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# Enteric Methane Emissions and Rumen Fermentation Profile Treated by Dietary Chitosan: A Meta-Analysis of *In Vitro* Experiments

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## ABSTRACT

**Chitosan is a natural compound obtained from deacetylation of chitin, which is a biopolymer present in the exoskeleton of crustaceans such as crabs and shrimp. The present study aimed to perform a meta-analysis from published studies regarding the effects of chitosan on methane emission and rumen fermentation profile of *in vitro* batch culture experiments. A total of 41 studies from 12 articles were integrated into a database. Parameters included were gas production, methane emission, rumen fermentation characteristics, microbial population, nutrient digestibility, and fatty acid profile. Data were analyzed according to mixed model methodology in which different studies were treated as random effects and chitosan addition levels were treated as fixed effects. Results showed that chitosan addition was able to reduce enteric methane emissions ($p<0.001$). Such methane decrease was accompanied by a decline in the protozoa population ($p<0.05$) and a tendency of methanogen reduction ($p<0.1$). The increasing chitosan level was associated with a decrease in total VFA and ammonia concentrations (both at $p<0.001$). Chitosan addition decreased acetate proportion ($p<0.001$) while elevated propionate proportion ($p<0.001$). Chitosan was associated with an increase of dry matter digestibility, crude protein digestibility, and neutral detergent fiber digestibility ($p<0.001$). Chitosan increased concentrations of $C_{18:3n3}$ ($p<0.05$), conjugated linoleic acid ($p<0.01$) and polyunsaturated fatty acids ($p<0.01$) while decreased concentration of saturated fatty acids ($p<0.001$). It can be concluded that chitosan addition can mitigate enteric methane emission and alters rumen fermentation profiles in a favorable direction.**

***Keywords: chitosan; additive; methanogenesis; rumen; meta-analysis***

## INTRODUCTION

Methane is a greenhouse gas that has more significant impact than carbon dioxide with regard to its ability to retain heat. Methane production from ruminant livestock is originated from synthesis during fermentation of feed in the rumen, which responsible for about 5% to 7% of feed gross energy (Hristov *et al.*, 2013). Thus, an approach of inhibiting $CH_4$ production in ruminants is considered to provide efficient use of feed energy, economic benefits, and reduce the effects of global greenhouse gases (Kaharabata *et al.*, 2015). Inhibition of $CH_4$ production in ruminants can be done by manipulating the rumen ecosystem. Several types of natural compounds that have antimicrobial properties can be used to manipulate the rumen microbial ecosystem. Some chemical feed additives, antibiotics, methane inhibitors, defaunation agents, and extracts from plants have been shown to increase rumen metabolism and growth of ruminant animals (Patra & Saxena, 2011; Jayanegara *et al.*, 2018a). However, chemical feed additives have been concerned about the presence of chemical residues in livestock products, the development of bacterial resistance to antibiotics and excessive toxicity, and the cost of some plant extracts that limited their use in ruminant diets (Wina *et al.*, 2005). As a result, ruminant nutrition scientists are still actively looking for alternative feed additives that can improve rumen function. One type of natural compound that has antimicrobial properties and has the potential to be used to manipulate rumen microbial ecosystems is chitosan.

Chitosan may be obtained from deacetylation of chitin, which is a biopolymer present in the exoskeleton of crustaceans such as crabs and shrimp. Chitosan is very interesting to study because it can change the profile of volatile fatty acids (VFA) by increasing propionate concentration ($C_3$) and thereby reducing the production of $CH_4$ (Haryati *et al.*, 2019). Furthermore, the reduction in $CH_4$ is related to the degree of deacetylation found in chitosan, which can modify the cell wall permeability of

methanogenic archaea (Zanferari *et al*., 2018). Previous studies have shown that the addition of chitosan can inhibit the synthesis of $CH_4$ *in vitro* when it is added to substrates at high concentrations (Goiri *et al*., 2009a). Furthermore, the addition of chitosan source from black soldier flies at a concentration of 2% of the substrate results in a sharp reduction effect on $CH_4$ emissions (Haryati *et al*., 2019). Although there have been a number of studies evaluating chitosan effects on rumen fermentation, to date, there is no study attempting to quantitatively summarize the effects by employing a meta-analysis approach.

This present study, therefore, aimed to perform a meta-analysis from published experiments regarding the effect of chitosan on methane emissions and rumen fermentation using *in vitro* batch culture experiments. All related parameters such as total gas, methane production, *in vitro* digestibility, rumen fermentation characteristics, rumen microbial profile, carboxymethyl cellulase (CMCase) enzyme activity, and rumen fatty acid profile were also evaluated to comprehensively assess the effect of chitosan on the rumen *in vitro* batch culture experiments.

## MATERIALS AND METHODS

### Database Development

The database was developed from studies reporting the use of chitosan to reduce enteric methane emissions from ruminants. Inclusion criteria for an article entered into the database were: (1) the article was published in English, (2) the concentration of chitosan in diet and $CH_4$ emissions were specified, and (3) the experiment was carried out by using *in vitro* batch culture systems with cattle or sheep as rumen fluid donors. A total of 41 studies from 12 articles were finally integrated into the database, as described in Table 1.

The parameters tabulated in this study were total gas production, methane production, *$H_2S$ production,* rumen fermentation characteristics, rumen microbial profile, CMCase enzyme activity, *in vitro* digestibility, and rumen fatty acid profile. The *in vitro* digestibility parameters were dry matter (DM) digestibility, organic matter (OM) digestibility, crude protein (CP) digestibility, and neutral detergent fiber (NDF) digestibility. The characteristics of rumen fermentation *in vitro* included

Table 1. *In vitro* batch culture experiments (Expt) included in the meta-analysis of the effect of chitosan levels (g/kg DM) on rumen fermentation and methane emissions

| Expt no. | Reference | Basal feed | Chitosan source | Deacetylation degree (%) | Chitosan level (g/kg DM) | Gas sampling* (h) | $CH_4$ method |
|---|---|---|---|---|---|---|---|
| 1 | (Goiri *et al*., 2009a) | Maize silage | Biolog, S:A | 75 | 0 and 0.75 | 12 | GC |
| | | | Biolog, S:A | 85 | | | |
| | | | Biolog, S:A | 85 | | | |
| | | | Biolog, S:A | 85 | | | |
| | | | FMC Biopolymer As | 75-90 | | | |
| | | | Trades S.A. | >95 | | | |
| 2 | (Goiri *et al*., 2009b) | Alfalfa hay and concentrate (80:20,50:50, and 20:80) | Biolog, S:A | 75 | 0-1.5 | 12 | GC |
| | | | Biolog, S:A | 85 | | | |
| | | | Trades S.A. | >95 | | | |
| 3 | (Goiri & Oregui, 2014) | Alfalfa hay and concentrate (50:50) | Trades S.A. | >95 | 0-5.96 | 12 | GC |
| 4 | (Li *et al*., 2013) | Alfalfa hay and concentrate (80:20,50:50 and 20:80) | Sigma-Aldrich, USA (Shrimp shell) | ≥75 | 0-1 | 24 | GC |
| 5 | (Wencelová *et al*., 2013) | Meadow hay and barley grain (80:20 and 50:50) | Sigma-Aldrich Co., St. Louis, MO, USA | Not specified | 0-0.1 | 24 | GC |
| | | Maize silage | | | | | |
| 6 | (Henry *et al*., 2015) | Concentrate | Pharma Nutrients, Inc., Lake Forest, IL | 90 | 0-10 | 24 | GC |
| 7 | (Gandra *et al*., 2016) | Sugarcane silage | Polymar Industria, Ceara, Brazil | 95 | 0 and 36.36 | Not specified | Not specified |
| 8 | (Belanche *et al*., 2016a) | Mixed diet | Insoluble chitosan and soluble chitosan | 80 | 0-2 | 24 | GC |
| | | | Nitta Gelatin India Ltd, Cocin, Kerala, India | >85 | | | |
| 9 | (Gandra *et al*., 2018) | Soybean whole-plant silage | Polymar Industria e Cia. Imp. And Exp. LTDA, Fortaleza, Brazil | 95 | 0 and 14.62 | Not specified | Not specified |
| 10 | (Haryati *et al*., 2019) | Setaria splendida grass and concentrate | Black soldier fly (Heredia illucens) | >61 | 0-20 | 24 | GC |
| 11 | (Pereira *et al*., 2019) | Hay forage and concentrate (100:0, 50:50, 40:60, and 20:80) | Not specified | >85 | 0 and 0.90 | Not specified | Not specified |
| 12 | (Seankamsorn *et al*., 2019) | Total mixed ration | Shrimp shell | 88 | 0-20 | 4 | GC |

Note: GC=gas chromatograph; DM=dry matter. *= Time of incubation when the gas was sampled for CH4 measurement.

were pH, total volatile fatty acids, the molar percentage of acetic ($C_2$), propionic ($C_3$), butyric ($C_4$), isobutyric (*iso*-$C_4$), valeric ($C_5$), isovaleric (*iso*-C5), and caproic ($C_6$) acids in total VFA, the ratio of $C_2$ to $C_3$, branch-chained volatile fatty acids (BCVFA) molar proportions, and total VFA to truly degraded substrate (TVFA:TDS) and ammonia concentration ($NH_3$). The *in vitro* rumen microbial parameters included were total bacteria, protozoa, *Fibrobacter succinogenes*, methanogen, and general anaerobic fungi. The fatty acid profile parameters included were $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{18:2n6}$, CLA, $C_{18:3n3}$, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA).

### Statistical Analysis

A meta-analysis of data was performed by using mixed model methodology according to St-Pierre (2001), in which different studies in the database were treated as random effects whereas chitosan addition levels in diets were treated as fixed effects. The number of publications included in the database reflected the population of such an *in vitro* batch study on chitosan addition from all periods. The mixed model procedure was employed with the following model:

$$Y_{ij} = B_0 + B_1X_{ij} + s_i + e_{ij}$$

where $Y_{ij}$ was the dependent variable, $B_0$ was overall intercept across all experiments (fixed effect), $B_1$ was linear regression coefficient of Y on X (fixed effect), $X_{ij}$ was the value of the continuous predictor variable (chitosan addition level), $s_i$ was random effect of experiment i, and $e_{ij}$ was the unexplained residual error. The variable of the experiment was declared in the class statement as it did not contain any quantitative information. Besides, the regression equations were also presented with p-value and root mean square error (RMSE). The statistical analysis was performed in SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA) by using mixed procedure (PROC MIXED).

## RESULTS

### Total Gas, Methane, and $H_2S$ Production

The effects of chitosan addition on total gas, methane, and $H_2S$ production *in vitro* batch culture study are shown in Table 2. An increase in the chitosan addition level was associated with a decrease in total gas production (p<0.001). Further, chitosan addition decreased enteric $CH_4$ emissions, both when expressed as $CH_4$/day and $CH_4$/DOM (p<0.001). However, increasing the chitosan addition level did not alter $H_2S$ production.

### Rumen Fermentation, Microbial Population, and CMCase Activity

The effects of chitosan addition on rumen fermentation, microbial population, and CMCase activity in the *in vitro* batch culture study are presented in Table 3. The addition of chitosan increased rumen pH (p<0.001) but decreased total VFA concentration (p<0.001). Rumen $NH_3$ concentration decreased due to chitosan addition (p<0.001). Concerning VFA composition, the proportions of $C_2$ and $C_4$ decreased due to the addition of chitosan (p<0.001). Similarly, the ratio of $C_2$ to $C_3$ and BCVFA decreased due to chitosan addition (p<0.001). On the contrary, the proportions of $C_3$, *iso*-$C_4$, $C_5$, and *iso*-$C_5$ increased (p<0.001) due to the addition of chitosan, but $C_6$ was unchanged. Chitosan addition resulted in an increase of TVFA:TDS ratio (p<0.001). The addition of chitosan reduced the protozoa population (p<0.05) but increased the total bacteria (p<0.01). Further, the addition of chitosan tended to reduce archaea methanogen (p<0.1) but did not change the populations of *Fibrobacter succinogenes* and anaerobic fungi. The addition of chitosan decreased CMCase enzyme activity (p<0.05).

### *In Vitro* Digestibility

The effect of chitosan addition on nutrient digestibility in the *in vitro* batch culture study is shown in Table 4. The increasing level of chitosan was associated with increasing dry matter digestibility (DMD) (P<0.001), crude protein digestibility (CPD) (P<0.01), and neutral detergent fiber digestibility (NDFD) (P<0.001).

### Rumen Fatty Acid Profile

The effect of chitosan addition on rumen fatty acid profile is presented in Table 5. The addition of chitosan was associated with decreasing concentrations of C14:0, C15:0, C16:0, C17:0, C18:0, and lower saturated fatty acid (SFA) (p<0.01), but increasing concentrations of C18:2n6 and C18:3n3 (p<0.05). Concentrations of *cis*9, *trans*11 C18:2, the main isomer of conjugated linoleic acid (CLA) in the rumen, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) increased with increasing level of chitosan addition (p<0.01).

Table 2. Gas production and enteric methane ($CH_4$) emission of *in vitro* batch culture study treated by chitosan addition (in g/kg DM)

| Response parameter | Unit | n | Parameter estimates | | | | Model statistics | |
|---|---|---|---|---|---|---|---|---|
| | | | Intercept | SE intercept | Slope | SE slope | p-Value | RMSE |
| Gas production | mL/g DM | 42 | 199.1 | 15.2 | -0.657 | 0.695 | <.001 | 28.9 |
| $CH_4$ | mmol/d | 65 | 1.03 | 0.13 | -0.072 | 0.017 | <.001 | 0.17 |
| $CH_4$ | mmol/g DOM | 47 | 3.58 | 0.41 | -0.050 | 0.055 | <.001 | 1.76 |
| $H_2S$ | µmol/d/g DM | 6 | 5.55 | 3.72 | 0.121 | 0.046 | 0.376 | 0.79 |

Note: DM= dry matter; DOM= digested organic matter; n= number of treatment; RMSE= residual mean square error; SE= standard error.

Table 3. Rumen fermentation, microbial population, and enzyme CMCase activity in the *in vitro* batch culture study treated by chitosan addition (in g/kg DM)

| Response parameter | Unit | n | Parameter estimates | | | | Model statistics | |
|---|---|---|---|---|---|---|---|---|
| | | | Intercept | SE intercept | Slope | SE slope | p-Value | RMSE |
| pH | | 82 | 6.40 | 0.05 | 0.002 | 0.004 | <.001 | 0.12 |
| $NH_3$ | mg/dL | 82 | 25.2 | 1.43 | -0.044 | 0.243 | <.001 | 8.24 |
| Total VFA | mmol/L | 89 | 67.8 | 3.19 | -0.228 | 0.366 | <.001 | 7.49 |
| $C_2$ | % | 92 | 62.7 | 0.98 | -0.024 | 0.071 | <.001 | 2.29 |
| $C_3$ | % | 92 | 20.4 | 0.68 | 0.304 | 0.155 | <.001 | 5.42 |
| $C_4$ | % | 94 | 11.5 | 0.47 | -0.199 | 0.086 | <.001 | 2.97 |
| iso-$C_4$ | % | 25 | 0.95 | 0.16 | 0.001 | 0.004 | <.001 | 0.07 |
| $C_5$ | % | 27 | 2.13 | 0.17 | 0.016 | 0.009 | <.001 | 0.17 |
| iso-$C_5$ | % | 25 | 1.86 | 0.33 | 0.003 | 0.011 | <.001 | 0.20 |
| $C_6$ | % | 6 | 0.20 | 0.22 | 0.004 | 0.002 | 0.527 | 0.04 |
| $C_2$:$C_3$ | | 92 | 3.23 | 0.14 | -0.041 | 0.024 | <.001 | 0.81 |
| BCVFA | | 25 | 3.39 | 0.44 | -0.314 | 0.082 | <.001 | 0.87 |
| TVFA:TDS | mmol/g DM | 36 | 8.75 | 0.53 | 1.80 | 0.227 | <.001 | 1.33 |
| Bacteria | $\times 10^{10}$/g | 12 | 0.98 | 0.06 | 0.018 | 0.050 | 0.004 | 0.13 |
| Methanogen | $\times 10^3$/g | 12 | 3.86 | 1.12 | -2.76 | 1.475 | 0.075 | 3.81 |
| *Fibrobacter succinogenes* | $\times 10^3$/g | 12 | 2.26 | 1.50 | 6.20 | 1.849 | 0.272 | 4.77 |
| Anaerobic fungi | $\times 10^3$/g | 12 | 1.86 | 0.84 | 0.014 | 0.303 | 0.158 | 0.78 |
| Protozoa | $\times 10^2$/g | 12 | 4.96 | 0.87 | -0.636 | 0.821 | 0.030 | 2.12 |
| CMCase | U/mL | 12 | 0.56 | 0.10 | -0.107 | 0.033 | 0.030 | 0.09 |

Note: $C_2$= acetate; $C_3$= propionate; $C_4$= butyrate; $C_5$= valerate; $C_6$= caproate; $NH_3$= ammonia; DM= dry matter; n= number of treatment; BCVFA= branch-chain volatile fatty acids; TVAS:TDS= total VFA-to-truly degraded substrate rasio; RMSE= residual mean square error; SE= standard error.

Table 4. Nutrient digestibility in the *in vitro* batch culture study treated by chitosan addition (in g/kg DM)

| Response parameter | Unit | n | Parameter estimates | | | | Model statistics | |
|---|---|---|---|---|---|---|---|---|
| | | | Intercept | SE intercept | Slope | SE slope | p-Value | RMSE |
| DMD | g/kg | 51 | 651 | 24.2 | 0.966 | 0.971 | <.001 | 71.6 |
| CPD | g/kg | 10 | 503 | 95.2 | 3.60 | 2.999 | 0.006 | 98.7 |
| NDFD | g/kg | 15 | 601 | 15.0 | 1.98 | 0.955 | <.001 | 73.9 |

Note: DMD= dry matter digestibility; OMD= organic matter digestibility; CPD= crude protein digestibility; NDFD= neutral detergent fiber digestibility; n= number of treatment; SE= standard error; RMSE= residual mean square error.

## DISCUSSION

The *in vitro* rumen fermentation process produces total gas in the form of $CO_2$, $CH_4$, and small amounts of $H_2$, $N_2$, and $O_2$. Total gas is produced from degradation and fermentation of substrate through the action of rumen microbes. Among the macromolecules, carbohydrate is the primary nutrient that contributes significantly to total gas production as compared to protein (Jayanegara *et al*., 2018b). In this study, the addition of chitosan can reduce total gas production and methane emission, but it has no effect on $H_2S$ production. This study was in agreement with previous research which reported that increasing level of chitosan was associated with a decrease in total gas production (Li *et al*., 2013; Wencelová *et al*., 2013; Henry *et al*., 2015; Haryati *et al*., 2019), but different from other reports stating that the addition of chitosan level did not affect the accumulation of gas production in an *in vitro* batch culture (Seankamsorn *et al*., 2019; Belanche *et al*., 2016a). A number of studies observed that dietary chitosan could reduce methane emission in the *in vitro* rumen fermentation system (Belanche *et al*., 2016a; Goiri *et al*., 2009a; Goiri *et al*., 2009b; Goiri & Oregui, 2014; Seankamsorn *et al*., 2019; Haryati *et al*., 2019; Henry *et al*., 2015; Li *et al*., 2013), which were in agreement with the present meta-analysis. In this study, increasing the chitosan level did not affect $H_2S$ production, but in a previous study reported that chitosan increased $H_2S$ production in low concentrate substrate under *in vitro* rumen environment (Henry *et al*., 2015).

Chitosan is a natural, non-toxic, and biodegradable biopolymer that commonly used as a broad-spectrum antimicrobial component (Kong *et al*., 2010; Vendramini *et al*., 2016). The decrease in methane production can be caused by inhibition of methanogenesis by decreasing the use of $H_2$ as a substrate for $CH_4$ formation (Janssen, 2010). Furthermore, chitosan is likely to reduce methanogenic archaea, the main microbial group responsible for methane formation. Another plausible explanation regarding such lower methanogenesis due to chitosan addition is through the reduction of the protozoa population, particularly the *Entodinium spp*. (Wencelová *et al*., 2013). A certain number of methanogen lives symbi-

Table 5. Rumen fatty acid profile in the *in vitro* batch culture study treated by chitosan addition (in g/kg DM)

| Response parameter | Unit | n | Parameter estimates | | | | Model statistics | |
|---|---|---|---|---|---|---|---|---|
| | | | Intercept | SE intercept | Slope | SE slope | p-Value | RMSE |
| $C_{14:0}$ | % | 18 | 2.26 | 0.34 | -2.01 | 1.68 | <.001 | 1.13 |
| $C_{15:0}$ | % | 18 | 2.49 | 0.32 | -2.70 | 1.38 | <.001 | 0.93 |
| $C_{16:0}$ | % | 18 | 22.7 | 2.51 | -18.1 | 8.38 | <.001 | 5.62 |
| $C_{17:0}$ | % | 18 | 1.45 | 0.27 | -0.858 | 0.702 | <.001 | 0.47 |
| $C_{18:0}$ | % | 18 | 36.4 | 1.67 | -39.9 | 21.1 | <.001 | 14.2 |
| $C_{18:2n6}$ | % | 18 | 3.34 | 1.43 | 3.15 | 3.74 | 0.048 | 2.51 |
| CLA | % | 18 | 0.74 | 0.22 | 0.471 | 1.03 | 0.009 | 0.69 |
| $C_{18:3n3}$ | % | 18 | 0.74 | 0.31 | 2.39 | 1.78 | 0.043 | 1.19 |
| SFA | % | 18 | 75.4 | 5.33 | -28.1 | 27.3 | <.001 | 18.3 |
| MUFA | % | 18 | 19.2 | 4.38 | 23.1 | 24.3 | 0.002 | 16.3 |
| PUFA | % | 18 | 5.53 | 1.57 | 4.11 | 6.38 | 0.008 | 4.28 |

Note: CLA= *cis*9, *trans*11 C18:2; SFA= saturated fatty acid; MUFA= monounsaturated fatty acid; PUFA= polyunsaturated fatty acid; n= number of treatment; RMSE= residual mean square error; SE= standard error.

otically with protozoa and takes advantage of the fauna. Therefore, any reduction of the protozoa population is expected to reduce methanogen as well and, probably, its methanogenesis activity. The ability of chitosan to decrease methanogen and protozoa populations is apparently related to its property for changing their cell permeability due to the interaction between polycationic chitosan and the electronegative charge on the microbial surface (Muxika *et al.*, 2017). Supporting the argument, such a positive charge of chitosan is thought to be responsible for its antimicrobial activity through interactions with cell membranes with negatively charged microorganisms (Cazón *et al.*, 2017). The decrease in total protozoa increases the total bacteria population in the rumen since protozoa possess predatory activity on bacteria in the rumen (Newbold *et al.*, 2015).

This present study was in agreement with previous research which reported that increasing level of chitosan was associated with an increase of rumen pH (Goiri *et al.*, 2009a; Goiri *et al.*, 2009b; Goiri & Oregui, 2014; Wencelová *et al.*, 2013; Pereira *et al.*, 2019; Li *et al.*, 2013; Henry *et al.*, 2015). However, there was a study reported that chitosan had no effect on rumen pH (Belanche *et al.*, 2016a). Aranaz *et al.* (2009) thought that the possibility of chitosan could increase pH was due to physical hydrogels and ammonia gas, which neutralized $H^+$ in solution. Another theory that can explain the phenomenon is that formate may diffuse to rumen liquid phase to form $HCO_3^-$ and $H_2$ and formation of the former product may increase the buffering capacity of rumen fluid (Leng, 2014). Concerning nitrogen metabolism, chitosan was reported to not affect rumen ammonia N concentration (Belanche *et al.*, 2016a; Goiri *et al.*, 2009a; Goiri *et al.*, 2009b; Seankamsorn *et al.*, 2019; Li *et al.*, 2013; Henry *et al.*, 2015). However, another study stated that chitosan increased ammonia levels in the *in vitro* rumen batch culture (Pereira *et al.*, 2019). Chitosan is a nitrogenous compound that can be degraded in the rumen by microbes, so the higher concentration of ammonia in the chitosan diet is possibly due to amine group ($R\text{-}NH_2$) conversion into ammonia (Beier & Bertilsson, 2011). However, there was a study that stated that chitosan reduced the concentration of ammonia in the rumen (Goiri *et al.*, 2014). The possibility of ammonia reduction is associated with a reduction in amino acid degradation through a mechanism of protection from ruminal degradation in a way that under the pH condition of the rumen, the positively charged $-NH_2^+$ groups of chitosan could interact electrostatically with the negatively charged carboxyl groups in amino acid (Chiang *et al.*, 2009). In the latter case, the chitosan effect is likely similar to tannin that can protect the protein from degradation by rumen microbes (Kondo *et al.*, 2014).

Belanche *et al.* (2016a) reported that the addition of chitosan to diets increased the proportion of propionate ($C_3$) and decreased the proportion of butyrate ($C_4$) in the rumen. Another study observed that the addition of chitosan increased propionate ($C_3$) and valerate ($C_5$) proportions, but decreased total VFA concentration, the proportion of acetate ($C_2$), the ratio between acetate and propionate ($C_2$:$C_3$), and BCVFA (Goiri *et al.*, 2009a). Other studies also confirmed such an increase in the proportion of propionate and a decrease in the acetate proportion in the addition of chitosan (Goiri & Oregui, 2014; Seankamsorn *et al.*, 2019; Li *et al.*, 2013). The proportion of VFA is greatly influenced by the ratio of forage and concentrate in the ration, microbial population structure in the rumen, long-chain fatty acids released from lipids, and many other end products resulting from microbial degradation from small components of the feed (Krehbiel, 2014). Such elevated propionate proportion by the addition of chitosan is apparently related also to the reduction of the protozoa population. It was reported that the defaunation of protozoa increased the molar proportion of propionate in the rumen and decreased the proportions of butyrate and acetate (Morgavi *et al.*, 2010).

This present study was in agreement with previous research, which reported that adding chitosan in the whole soybean and sugarcane silage increased DMD, CPD, and NDFD (Gandra *et al.*, 2016; Gandra *et al.*, 2018). However, other studies reported conversely that the addition of chitosan reduced DMD in the *in vitro* rumen batch culture (Li *et al.*, 2013; Wencelová *et al.*, 2013). Some other studies even reported that chitosan addition had no effect on DMD, OMD, CPD, and NDFD (Henry

*et al*., 2015; Pereira *et al*., 2019; Seankamsorn *et al*., 2019). In the present meta-analysis study, across all different experiments, chitosan was found to increase nutrient digestibility. Such an increase in the nutrient digestibility is apparently related to the alteration of microbial population structure following chitosan addition. Chitosan reduces the protozoa population, decreases predation intensity of protozoa on bacteria, and in turn, elevates the total bacteria population that greatly responsible for nutrient degradation and fermentation. Although chitosan has a broad spectrum anti-microbial property, apparently protozoa are generally more sensitive to the compound in comparison to those of rumen bacteria.

With regard to the influence of chitosan on fatty acid metabolism in the rumen, the current results supported the finding that chitosan reduced ruminal fatty acid biohydrogenation by simultaneously increasing the proportion of CLA and reducing $C_{18:0}$ regardless of the dietary fatty acid source (Goiri *et al*., 2010). Apparently, chitosan selectively inhibits microbial species involved in the lipolysis and biohydrogenation steps of fatty acids. Accordingly, there are three main groups of microbes involved, namely *Anaerovibrio lipolytica* that liberates fatty acids from their glycerol backbones, *Butyrivibrio fibrisolvens* that biohydrogenates PUFA to vaccenic acid, and finally *Butyrivibrio proteoclasticus* that plays a role in the terminal step of biohydrogenation, i.e., the conversion of vaccenic acid to stearic acid, the C18 saturated fatty acid (Jenkins *et al*., 2008; Lourenço *et al*., 2010; Toral *et al*., 2018; Vasta *et al*., 2019). A study of Belanche *et al.* (2016b) showed that chitosan addition at 5% DM decreased the relative abundance of both *Anaerovibrio* sp. and *Butyrivibrio* sp. in the rumen simulation technique system, which was measured by employing the Next Generation Sequencing method. The result, therefore, indicates that chitosan may be used to modulate fatty acid metabolism in the rumen by elevating beneficial fatty acids for human health such as PUFA, omega 3 fatty acids, and CLA. However, their appearance in animal products requires further *in vivo* investigation.

## CONCLUSION

Chitosan seems to be suitable for use as a feed additive in ruminant diets. Chitosan addition is able to mitigate enteric methane emission, alters rumen fermentation profiles toward a favorable direction, and improves nutrient digestibility. Further, chitosan plays a role in inhibiting biohydrogenation of fatty acids in the rumen as indicated by the increase of PUFA and the decrease of SFA.

## CONFLICT OF INTEREST

Anuraga Jayanegara, Nahrowi, and Sri Suharti serve as editors of the Tropical Animal Science Journal, but has no role in the decision to publish this article. We also certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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