

In Vitro* Evaluation of Rumen Methane Production in Cattle Feed Supplemented with Effective Microorganisms*Shaill Rathnayake R.M.H.^{1,2}, Nayananjalie W.A.D.^{2*}, Adikari A.M.J.B.², Mangalika U.L.P.³**¹*Postgraduate Programme, Faculty of Agriculture, Rajarata University, Mihinthale, Sri Lanka*²*Department of Animal and Food Sciences, Faculty of Agriculture,**Rajarata University of Sri Lanka, Puliyankulama, Anuradhapura, Sri Lanka*³*Department of Animal Production and Health, Getambe, Peradeniya, Sri Lanka***deepthin@agri.rjt.ac.lk***Abstract**

Effective microorganisms (EM) are a consortium of beneficial microbes that are used in livestock waste management and as a feed additive to improve animal nutrition. This study was conducted to evaluate the effect of EM supplementation on *in vitro* methane production using rumen fluid collected from dairy cows. Rumen fluid was collected from freshly slaughtered cows at a local abattoir into prewarmed vacuum flasks and immediately transported to the laboratory. The 200 mL of fluid was filtered through clean cheesecloth and transferred into prewarmed conical flasks. Each flask was assigned either 1 g of feed alone (control) or 1 g of feed supplemented with 1 mL of EM solution (treatment), with six replicates per treatment. The flasks were sealed with rubber stoppers connected to 0.5 M NaOH for gas trapping and incubated in a 39 °C water bath at 70 rpm for 24 hours. Methane volume was measured using the syringe plunge displacement method at 2,4,6,12 and, 24 hours of incubation. Data were analysed using the mixed procedure in SAS software. Results revealed that time had a significant effect on methane production ($p < 0.05$), while the overall treatment effect was not significant (Con: 16.97 ± 0.70 cm³; EM: 18.83 ± 0.70 cm³). However, the treatment-time interaction was significant ($p < 0.05$), indicating that EM affected the temporal pattern of methane production. In the first 2 hours, EM-treated samples produced a higher volume of methane (40.33 ± 1.42 cm³) than the control (27.17 ± 1.42 cm³), whereas at 24 hours later, methane production was higher in the control (18.00 ± 1.42 cm³) than in EM-treated samples (12.50 ± 1.42 cm³). These results demonstrate that EM influenced the kinetics of fermentation rather than the total cumulative methane production. The findings suggest that although EM supplementation does not significantly alter total methane production, it modifies the fermentation dynamics, influencing early and late methane production differently. This time-dependent effect shows the importance of assessing temporal patterns when evaluating feed additives for ruminants.

Keywords: *Feed additives, Gas kinetics, Methane, Microbial inoculants, Ruminant nutrition*