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Research Article
Effect of the Addition of Sodium Nitrate in a Total Mixed Ration with Fermented Tofu Waste on Methane Production from the Rumen Fluid

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Abstract

Background and Objective: This study aimed to examine the effect of sodium nitrate inclusion in a total mixed ration based on tofu waste on *in vitro* methane production. Ruminant methane production reflects livestock feed energy loss, indicating low feed energy utilization. Thus, a reduction in ruminal methane production is required to improve feed utilization and livestock productivity. **Materials and Methods:** The total mixed ration based on tofu waste was treated with the addition of sodium nitrate at levels of 0.0, 1.0, 2.5, 5.0, 7.5 and 10.0 mM. The ruminal gas production of each sample was observed *in vitro* for 48 h at 39°C. Three replicates of the samples were used. At the end of incubation, microbial protein synthesis, the volatile fatty acid (VFA) concentration and methane production were determined. The data were analysed statistically by using one-way ANOVA. **Results:** The addition of up to 10.0 mM sodium nitrate did not affect pH, total gas production, CGA, ammonia, rumen microbial synthesis and the protozoa population ($p<0.05$). Nevertheless, the addition of sodium nitrate tended to reduce methane production ($p = 0.057$). **Conclusion:** The addition of up to 10 mM sodium nitrate in a total mixed ration based on tofu waste did not affect the nature of microbial fermentation in the rumen but tended to reduce methane production.

Key words: Methane, rumen microbial protein, sodium nitrate, tofu waste, volatile fatty acid

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Methane (CH_4) that is produced by ruminants has an effect on the environment and animal productivity. Methane, which is produced in the rumen (the first stomach of a ruminant), reflects the energy lost from the consumed feed, indicating a low efficiency of feed utilization by livestock[1]. This ruminal methane production is also highly associated with environmental issues and has the effect on the greenhouse[2]. Thus, a reduction in ruminal methane production will significantly contribute to alleviating the negative effects of greenhouse gases and improve livestock productivity as well. Feed is one of the important factors that support a successful farm industry. Sufficient feed nutrient contents are required to support livestock growth and productivity. The nutrient contents of forage in the tropics are not as high as those of forage in the subtropics. Tropical forage tends to have a lower protein content and higher crude fibre content.

Low-quality forage is one obstacle to increase livestock productivity; therefore, alternative feedstuffs that have higher nutrient contents are needed and as a result, these alternative feedstuffs will support livestock needs. Industrial tofu waste has become a potential feedstuff for the animals. However, it has a high water content, which makes it perishable. Fermentation using lactic acid bacteria (LAB) can be conducted to improve the quality of tofu waste. A total mixed ration based on tofu waste is expected to complement the nutritional value of the forage that is fed, although this waste would increase production cost.

In addition to providing fermented feed, a decrease in methane production can be accomplished by directly interfering with rumen fermentation through supplementing ionophores, increasing propionate, stimulating acetogens, adding oxidative agents, increasing defaunation, adding probiotics and giving immunizations[3]. One of the alternative ways to decrease methane production is by adding the feed additive sodium nitrate ($NaNO_3$). The addition of nitrates to the feed can reduce methane production by ruminants[4].

The reduction in methane production through adding oxidizers has not been widely investigated. Therefore, through adding $NaNO_3$, as a compound that has a role as an electron acceptor from CO_2, it was expected that methane production would decrease without affecting the nature of rumen microbial fermentation.

The objective of the present study was to investigate the effect of the addition of $NaNO_3$ as an electron acceptor on ruminal pH, total gas production, methane production, VFAs, ammonia, rumen microbial protein and the rumen protozoa population.

MATERIALS AND METHODS

This research was conducted from June to August 2015 in the Nutritional Biochemistry Laboratory, Animal Nutrition and Feed Department of the Faculty of Animal Science, Universitas Gadjah Mada.

Materials: The materials that were used for this research included rumen fluid from Bali cattle with rumen cannulas; a fermented concentrate from tofu waste consisting of rice bran, pollard, *Lactobacillus plantarum*, minerals, vitamins and molasses; $NaNO_3$; chemicals to test the *in vitro* gas production and reagents for testing microbial protein based on Lowry's method.

Method

Lactic acid bacteria (LAB) production: The production of LAB as a starter was completed through the use of a medium of 40% fresh tofu waste, 30% rice bran and 30% pollard. Then, the medium was inoculated with pure *Lactobacillus plantarum* at 5% of the total medium and a total volume of 500 g of the inoculated medium was inserted into a plastic bag that would be vacuum sealed. The plastic bag, which had been filled with the medium for fermentation, was vacuum sealed by a vacuum sealer for 14 days[5].

Total mixed ration based on tofu waste: All the materials included tofu waste, rice bran, pollard, minerals, molasses and LAB starter which was already available and were fermented in silos with a total volume of 100 kg for 14 days[5].

Analysis of *in vitro* gas production: The treatments were different levels of $NaNO_3$ (0.0, 1.0, 2.5, 5.0, 7.5 and 10.0 mM) added into the medium. The treatments were analysed through an *in vitro* gas production technique, where three replicates of each treatment were analysed[6]. Rumen fluid was sampled from Bali cattle with rumen cannulas, which were fed dried rice stalks and a fermented concentrate based on tofu waste at a 70:30 ratio for one week before the rumen fluid was sampled.

The variables measured in this experiment were methane, volatile fatty acids and microbial protein from *in vitro* rumen fermentation.

After 48 h of *in vitro* rumen fermentation, the fermented gas and liquid samples were taken and analysed for the methane and volatile fatty acids produced using a chromatography gas method[7]. Microbial protein production after 48 h of *in vitro* rumen fermentation was analysed using Lowry's method[8].

Data analysis: The obtained data were analysed using one-way analysis of variance (ANOVA). The differences in the mean values were analysed by Duncan's new multiple range test[9].

RESULTS AND DISCUSSION

The production of CH_4 from *in vitro* gas production: Table 1 indicates that the addition of $NaNO_3$ up to 10 mM in a fermented concentrate based on tofu waste did not have any impact on decreasing CH_4; however, there was a tendency to decrease CH_4 by adding $NaNO_3$ at 2.5 and 5 mM compared to that of the control ($p = 0.057$). The amount of CH_4 produced in this research was approximately 16.71-25.57 mL mg^{-1} DM. This result is different from the result of a previous study, which showed that the use of nitrates in feed at 5-10 mM could be a way for the electron acceptor to decrease CH_4 production[10].

There was no decrease in CH_4 from adding $NaNO_3$ at 10 mM. This result was possibly due to the limited amount of feed, which included concentrates without additional forage. The use of nitrates can be effective in lowering the production of CH_4. Feeding a high proportion of forage and nitrates can reduce methane gas production from 54.71-35.91% and a feed with a high proportion of concentrate can reduce the production of methane from 60.24-53.03%[10].

Volatile fatty acid: This study indicates that the addition of $NaNO_3$ did not have any impact on the average levels of VFAs (acetate, propionate and butyrate), total VFAs, or ratio of C_2:C_3 as a result of *in vitro* rumen gas production for 48 h (Table 2). The totals levels of VFAs, which were the result of *in vitro* fermentation gas production from a concentrate with fermented tofu, ranged from 108.17-121.04 mM. There was no difference in the levels of total VFAs between the sample with added $NaNO_3$ and in the levels of total VFA in control. This result is in line with a previous study[11] which showed that the average level of total of VFAs from the *in vitro* fermentation of alfalfa hay and a concentrate (50:50) without the addition of nitrates was equal to 92.8 mM and that with the addition of 5 mM of nitrates was 90.1 mM. The addition of nitrates in this study did not significantly affect the levels of total VFAs[11].

Table 1: *In vitro* gas production (CH_4) by adding several levels of $NaNO_3$ to feed for 48 h of incubation (mL mg^{-1} DM)

	$NaNO_3$ level (mM)					
	0	1	2.5	5	7.5	10
1	20.26	25.70	21.29	20.36	24.66	25.78
2	20.46	28.10	8.95	13.74	26.03	18.95
3	21.32	21.62	20.79	16.04	26.01	18.74
Average	20.68±0.56	25.14±3.27	17.01±6.98	16.71±3.36	25.57±0.78	21.16±4.00

Table 2: VFA production after 48 h of in vitro rumen fermentation with the addition of several levels of $NaNO_3$

		Addition of $NaNO_3$ (mM)					
Fermented	Parameter repetition	0	1	2.5	5	7.5	10
Total	1	121.68	109.76	115.47	104.46	113.79	116.13
VFAs	2	117.74	102.19	129.43	123.22	112.8	110.52
	3	123.69	112.56	111.07	114.05	118.47	114.52
	Average	121.04±3.03	108.17±5.36	118.66±9.58	113.91±9.38	115.02±3.02	113.72±2.89
Acetate	1	84.81	75.87	80.71	72.66	79.17	83.86
	2	83.16	71.59	91.45	85.84	82.45	75.78
	3	85.55	79.15	78.07	80.82	81.58	78.72
	Average	84.51±1.22	75.54±3.80	83.41±7.08	79.77±6.65	81.06±1.69	79.45±4.09
Propionate	1	27.66	24.41	25.69	24.13	25.14	26.76
	2	26.67	22.25	27.07	26.77	25.32	26.13
	3	27.18	23.98	23.57	23.62	26.74	25.13
	Average	27.17±0.50	23.54±1.14	25.44±1.76	24.84±1.69	25.73±0.87	26.00±0.82
Butyrate	1	9.20	9.49	9.08	7.67	9.49	5.51
	2	7.90	8.35	10.91	10.62	5.03	8.61
	3	10.96	9.43	9.44	9.60	10.15	10.67
	Average	9.35±1.53	9.09±0.64	9.81±0.96	9.30±1.50	8.22±2.78	8.26±2.59
Acetate: propionate	1	3.07	3.11	3.14	3.01	3.15	3.13
	2	3.12	3.22	3.38	3.21	3.26	2.90
	3	3.15	3.30	3.31	3.42	3.05	3.13
	Average	3.11±0.04	3.21±0.10	3.28±0.12	3.21±0.21	3.15±0.10	3.06±0.13

Table 3: Microbial protein production from rumen fermentation by an in vitro gas technique after 48 h of incubation with the addition of several levels of $NaNO_3$ (mg/100 mL)

	$NaNO_3$ level (mM)					
	0	1	2.5	5	7.5	10
1	0.72	0.84	0.74	0.61	0.73	0.8
2	0.7	0.79	0.73	0.66	0.64	0.9
3	0.64	0.88	0.81	0.92	0.84	0.85
Average	0.69±0.04	0.84±0.04	0.76±0.04	0.73±0.17	0.74±0.10	0.85±0.05

The average proportion of acetate and propionate observed in this study was higher than the proportion of acetate and propionate in a previous study conducted by Bergman *et al.*[12]. These authors found that the proportion of acetate, propionate and butyrate ranged from 63-70%, 17-21% and 11-16%, respectively, in rumen fluid from sheep that were continuously fed a concentrate containing 89% DM, 10.6% CP and 4.23 cal g^{-1} [12]. The results of the present study are different from those of Bergman *et al.*[12]. because the cow rumen fluid used in the this study was from cows that were previously fed straw and a concentrate with fermented tofu waste and as a result, the fermentation liquid could have contained greater amounts of fibre and soluble carbohydrates than the fermentation liquid from Bergman *et al.*[12]. Glucose increases propionate production, while feed containing a large amount of fibre can increase acetate production.

The VFA levels in this study were correlated with the production of CH_4, which can be seen in the Table 2. The amount of VFA, which was not significantly different among the treatments, showed a pattern of carbohydrate fermentation which indicated that rumen fermentation functioned normally. When H_2 is more effectively eliminated, the concentration of NADH in the cell will be low and the concentration of NAD^+ will increase and triggering the formation of acetate and propionate, which will support a more rapid fermentation of carbohydrates[13].

Microbial protein production: The addition of different levels of $NaNO_3$ in a fermented concentrate based on tofu waste did not significantly increase microbial protein synthesis ($p<0.05$) (Table 3). The microbial protein level in the sample without added $NaNO_3$ was 0.69 mg mL^{-1} and with the addition of $NaNO_3$ up to 10 mM, the level of microbial protein was equal to 0.85 mg mL^{-1}.

The microbial protein in rumen is a main source of amino acids for the ruminants. Microbial protein contributes 1/3 to ½ of the total amount of protein used by the ruminants. Organic materials, which are from carbohydrates, are the main energy sources for microbial protein synthesis. The synthesis of microbial protein is affected by the feed that the animal consumes. Animals that consume a single type of forage protein have a microbial protein content of 13.0 g MCP/100 g from total digestible nutrients (TDN); a single mixed concentrate, 13.2 g and mixed forage-concentrate, 17.6 g MCP/100 g[14].

The addition of $NaNO_3$ was expected to provide an additional supply of NH_3 from the conversion of nitrates to nitrites and then to ammonia. Protein, which is degraded into amino acids, will be deaminated and produce ammonia, which functions as the main nitrogen source that is vital for microbial protein synthesis. Approximately 82% of microbial species are capable of using ammonia as a nitrogen source[15].

The present study suggests that addition of $NaNO_3$ up to 10 mM as an electron acceptor in feed tended to reduce methane production, as shown with an *in vitro* method. Further studies on $NaNO_3$ as an electron acceptor should include the addition of various forages to the ration, should be conducted on a larger scale and should be performed *in vitro* and *in vivo*.

CONCLUSION

Based on this research, it can be concluded that the addition of $NaNO_3$ up to 10 mM, as the alternative electron acceptor, in a concentrate based on fermented tofu waste did not influence the rumen fermentation conditions; however, the addition of $NaNO_3$ tended to decrease the production of CH_4 *in vitro*.

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