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Ratna Puspita Haryati, Anuraga Jayanegara, Erika Budiarti Laconi, Muhammad Ridla, and Pipih Suptijah

Evaluation of Chitin and Chitosan from Insect as Feed Additives to Mitigate Ruminal Methane Emission

Ratna Puspita Haryati[1, a)], Anuraga Jayanegara[2, b)], Erika Budiarti Laconi[2, c)], Muhammad Ridla[2, d)] and Pipih Suptijah[3, e)]

[1]*Graduate School of Nutrition and Feed Science, Faculty of Animal Science, Bogor Agricultural University, Jalan Agatis Kampus IPB Darmaga, Kec. Dramaga, Bogor, Jawa Barat 16680, Indonesia*
[2]*Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Jalan Agatis Kampus IPB Darmaga, Kec. Dramaga, Bogor, Jawa Barat 16680, Indonesia*
[3]*Department of Aquatic Products Technology, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Jalan Agatis Kampus IPB Darmaga, Kec. Dramaga, Bogor, Jawa Barat 16680, Indonesia*

[b)]Corresponding author: anuraga.jayanegara@gmail.com
[a)]ratna_iethaa@yahoo.com
[c)]elaconi@yahoo.com
[d)]hmridla@yahoo.com
[e)]suptijah@yahoo.com

Abstract. Greenhouse gases are produced through microbial activity in the digestive tract and feces of ruminants. The main gas is in the form of CH_4 which has an impact 25 times more than carbon dioxide to retain heat. Efforts to reduce methane emission have been carried out through feed and nutritional approaches. Natural compounds that potentially can be used are chitin and chitosan which can be found on the exoskeleton of the insects. This experiment aimed to investigate the effect of chitin and chitosan extracts from black soldier fly (BSF) on methane emission and rumen fermentation profile *in vitro*. This study consisted of two phases, the first was chitin extraction from insect and the second was chitin and chitosan evaluation by using an *in vitro* rumen fermentation method. Extraction of chitin was obtained through three steps, i.e., delipidation, deproteination, and demineralization. Conversion from chitin to chitosan was performed by deacetylation. Parameters measured in the *in vitro* evaluation were gas production kinetics, pH and methane emission. Extracted chitin and chitosan were used as feed additives and able to positively modulate rumen fermentation and mitigate methane emission.

INTRODUCTION

Greenhouse gases are produced through digestive activity and feces of ruminants. This gas is in the form of CH_4 which has an impact 25 times more dangerous than carbon dioxide [6]. The amount of methane emissions in the atmosphere is around 63% derived from anthropogenic emissions. Methane gas is the biggest contributor to gas in the atmosphere after carbon dioxide. Livestock largely contributes to methane emission. A large amount of methane originating from the livestock sector encourages the emergence of innovation in reducing methane gas through the feed. Generally, the fibrous feed will produce acetic acid and CH_4 higher than feed from concentrate based. Increased fiber levels in the diet affect the ratio of propionate acetate and higher CH_4 production [8]. The increase in CH_4 itself affects global warming which is an environmental problem.

Several alternatives for reducing methane emission have been found such as tannins, antibiotics, methane inhibitors, defaunation agents, and feed additives [9]. Other compounds that can potentially reduce methane are chitin and chitosan. Chitin and chitosan can be extracted from black soldier fly (BSF, *Heredia illucens*) insects. The

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composition of chitin in the BSF is 8.7% DM [5]. Chitosan was found to be more reactive than chitin because it has a higher degree of deacetylation. This experiment aimed to investigate the effects of chitin and chitosan extracts from BSF on methane emission and rumen fermentation profile in vitro.

EXPERIMENTAL DETAILS

Experimental Ration and Composition Determination

The ration consisted of *Setaria splendida* grass and concentrate. The grass was taken from the Laboratory of Agrostology Faculty of Animal Science, Bogor Agricultural University while for the concentrate was purchased from a commercial source. The ration was determined for proximate [1] and Van Soest fiber fraction (Table 1). There were five treatments for this experiment. The basal ration was grass: concentrate 60:40 w/w. The treatments ware as follow; T1 (control ration in the form of Setaria splendia + concentrate 60:40, T2 (control ration + chitin BSF 1%), T3 (control ration + chitin BSF 2%), T4 (control ration + chitosan BSF 1%), and T5 (control + chitosan BSF 2%).

TABLE 1. Chemical composition of *Setaria splendida* grass and concentrate

Composition	*Setaria splendida*	Concentrate
Crude protein	14.54	11.08
Crude fibre	32.29	32.19
Crude fat	3.9	2.68
Ash	20.21	24.41
NDF	71.06	79.89
ADF	45.04	55.65
NDICP	51.31	30.69
ADICP	21.77	46.11
NFE	29.03	29.63

NDF, neutral detergent fiber: ADF, acid detergent fiber: NDIC, neutral detergent insoluble crude protein: ADICP, acid detergent insoluble crude protein: NFE, nitrogen free extract.

In Vitro Rumen Fermentation

All the experimental dietary treatments were incubated in an in vitro rumen fermentation system. The rumen, ration, and McDougall buffer were incubated at 39 °C under anaerobic condition. Gas production was observed at regular time point interval, i.e., at 2, 4, 6, 8, 10, 12, 24, 48, and 72 h after the incubation. Methane collection was carried out during the first 24 h. The methane concentration was measured using the Shimadzu 8A GC with a flame ionization detector (FID).

Statistical Analysis

The environmental design used in this study was a randomized block design. The data obtained were then analyzed using analysis of variance according to [11]. Furthermore, if the results of the analysis were significantly different at least at ($P < 0.05$), then the analysis was continued using the Duncan Multiple Range Test (DMRT) test.

RESULTS AND DISCUSSION

The rumen pH value can undergo changes along with rumen microbial activity. If found high rumen activity will be caused by pH decreases. This change is influenced by rumen microbial growth and its activity produces VFA and NH_3 [7]. The initial rumen pH before treatment has a pH value of around 7-7.65. This value is very high compared to the optimal pH for archaea microbial growth namely 6.7-7.4. After incubation for 24 h pH changes occur. These changes can be seen in Table 2. This study also measured gas production for 24 and 72 h in which the results are presented in Table 2.

The addition of feed additives in the form of chitin and chitosan show a significant effect on total gas production. There is a decrease with the addition of feed additive chitin and chitosan. The value obtained in the addition of 2%

chitosan shows the lowest value compared to other treatments. Followed by chitosan 1%, chitin 2%, control and finally chitin 1%. The addition of chitosan had an effect on the decrease in rumen bacteria [2]. This can cause a decrease in digestibility of dry matter or organic matter after being given an additional feed of chitin and chitosan. Decreasing digestibility will cause a decrease in gas production by livestock.

TABLE 2. The pH value of rumen fluid and gas production of the experimental treatments

Treatment	Parameter		
	pH	**Gas production in 24 h (mL)**	**Gas production in 72 h (mL)**
T1	7.10 ± 0.12	43.64ab	76.83ab
T2	7.11 ± 0.10	46.13b	78.69b
T3	7.07 ± 0.12	41.06a	74.00ab
T4	7.00 ± 0.15	41.25a	74.94ab
T5	7.05 ± 0.16	39.88a	72.88a

Superscript letters that are not the same on the same line show significantly different ($P < 0.05$).

The addition of chitosan also caused changes in the composition of acetate and propionate [2]. The content of acetate will decrease while for propionate it will increase. Periodic decreases in acetate content will cause changes in hydrogen production that main substrate for methane production. The production of methane gas in ruminants is formed from the microbial activity of the rumen during feed fermentation. Some of the results of this process are carbon dioxide, hydrogen, and microbial mass. Methane production can be seen from the total gas per unit of organic matter digested after 24 h incubation. Addition of chitin and chitosan feed additives at level 1 and 2% didn't have significant value ($P > 0.05$). After gas collection for 24 h, then the concentration of CH_4 is measured and obtained as follows in Fig. 1.

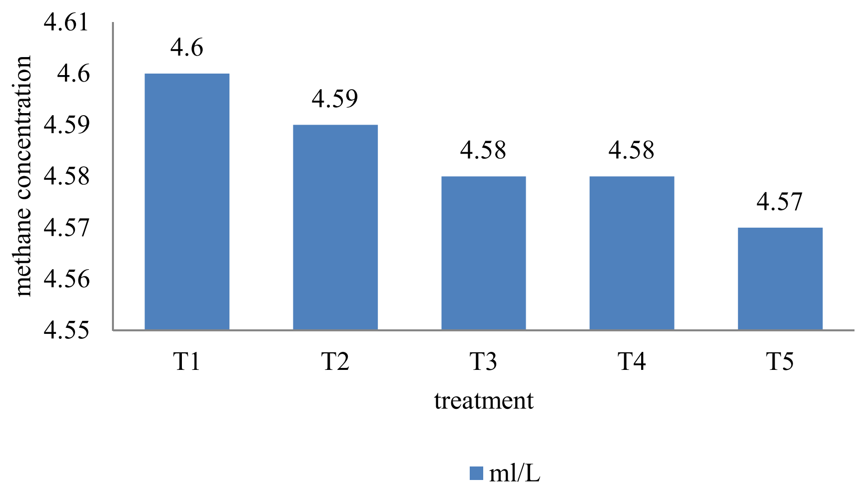

FIGURE 1. Methane concentration of the experimental treatments

The results of the research in each treatment produced an average of 4.58 ml. The addition of chitosan flour with a level of 2% showed the lowest methane gas production compared to other treatments. It can be identified that chitin and chitosan affect the proportion of acetate and propionate in the rumen. If the acetate in the rumen decreases, the production of H_2 and CO_2 will decrease. This causes the production of methane gas to decrease. The addition of chitosan as supplementation to feed caused a decrease in methanogenic bacteria [2]. Kong *et al.* stated that chitosan has a role in antimicrobial materials. Chitosan can increase the proportion of propionate [4].

Chitosan has a higher activity compared to chitin. This is due to the high degree of acetylation. Chitosan added to treatments 4 and 5 has a deacetylation degree of 61.63%. This value is greater than the degree of chitin deacetylation that reaches 33.41%. The higher the level is given in the ration, the higher the influence of chitin and chitosan. Through the treatment, there was no significant difference ($P > 0.05$), but the value of the mean gas content after being given 2% chitosan treatment has a lower value compared to other treatments. The 2% chitin level can only surpass 1% chitin level and control treatment.

The addition of chitosan has an influence in influencing the fermentation pattern of acetate as a hydrogen producer [8]. Hydrogen is a methane-producing substrate so that with a decrease in hydrogen the production of methane will decrease. These results cannot be guessed that the most potential impact as a decrease in methane gas is chitosan. This is also supported by the characteristics of the degree of deacetylation of chitosan formed. Increased levels of chitin or chitosan administration have an influence on the interaction between chitosan glucosamine and bacterial cell walls [3]. This interaction will cause bacteria to experience changes in cell wall permeability [10]. Changes in the permeability of bacterial cell walls will cause bacteria to experience a decline in population.

SUMMARY

Adding chitin and chitosan with level 1 and 2% have a significant impact on gas production and methane production. The value of feed additives is able to reduce gas production but has an impact on decreasing digestibility of organic matter and dry matter. Chitin and chitosan can be used as methane-reducing agents because of they able to give an effect in antimicrobial and influence the fermentation process.

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