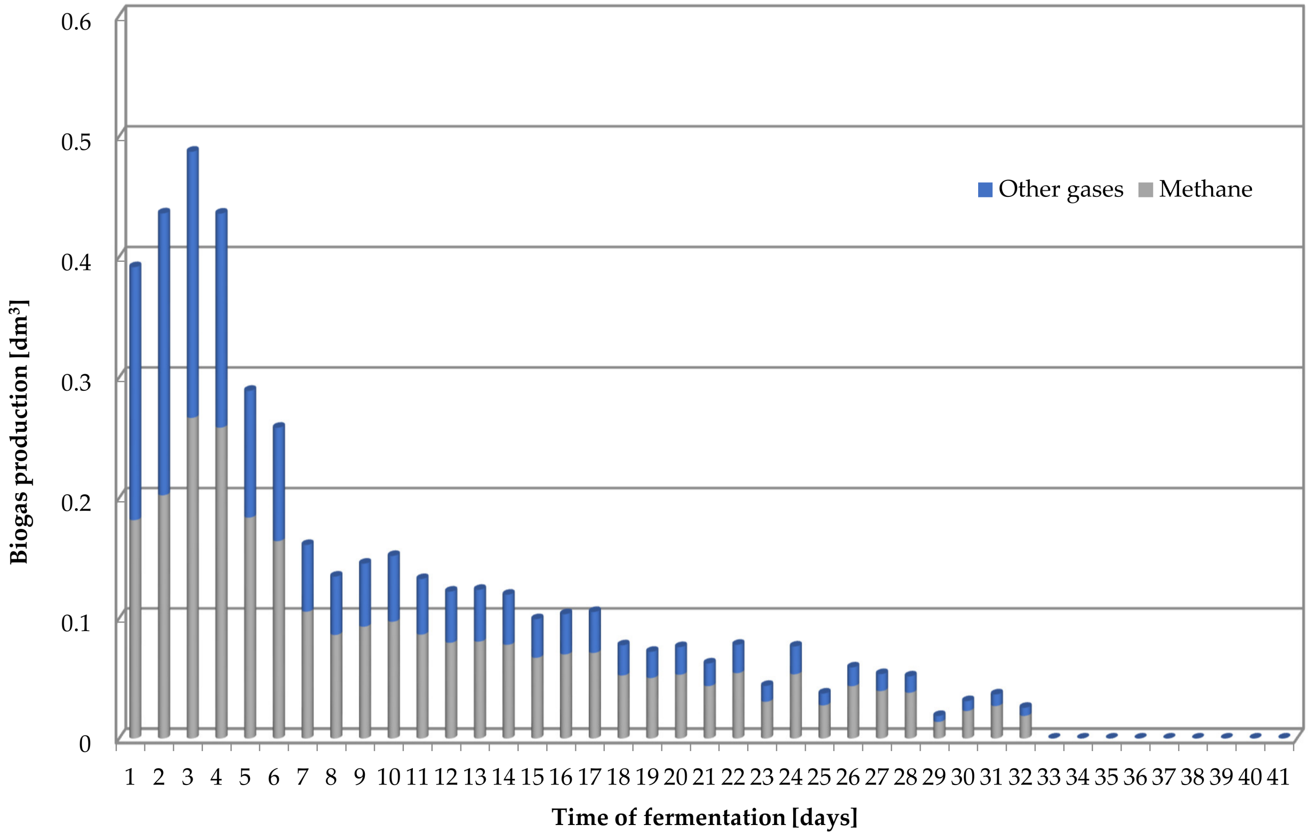

Figure 2. Graph of daily volume increments of biogas produced.

The volumes of the biogas produced were measured with a Geotech GA5000 gas analyzer (QED Environmental Systems Ltd., Coventry, UK). The measurement ranges of the Geotech analyzer are as follows: O_2 0–25%, CO_2 0–100%, CH_4 0–100%, NH_3 0–1000 ppm, H_2S 0–10,000 ppm. The GA5000 was calibrated as standard once a week using calibration gases (from Air Product).

To determine the significance of differences from the averages for individual results (pH, DM, DOM, CH_4 content, and biogas and methane yield), a standard Student's *t*-test for independent variables was performed for the CON and AC groups. Additionally, the standard deviation was also provided. Calculations were performed using Excel Microsoft Office (ver. Excel 2019, Microsoft Corporation, One Microsoft Way, Redmond, WA, USA) statistical functions.

3. Results

3.1. Physical Analysis Results

Characteristics of substrates from a given batch, including dry matter (DM) and organic dry matter (DOM as % of DM) values, as well as pH, are listed in Table 2.

Table 2. Initial parameters of the substrates.

Parameters	pH [-]	Dry Matter [%]	Dry Organic Matter [%]
	Control group–"CON"		
Average value and standard deviation	7.01 ± 0.05	13.19 ± 0.83	84.23 ± 2.83
	Experimental group (with CA)–"CA"		
Average value and standard deviation	7.06 ± 0.012	13.88 ± 1.77	82.54 ± 4.05
CON vs. CA *p*-value from a *t*-test	−0.6 0.19	−0.69 0.30	1.70 0.32

Based on the results, it can be concluded that (for all samples) the substrates had a neutral pH, dry matter content was in range 12–17%, dry organic matter content ranged from 76% to 88%, and the standard deviation for the CA group was almost twice as high in each indicator.

The experimental group had a slightly higher average dry matter share in the substrate than the conventional group, and in the case of dry organic matter, the control group had a higher content of organics than the group with the CA additive. It should be noted that the differences were statistically insignificant (p-value > 0.05).

3.2. Methane Fermentation Tests Results

The average values from subsequent repetitions in a given series, including the share of methane in biogas, the production of biogas and methane in terms of fresh matter (FM), dry matter (DM), and dry organic matter (DOM), and the result of the t-test are presented in Table 3.

Table 3. Average values of biogas productivity.

Parameters/Test No.	CH_4 Content (%)	CH_4 m^3/Mg FM	Biogas m^3/Mg FM	CH_4 m^3/Mg DM	Biogas m^3/Mg DM	CH_4 m^3/Mg DOM	Biogas m^3/Mg DOM
Control group–"CON"							
Average value and standard deviation	59.38 ± 0.7	19.46 ± 2.3	32.75 ± 3.11	147.22 ± 11.24	247.79 ± 16.86	175.21 ± 15.84	294.91 ± 23.88
Experimental group (with CA)–"CA"							
Average value and standard deviation	59.98 ± 0.7	22.95 ± 1.15	38.28 ± 2.06	166.70 ± 17.66	278.24 ± 32.69	201.51 ± 13.99	336.21 ± 27.06
CON vs. CA	1.0%	15.2%	14.4%	11.7%	10.9%	13.1%	12.3%
p value *	0.09	0.001	0.001	0.01	0.02	0.001	0.001

* the p-value of averages of two groups.

The data shown in Table 3 enable a conclusion that all analyzed substrates had a methane share in biogas at the level of the tested materials, and that methane concentration was very similar, approximately 59.7% (p-value > 0.05 indicates a small significance of the difference at the level of statistical tendency). The indicators of biogas (methane) yield, in contrast, show statistically significant differences (p-value < 0.05). Thus, for a given group of samples, methane (CH_4) production from 1 m^3 of fresh matter for the control and experimental groups was 19.46 Mg and 22.95 Mg, respectively.

Therefore, for a given group of samples, methane (CH_4) production from 1 m^3 of fresh matter in the control and experimental groups was 19.46 Mg and 22.95 Mg, respectively. Higher methane yields in the CA group were also obtained in relation to dry matter and dry organic matter, i.e., 147.22 m^3/Mg and 175.21 m^3/Mg, respectively, while the averages for the CON group were 166.70 m^3/Mg and 201.51 m^3/Mg, respectively.

Upon analyzing the individual differences in the average biogas production in the analyzed groups, it can be concluded that in the experimental groups, 15.2% and 14.4% more methane and biogas were generated in terms of fresh matter than in the control group, respectively. In relation to dry matter, the figures were higher by 11.7% and 10.9%, respectively; when comparing the yields from organics (DOM), a difference of 13.1% for methane and 12.3% for raw biogas has been recorded. It should be noted that significantly higher productivity was achieved in relation to biogas and methane from dry organic matter, despite the fact that its content in the feces of the group with the addition of CA was as much as 1.7% lower than in the control group (Table 2).

4. Discussion

The discussion should commence with mentioning that the commercial additives dedicated to mitigating methane emissions from the dairy sector are not popular strategies in certain countries, such as Poland, compared to Western European countries. However, due to preparations for the European Union Methane Action Plan to meet obligations in the EU [65], the mitigation of methane emissions from the dairy sector has become a very urgent topic.

Based on the tests, of which the results are presented in this paper, it can be stated that the dietary supplementation of commercial additives consisting of an essential oil blend and minerals increased the yield of methane by 15.2% (from 19.46 m^3/Mg to 22.95 m^3/Mg) on a fresh matter basis in feces. The increase in methane production amounted to 11.7% (from 147.22 m^3/Mg to 166.70 m^3/Mg) in terms of dry matter. In turn, when converted to dry organic matter, the increase was 13.1% (from 175.21 m^3/Mg to 201.51 m^3/Mg). It should be emphasized that the applied CA supplement statistically decreased enteric methane daily production (−10%; $p < 0.01$; from 429 vs. 388 g/day), per dry matter intake (−12%; $p < 0.001$; from 18.3 vs. 16.3 g/kg dry matter intake), while increasing the dry matter intake of the applied dose (+2.4%; $p < 0.03$; 23.4 vs. 23.9 kg dry matter intake, which resulted in a numerical increase in the amount of feces excreted from 8.6 to 8.8 kg dry matter/day; unpublished data). This, together with the increase in the efficiency of biogas and methane from feces fermentation, confirmed the positive theory that such a comprehensive approach (reducing enteric emission and increasing biogas production from feces fermentation) is a good strategy for mitigating the negative aspects of greenhouse gas emissions.

The biogas yields, among agricultural substrates (energy crops or byproducts and waste products), are so diverse that they can range from 20 m^3/Mg to over 600 m^3/Mg with fresh matter, which would mean a 30-fold difference [66,67]. In the case of methane recovery, the values range from 178 m^3/Mg to 191 m^3/Mg for dry organic matter (DOM) [66,68]. When introducing substrates other than manure into the fermenter, productivity of almost 290 m^3/Mg per DOM can be expected. Similarly high CH_4 yields can be found in studies analyzing the impact of organic and conventional feeding of dairy cows on biogas production. Indeed, one of the literature sources demonstrated that the standard feed enables the production of methane at the level of 296 m^3/Mg DOM, and the organic feed at 234 m^3/Mg DOM [69,70]. By comparing the obtained indicators of biogas and methane efficiency from cow feces acquired with these studies with other agricultural sources, their productivity was relatively low, amounting to less than 23 m^3/Mg and 39 m^3/Mg of methane and biogas, respectively.

Studies involving the analysis of raw manure in relation to fresh matter show that the yield of biogas and the share of CH_4 in biogas are, respectively, 79.90–80.49 m^3/Mg and 56.30–56.84% [51,52,71]. The results presented in this study are twice as low (although with a higher content of CH_4 in biogas). However, it is important to note that our results come from pure feces, while the cited studies refer to manure, i.e., a mixture of feces, urine, and litter. As the analyses of biogas profitability indicate, wheat straw (as a litter) can provide as much as five times higher biogas productivity, i.e., 468.49 m^3/Mg FM [71].

Comparisons of biogas produced from different manure reveal that cow dung has a maximal potential of 204 m^3/Mg DOM [72,73]. In cases of very high loads, the maximum values obtained in the referred study reached approximately 192 m^3/Mg DOM [72], which corresponds to the values from this study of 175–201 m^3/Mg DOM. The data indicate that applying CA supplements enables achieving potentially maximum methane yields from the feces of dairy cows, which confirms that such a strategy will be more sustainable for the environment.

In the case of feed additives, it can be assumed, based on a meta-analysis of the available literature sources, that only *Asparagopsis* (a species of red seaweed) seems to meet the expectations in terms of reducing enteric methane emissions [60]. Similar studies involving the addition of *Asparagopsis* to feed showed that enteric methane emissions decreased by 61% and that the feces productivity of dairy cows with and without supplements was at a similar level, which in the context of chemical-form energy (CH_4) recovery within methane fermentation processes should be considered positive [74]. Overall, the implementation of

Asparagopsis in cow rearing significantly reduced CH_4 emissions from feces (as much as 44% compared to raw feces), which is crucial for the possible storage of dung/manure in uncovered heaps before application in the fields.

In the case of essential oil supplements, i.e., additives that do not constitute an energy input in animal nutrition, reductions in ruminal emissions are estimated at a real level (in-vivo studies) of 8.8–9.8%, with no impact of additives on feces composition [57,75,76]. Therefore, it is important for the comprehensive treatment of the use of essential oils in the nutrition of ruminants to enable implications introducing sustainable principles in the production of ruminants.

We have demonstrated for the first time that the use of CA containing essential oils has a positive effect both on the emission from the rumen (a lower emission) and on the subsequent processing of feces (more methane output for biogas).

Nevertheless, it would be advisable to conduct further research on the chemical composition of feces and the emissions of gases other than methane, such as hydrogen sulfide, the emissions of which may be significant and may negatively affect the processes of purifying biogas into methane.

5. Conclusions

Supplementation of CA into cow's diet, particularly in the context of the growing global importance of methane from manure, should be taken into account when recommending nutritional options to reduce methane emissions from dairy cows and in implementing a comprehensive, sustainable strategy regarding GHG emission from dairy sectors. It can be concluded that the introduction of a CA (consisting of blended essential oils and minerals) into cattle diet significantly increases the energy value of fermented feces. Nevertheless, in vivo studies of CA addition are highly desirable to confirm the long-term effect and the impact on ruminal and intestinal CH_4 emissions. Finally, the impact, both environmental and economic, of CA's carbon footprint on an investment in a potential biogas plant should be analyzed.

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Institutional Review Board Statement: All investigations were performed according to the rules accepted by the National Ethical Commission for Animal Research (Ministry of Science and Higher Education, Poland). Additionally, the Local Ethical Commission for Animal Research (permission No. 44/2023) approved the presented study.

Data Availability Statement: Data are available upon reasonable request to the corresponding author.

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