

THE INFLUENCE OF PROBIOTIC SUPPLEMENTATION ON NITROUS OXIDE AND CARBON DIOXIDE EMISSIONS OF GROWING MALE DAIRY BUFFALOES (*Bubalus bubalis* L.)

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ABSTRACT

The influence of probiotics on the nitrous oxide (N₂O) and carbon dioxide (CO₂) emissions was evaluated on four (4) growing male dairy buffaloes (*Bubalus bubalis* L.) with similar body weight (250.38 ± 10.38 kg), age (15-16 mos). The animals were allocated into four treatments following a Latin Square design. Treatment 1 (T1) was the control with no probiotic supplement, Treatment 2 (T2) was supplemented with 5 ml probiotics, Treatment 3 (T3) was offered 10 ml and Treatment 4 (T4) was given 15 ml. The range of accumulated N₂O was from 0.4047- 0.5137 g d⁻¹ and the animals offered with T2 had the lowest emission (p-value < 0.05). The range of accumulated CO₂ was from 3656.27- 4122.50 g d⁻¹, however, it was not affected by probiotic supplementation. While no significant correlation (p-value > 0.05) was found between N₂O emissions and digestibility parameters, CO₂ emissions showed a significant correlation (p-value < 0.05) with most digestibility parameters (GE, NDF, CP and DM). This study demonstrates the potential of probiotic supplementation to reduce N₂O emissions and emphasizes the role of digestibility parameters in estimating CO₂ emissions.

Keywords: CO₂ emissions, Greenhouse gas, microbial supplement, ruminant, N₂O emission

INTRODUCTION

The environmental impact of agriculture is a pressing concern in the context of global climate change, with dairy buffaloes (*Bubalus bubalis* L.) being significant contributors to greenhouse gas (GHG) emissions (Reddy *et al.*, 2019; Valiente *et al.*, 2023). The emissions of nitrous oxide (N₂O) and carbon dioxide (CO₂) from livestock production are problematic due to their high global warming potential. N₂O is a potent GHG with a global warming potential of approximately 273-298 times that of CO₂ over a 100-year period (IPCC, 2014; US-EPA, 2024). In Asia, agriculture is a major source of GHG emissions. The region is responsible for a substantial proportion of global livestock-related emissions due to its large population of ruminants and intensive farming practices. According to DENR-EMB (2011), manure

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management is the main source of nitrous oxide in livestock production in the Philippines. As for CO₂, there are limited references on the emission of this GHG. Nonetheless, these emissions are driven by the continuous increase in livestock production. The most recent livestock inventory reported over 22 million heads in 2020, which includes carabao, cattle, swine, and goats (PSA, 2021). Addressing these emissions is essential for the Philippines to meet its climate commitments and ensure sustainable agricultural practices through a 30-45% reduction from the baseline emission by 2030 (UNDP, 2023).

Mitigation strategies to decrease GHG emissions in the livestock industry include improvements in manure management, optimization of feed efficiency, and the use of microbial supplements such as probiotics. Probiotics provide health benefits to the host when administered in adequate amounts, and have been shown to improve gut health, nutrient utilization, and overall animal performance (FAO/WHO, 2001; Hill *et al.*, 2014). In ruminants, probiotics can modulate the gut microbiota, enhance digestive efficiency, and reduce methane emissions (Jeyanathan *et al.*, 2014). A study by Gerber *et al.* (2013) outlines several mitigation strategies, including improved grazing management and the use of feed additives to potentially reduce methane and N₂O emissions. Similarly, Hristov *et al.* (2013) showed the effectiveness of feed management practices and the incorporation of supplements to reduce N₂O and CO₂ emissions. However, the impact of probiotics on N₂O and CO₂ emissions remains underexplored, particularly in dairy buffaloes. As previously mentioned, carabao (swamp buffaloes) are one of the primary livestock utilized in the Philippines, and this study is essential to support mitigation strategies for GHG emissions from this industry. In line with the use of feed additives in prior experiments, a commercially available probiotic supplement with blended strains of *Lactobacillus*, *Bacillus*, and *Phenibacillus* was tested in the study to estimate its effect on various digestibility parameters and its influence on N₂O and CO₂ levels, quantified by a gas analyzer.

Despite the promising effects of probiotics in ruminant health, there is a lack of sufficient research on the mitigating effects of probiotics in N₂O and CO₂ emissions in the Philippine dairy buffaloes. Therefore, this study was conducted to evaluate the influence of probiotics on N₂O and CO₂ emission of growing dairy buffaloes, in particular: (1) to estimate the nutrient digestibility of probiotics-supplemented diets in growing dairy buffaloes; (2) to assess the mitigation potential of probiotics on the N₂O and CO₂ emission; (3) to analyze the relationship of nutrient digestibility parameters with N₂O and CO₂ emission.

MATERIALS AND METHODS

Experimental Animals, Design and Treatments

The experiment took place at the Philippine Carabao Center National Headquarters and Gene Pool, Science City of Muñoz, Nueva Ecija. It ran from August 2022 to March 2023. The research followed guidelines set by the University of the Philippines Los Baños (reference number UPLB-2022-045) for animal care and use.

The study used four growing male buffaloes of similar age (15-16 mos) and body weights (250.38 ± 10.38 kg). Each animal received corresponding treatments: control-T1, and probiotic doses of 5ml-T2, 10ml-T3, and 15ml-T4 daily over four periods following the Latin Square design. The individual buffaloes and the period of measurement served as blocking factors to isolate the effect of treatment. The animals were given a preset dietary treatment that rotated per period, such that the animals would have consumed four dietary

treatments within four periods, with a wash-out in between. A feeding regime followed throughout the experiment is as follows: adaptation phase (day 1-12), an experimental phase (day 13-14), and a wash-out phase (day 15-24) per period (Montoya-Flores *et al.* 2020).

The buffaloes were housed in polytunnels and provided a daily diet of 70:30 para grass-to-concentrate ratio that was offered twice daily at 7:00 h and 15:00 h. Dietary treatments with probiotics (MagikGRO™, Dionysus Ent., Philippines), a blended strains of *Lactobacillus*, *Bacillus*, and *Phenibacillus* at a minimum of 10¹² cfu /ml, were prepared by mixing with the concentrate depending on the dose of the treatment (Table 1).

Table 1. Dietary treatments of growing dairy buffaloes fed with probiotic supplement.

Treatment 1	Treatment 2	Treatment 3	Treatment 4
Basal Diet*	Basal Diet* + 5ml probiotic	Basal Diet* + 10ml probiotic	Basal Diet* + 15ml probiotic

*Control diet consisting of paragrass and buffalo grower concentrate pellet with the following ingredients: yellow corn, wheat, soybean meal, rice bran D1, copra meal, wheat pollard, palm kernel, limestone powder, molasses, toxin binder, salt, pellet binder, mold inhibitor, vitamins, and minerals

Nutrient Composition and Analysis

Individual samples of commercial grower concentrate and paragrass (*Brachiaria mutica*) forages were collected to determine its nutrient composition, such as percent dry matter, ash, crude protein, and ether extract (EE), following the standard procedures of AOAC (2006), while NDF and ADF were calculated based on the procedures of Van Soest *et al.* (1991). Fecal samples were also subjected to proximate analysis to determine dry matter (DM), gross energy (GE), organic matter (OM), and crude protein (CP) content. The neutral detergent fiber (NDF) content of the fecal samples was calculated based on the procedures of Van Soest *et al.* (1991). All the analyses were conducted at the Production System and Nutrition Section (PSNS) of the Philippine Carabao Center National Headquarters and Gene Pool, Science City of Muñoz, Nueva Ecija.

Nutrient Digestibility

Total tract apparent digestibility of nutrients was estimated using the total collection method during the experimental period. The dry matter intake per animal was estimated at the start of the experimental phase at 8:00 h. Pooled samples from feed offered and feed refusal were collected to estimate the intakes of DM, OM, CP, NDF and GE. The weight of total fecal output was obtained at around 8:00 h the next day to cover a 24-hour digestibility trial that was repeated 4 times for each period. Pooled fecal samples were analyzed to determine DM, OM, CP, GE and NDF content (Givens *et al.*, 2000). The difference between intake and fecal nutrient content was used to estimate the coefficient of digestion.

Nitrous oxide and Carbon dioxide Concentration Measurement

The method of N₂O and CO₂ concentration estimation using the portable FTIR gas analyzer Gasmet DX404™ was adapted from the studies of Gaviria-Uribe *et al.* (2020). Four (4) individual polytunnels measuring 2.8m x 4m x 2.25m were constructed on site.

Polytunnels were installed with orbit fans and exhaust fumes to contain methane emissions. Hygro-thermometers were installed in and out of the polytunnels to monitor changes in temperature and relative humidity.

Growing buffaloes were allowed to adapt inside the polytunnel and the experimental diet for 12 days every period before the start of the experimental phase. On day 13 at exactly 8:00 h, N₂O and CO₂ concentration estimation started using GASMET DX4040™. N₂O and CO₂ were allowed to accumulate inside each polytunnel for 1 hour by closing the chamber airtight. After accruing N₂O and CO₂, the exhaust fume was operated to collect the gas inside, siphoning it into the inlet tube of GASMET DX4040™ to estimate real-time N₂O and CO₂ concentration. The GASMET DX4040™ was configured such that every estimation will last for 2 minutes with a recording interval of 5 seconds. After gas collection, the polytunnel's gate was opened for 3-5 minutes to release the confined gases in preparation for another round of sampling. This ran for 24 hours per period to document 24 readings.

Statistical Analysis

The PROC GLM procedure of SAS ver. 9.1.3 (SAS Inst. Inc., Cary, NC) in a one-way ANOVA was used to analyze N₂O and CO₂ concentration and digestibility parameters following the Latin Square Design experiment. Homogeneity of variances and test of normality was also conducted using Levene's and Kolmogorov-Smirnov tests. Treatment means were compared using the Tukey-Kramer test at 95% confidence interval. The relationship of digestibility parameters to the N₂O and CO₂ emission were examined using the CORR procedure of SAS ver. 9.1.3 with the significance level set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Nutrient Intake and Apparent Total Tract Digestibility

Table 2 highlights the nutrient composition of forage and concentrate offered to growers while Table 3 summarizes the nutrient intake, digested and coefficient of digestion of growers as influenced by probiotics supplementation. There were no significant differences in the coefficient of digestion, intake, and output for DM, GE, OM, CP, and NDF across treatments.

Table 2. Nutrient composition of the concentrate and forage.

Nutrient, %	Concentrate	Forage
Dry Matter	88.00	30.99
Organic Matter	91.78	88.85
Ash	8.22	11.15
Crude Protein (N x 6.25)	20.62	8.52
Crude Fiber	10.80	37.74
Ether Extract	8.03	2.42
ADF	20.18	45.47
NDF	40.68	71.69
GE (MJ kg ⁻¹)	19.00	16.20

Table 3. Total nutrient intake, digested and Coefficient of Digestion (COD) for DM, GE, OM, CP and NDF of growing dairy buffaloes fed with probiotics supplement.

Parameters ¹	TREATMENT				SEM	P-Value
	Control	Basal Diet + 5ml probiotic	Basal Diet + 10ml probiotic	Basal Diet + 15ml probiotic		
DMI, kg	8.75	8.65	8.08	8.34	0.40	0.8130
DM digested, kg	6.35	5.85	5.51	5.75	0.43	0.8099
Apparent DM COD, %	72.40	66.29	67.31	67.23	2.29	0.7168
GE intake, MJ	144.73	143.45	134.71	138.09	6.55	0.8257
GE digested, MJ	107.98	100.57	95.67	98.19	6.95	0.8269
Apparent GE COD, %	74.47	68.86	70.14	69.58	2.08	0.6998
OM intake, kg	7.65	7.58	7.11	7.29	0.36	0.8240
OM digested, kg	4.06	3.72	3.50	3.56	0.28	0.8111
Apparent OM COD, %	73.85	67.96	69.36	68.82	2.18	0.7129
CP intake, kg	0.97	0.97	0.92	0.94	0.06	0.7583
CP digested, kg	0.71	0.66	0.62	0.66	0.06	0.7465
Apparent CP COD, %	72.73	66.29	66.56	67.92	2.40	0.6356
NDF intake, kg	5.57	5.49	5.10	5.27	0.25	0.8290
NDF digested, kg	4.06	3.72	3.50	3.56	0.28	0.8111
Apparent NDF COD, %	72.64	66.26	67.40	65.74	2.49	0.7072

¹DMI: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, GE: gross energy, COD: coefficient of digestion

Probiotic supplementation in ruminants, particularly with lactic acid bacteria, enhances nutrient digestibility and performance by optimizing rumen fermentation. Probiotics stabilize rumen pH, promote beneficial microbial populations, and reduce methane production, improving fiber digestion and energy efficiency. These microbes produce digestive enzymes, boost microbial protein synthesis, and enhance gut health, leading to better nutrient absorption and feed utilization. These effects increase feed efficiency, support growth, and improve milk production and composition (Reuben *et al.*, 2022).

Similar to the current study, Elam *et al.* (2003) used a combination of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* as a supplement to the diet of steers and found that it had no effect on ADG, intake, and carcass characteristics. Another trial on Holstein cows given a diet with *L. acidophilus*, *P. freudenreichii*, and lactose did not stimulate nutrient digestibility and ruminal fermentation (Raeth-Knight *et al.*, 2007). Likewise, Masucci *et al.* (2011) found out that using *Enterococcus faecium* on the diet of buffalo calves did not affect DMI and OM, CP and NDF total tract digestibility. Philippeau *et al.* (2017) concluded that the cellulase activity could have been minimal to elicit a change in the total tract digestibility of nutrients.

In contrast, the feed efficiency of lactating Holstein cows improved upon supplementation of *Pbat* 6×10^{11} (Weiss *et al.* 2008). The result of the study of Huck *et*

al. (2000) proved otherwise that adding *L. acidophilus* and *P. freudenreichii* to the feeds offered to heifers yielded a significant increase in ADG and feed efficiency. Further, when *Bacillus licheniformis* was added to sheep's diet, the apparent total tract digestibility of DM, OM, nitrogen, and NDF increased in the treated groups causing an improvement in both N utilization efficiency and energy metabolizability (Deng *et al.*, 2018).

Both Deng *et al.* (2018) and Masucci *et al.* (2011) claimed that the differences in the results of several studies can be attributed to the species, diet offered, strain, concentration of probiotics, level of administration, and physiological state of the animal.

Nitrous Oxide Emission

Table 4 describes the N₂O emissions of buffaloes under varying doses of probiotics. The lowest total emission and CO₂-eq kg yr⁻¹ was observed from T2 with 5 ml probiotic supplementation (*p-value* < 0.05). However, N₂O produced per nutrient intake and digested (DMi, OMi, CPi, NDFi, DMd, OMd, CPd, NDFd) showed similar results (*p-value* > 0.05). Figure 1 indicates the steady increase in N₂O emissions per period within 24-hour monitoring with T1, T3 and T4 securing the highest while T2 obtaining the lowest accumulated N₂O emission.

Table 4. Total nitrous oxide emissions and nitrous oxide per nutrient intake and digested of growing dairy buffalo fed with microbial supplement.

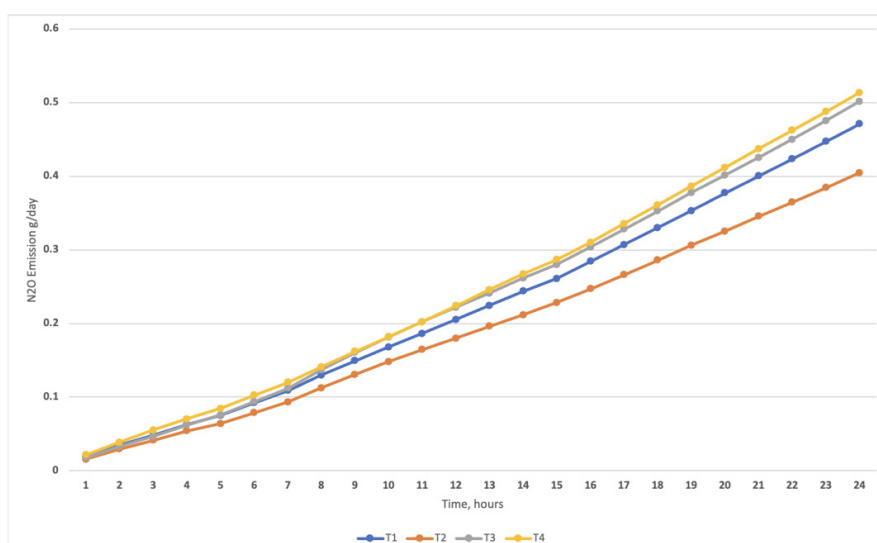
Parameters ¹	TREATMENT				SEM	P-Value
	Control	Basal Diet + 5ml probiotic	Basal Diet + 10ml probiotic	Basal Diet + 15ml probiotic		
Total N ₂ O, g	0.4762 ^a	0.4047 ^b	0.5018 ^a	0.5137 ^a	0.0133	0.0029
N ₂ O g DMi kg ⁻¹	0.0543	0.0489	0.0669	0.0632	0.0039	0.3624
N ₂ O g OMi kg ⁻¹	0.0621	0.0560	0.0766	0.0722	0.0046	0.3976
N ₂ O g CPi kg ⁻¹	0.4992	0.4479	0.5905	0.5734	0.0362	0.5136
N ₂ O g NDFi kg ⁻¹	0.0850	0.0768	0.1071	0.0997	0.0064	0.3543
N ₂ O g DMd kg ⁻¹	0.0756	0.0765	0.1019	0.0988	0.0077	0.5442
N ₂ O g OMd kg ⁻¹	0.0846	0.0855	0.1131	0.1096	0.0086	0.6115
N ₂ O g CPd kg ⁻¹	0.6948	0.7047	0.9109	0.8921	0.0710	0.6115
N ₂ O g NDFd kg ⁻¹	0.1178	0.1214	0.1642	0.1611	0.0135	0.5096
CO ₂ -eq kg yr ⁻¹	51.8014 ^a	44.0187 ^b	54.5852 ^a	55.8724 ^a	1.4463	0.0029

^{a-b}Values in the same row with different superscript letters are different (*P* < 0.05)

¹DMI: dry matter intake, OMi: organic matter intake, CPi: crude protein intake, NDFi: neutral detergent fiber intake, DMd: dry matter digested, OMd: organic matter digested, CPd: crude protein digested, NDFd: neutral detergent fiber digested, CO₂-eq: Carbon dioxide equivalent

There are two pathways in the synthesis of N₂O from feces and urine of animals; Nitrification converts ammonium to nitrate forming N₂O as a byproduct while denitrification reduces nitrate to nitrogen gas, when this process is halted, it produces N₂O instead (Kebreab

et al., 2006). The main contributor to N_2O synthesis is the amount of N deposited in the feces and urine as excess nutrients. This surplus of N can be mitigated by probiotics through improved ruminal fermentation, allowing thorough utilization of nutrients while decreasing residue in feces and urine. (Rivera & Chara, 2021; McAllister & Newbold, 2008). Probiotics, such as lactic acid bacteria (LAB), have been shown to modulate rumen fermentation patterns, potentially leading to reduced methane production. For instance, LAB supplementation can improve fermentation quality and fiber digestibility, which may indirectly influence nitrogen utilization in the rumen. Enhanced nitrogen utilization could theoretically result in reduced nitrogen excretion in manure, thereby potentially decreasing N_2O emissions during manure decomposition (Doyle *et al.* 2019). Likewise, a rise in soluble carbohydrates from effective fiber digestion generated by probiotic supplementation can augment the energy needs for microbes to form microbial protein, increasing the utilization of dietary N (McAllister & Newbold, 2008; Balvert & Lou, 2017). However, the current study had the same protein digestibility across treatments and thus reduction of N_2O emission as affected by T2 might not be because of the abovementioned mode of action of probiotics. The complexity of N metabolism and the accounting for specific N intake and digestion can be a valuable representation to prove this cause.



*Trt 1- Control; Trt 2- Basal diet + 5 ml probiotic; Trt 3- Basal diet + 10 ml probiotic; Trt 4- Basal diet + 15 ml probiotic

Figure 1. Total nitrous oxide emissions from growing dairy buffaloes fed with probiotics supplement.

Evidence is scarce regarding specific research using probiotics to abate N_2O emission; thus, the values from the current experiment were compared with greenhouse gas profiling studies in ruminants. The survey by Waldrip *et al.* (2020) reported an average of $1.75 \text{ g hd}^{-1} \text{ d}^{-1}$ N_2O emission from beef cattle farms, approximately thrice the emission observed from the current study. Estimates of N_2O emission that were close to the observed value are from the study of Mitloehner *et al.* (2009) on dairy cows amounting to $0.48 \text{ g hd}^{-1} \text{ d}^{-1}$ when fed with a mixture of grain mix, alfalfa and almond hulls at 17% CP. Stackhouse

et al. (2011) reported an average of 0.477 g hd⁻¹ d⁻¹ from Angus steers fed with TMR predominantly with steam-flaked corn and alfalfa with 13.1%, while de Melo Coelho *et al.* (2022) recounted approximately 0.628 g hd⁻¹ d⁻¹ from finishing cattle offered with 70% corn silage and 30% concentrate with an estimated CP of 15-16%. The forages offered were different from what was given to growers of the current study but the CP content of forage mixed with concentrates are relatively similar.

Carbon Dioxide Emission

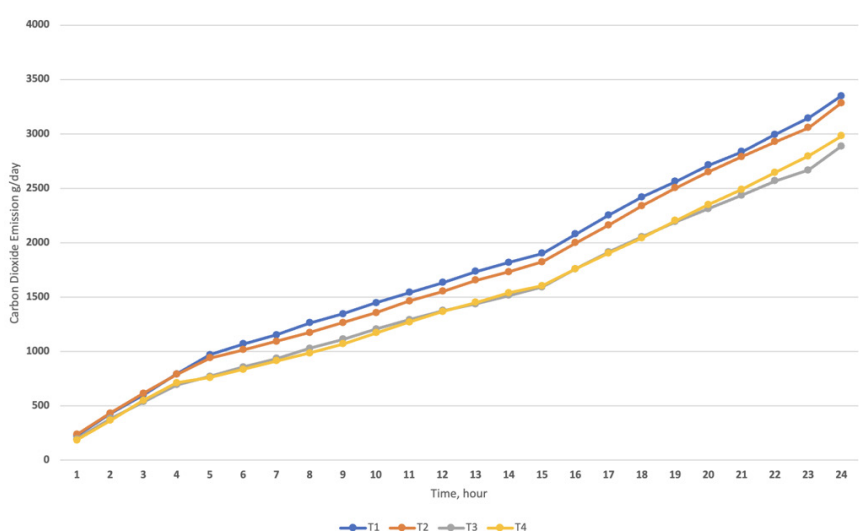
Table 5 presents the CO₂ and N₂O emissions of buffaloes given different doses of probiotics. Increasing the level of probiotics resulted in a gradual decrease in CO₂ production however, no significant differences were observed across treatments (*p-value* > 0.05). Moreover, CO₂ produced per nutrient intake and digested (DMi, OMi, CPi, NDFi, DMd, OMd, CPd, NDFd) showed similar results (*p-value* > 0.05). Figure 2 indicates the steady increase in CO₂ emissions per period within 24-hour monitoring. The expected trend was seen from the graph with T1 having the highest and T4 the lowest CO₂ emissions.

Table 5. Total carbon dioxide emissions and carbon dioxide per nutrient intake and digested of growing dairy buffalo fed with microbial supplement.

Parameters ¹	TREATMENT				SEM	P-Value
	Control	Basal Diet + 5ml probiotic	Basal Diet + 10ml probiotic	Basal Diet + 15ml probiotic		
Total CO ₂ , g	4122.50	4053.58	3924.37	3656.27	161.00	0.7861
CO ₂ g DMi kg ⁻¹	472.81	481.26	520.13	452.64	30.24	0.9059
CO ₂ g OMi kg ⁻¹	541.77	551.01	595.72	517.58	36.12	0.9142
CO ₂ g CPi kg ⁻¹	4345.26	4365.35	4573.83	4091.30	278.55	0.9566
CO ₂ g NDFi kg ⁻¹	740.88	757.23	832.34	713.71	49.88	0.8814
CO ₂ g DMd kg ⁻¹	652.33	745.99	782.67	706.98	57.19	0.8945
CO ₂ g OMd kg ⁻¹	731.65	834.41	871.24	785.57	65.43	0.9085
CO ₂ g CPd kg ⁻¹	5975.39	6818.14	6980.30	6378.38	533.73	0.9280
CO ₂ g NDFd kg ⁻¹	1018.43	1186.25	1262.07	1154.17	101.35	0.8858
CO ₂ -eq kg yr ⁻¹	1504.71	1479.56	1432.40	1334.54	58.76	0.7861

¹DMi: dry matter intake, OMi: organic matter intake, CPi: crude protein intake, NDFi: neutral detergent fiber intake, DMd: dry matter digested, OMd: organic matter digested, CPd: crude protein digested, NDFd: neutral detergent fiber digested, CO₂- eq: Carbon dioxide equivalent

Carbon dioxide is produced through cellular respiration and ruminal fermentation. Glucose is cleaved and converted to metabolites to harness energy with concomitant release of CO₂ and water in cellular respiration whereas CO₂, methane and VFAs are synthesized from the fermentation of carbohydrates in the rumen (Lee *et al.*, 2017). As proposed earlier, probiotics can lessen gas emissions through improved ruminal fermentation, allowing thorough utilization of nutrients while decreasing CO₂ output. Nevertheless, the supplementation of probiotics in the current study did not affect CO₂ emissions.



*Trt 1- Control; Trt 2- Basal diet + 5 ml probiotic; Trt 3- Basal diet + 10 ml probiotic; Trt 4- Basal diet + 15 ml probiotic

Figure 2. Total carbon dioxide emissions from growing dairy buffaloes fed with probiotics supplement.

Similar to the current study, direct-fed microbial and exogenous enzymes mixed with the diet had no significant effect on CO₂ emissions of lactating dairy cows (Oh *et al.* 2018). The CO₂ emission of dairy cows is within 11, 841- 12, 219 g hd⁻¹ d⁻¹ which is more than the figures from the current experiment as is expected because of the discrepancy in weight and physiological state of growers and lactating animals. However, the CO₂ emission obtained from Holstein bull calves offered with a plant-derived feed supplement is close to the current study with an estimated 4,841- 5,081 g hd⁻¹ d⁻¹ (Brand *et al.* 2021). When *Moringa oleifera* extract and *Saccharomyces cerevisiae* were added to the basal diet incubated in an *in vitro* trial, no significant differences were perceived in CO₂ fermentation kinetics and proportional CO₂ production (Pedraza-Hernandez *et al.*, 2019). In contrast, an *in vitro* study conducted by Direkvandi *et al.* (2021), through the application of microbial additives consisting of *S. cerevisiae*, *L. fermentum*, *L. plantarum*, and *Megasphaera elsdenii* decreased the proportional CO₂ emissions. Another *in vitro* trial tested the combination of *Sc cerevisiae* and xylanase in goat, sheep and steer inoculum and found out that irrespective of the rumen fluid source, proportional CO₂ production decreased (Vallejo-Hernandez *et al.*, 2018).

Relationship of parameters with nitrous oxide and carbon dioxide emissions

The relationship between CO₂, N₂O emissions and nutrient digestibility of growing dairy buffaloes are summarized in Table 6. All digestion coefficients had a significant positive correlation with total CO₂ emissions, while nutrient intake did not influence CO₂ emissions. A rise in these variables would lead to a concomitant increase in CO₂ production and emission. No significant relationship was observed between N₂O emissions and nutrient digestibility.

Table 6. Pearson correlation coefficients (r) between total nitrous oxide and carbon dioxide digestibility and production parameters of grower dairy buffalo fed with microbial supplement.

Parameters	Total N ₂ O (r)	Total CO ₂ (r)
Total GE Intake, MJ	-0.0560	0.2620
Apparent GE COD, %	0.0424	0.5238*
Total NDF Intake, kg	-0.0667	0.2767
Apparent NDF COD, %	0.0176	0.5009*
Total CP Intake, kg	-0.0569	0.4166
Apparent CP COD, %	0.0275	0.5152*
Total DM Intake, kg	-0.0626	0.2760
Apparent DM COD, %	0.0441	0.5603*
Total OM Intake, kg	-0.0551	0.2556
Apparent OM COD, %	0.0472	0.5152

*P < 0.05

The byproduct of cellular respiration and fiber digestion is ultimately CO₂ and an increase in available nutrients for assimilation will favor this process and thus yield more of these gases (Lee *et al.*, 2017). However, improved feed efficiency as influenced by probiotics could have supported the animal's satiety in terms of energy requirement and has the potential to decrease feed intake thereby limiting CO₂ emissions. A study conducted by Moreira *et al.* (2019) proved that probiotic supplementation in beef cattle diet improved feed efficiency and weight gain of treated groups while having a comparable intake to the control animals. It was hypothesized that nutrients were sequestered proficiently including carbon atoms and thus limits excess formation of CO₂ from normal metabolism.

CONCLUSION

Probiotic supplementation at 5 ml decreased the total N₂O (p-value <0.05) but did not affect the N₂O emission per nutrient intake and digested in growing dairy buffaloes. Total CO₂ emission and CO₂ emission per nutrient intake were also not affected by probiotic supplementation. Most of the digestion coefficients had a significant positive correlation with total CO₂ emissions (p-value <0.05), whereas N₂O emission had no relationship with these digestibility parameters. In this study, we showed that probiotics can mitigate N₂O emission of growing dairy buffaloes, while the observed functional relationship between total CO₂ emission and digestibility parameters can be a valuable input for the greenhouse gas emission inventory data in the Philippines.

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