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# Chitosan as a feed additive: Its modulatory effect on methane emission and biohydrogenation under artificial rumen system

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**Abstract**. This study aimed to determine the effects of chitosan addition on methane emission, fatty acid biohydrogenation and fermentation profile of rumen simulation technique (rusitec). Four studies reported chitosan use as a feed additive in the rusitec system were integrated into a database. Treatments were categorized into control (no chitosan addition) and chitosan addition at different levels. Various studies were treated as random effects whereas treatments were considered as fixed effects. Data were analyzed by using mixed model methodology. Significance was declared when $P<0.05$ and tendency was stated when $P<0.1$. Results showed that chitosan addition reduced methane emission in comparison to control by as much as 28% ($P<0.05$). Total volatile fatty acid concentration was not altered due to chitosan, but acetate proportion was decreased ($P<0.05$) while propionate proportion tended to elevate in the addition of chitosan ($P<0.1$). Chitosan did not influence ruminal pH, ammonia concentration and nutrient digestibility (organic matter, crude protein and neutral detergent fiber) of substrate. Proportions of vaccenic acid ($P<0.01$) and conjugated linoleic acid ($P<0.001$) were increased by the addition of chitosan. In conclusion, chitosan may provide beneficial effects in the rumen by mitigating methane emission and elevating fatty acids contributing to human health.

## 1. Introduction

Chitosan is a natural biopolymer that obtained from deacetylation of chitin. Chitin itself is a long-chain polymer of N-acetylglucosamine and it naturally present in the exoskeleton of crustaceans and insects [1,2]. Chitin is considered as an anti-nutritive factor, i.e., inhibits degradation and absorption of nutrients in the digestive tract when ingested by animals [3]. Its anti-nutritive effect is apparently similar to plant tannin that interact with nutrients thus preventing the action of digestive enzymes to degrade the nutrients [4]. However, when chitin is converted to chitosan by removing an acetyl group within its chemical structure, it may provide a number of beneficial effects for animals both for monogastrics and ruminants [5]. Therefore, chitosan may be extracted from its matrix and potentially utilized as a feed additive.

A number of experiments had tested the effects of chitosan addition under artificial rumen system to modify its fermentation pattern towards a desirable direction [6-8]. However, these experiments have not been integrated in order to generate a more robust conclusion regarding the effectiveness of chitosan as a feed additive. This study aimed to determine the effects of chitosan addition on methane emission, fatty acid biohydrogenation and fermentation profile of rumen simulation technique (rusitec) by employing a statistical meta-analysis approach.

## 2. Materials and methods

Four studies from three papers that reported chitosan use as a feed additive in the rusitec system were integrated into a database [6-8]. These papers were identified from Scopus database by using "chitosan" and "rumen" as the keywords. Treatments were categorized into control (no chitosan addition) and chitosan addition at different levels. Duration of rusitec experiments ranged from 14-18 days per run, and each run was replicated three to four times. Chitosan was added at the concentration between 50 to 138 g/kg dry matter of basal ration. Parameters observed were pH, ruminal ammonia concentration, volatile fatty acid (VFA) concentration, i.e., acetate (C2), propionate (C3), butyrate (C4), valerate (C5) and branched chain volatile fatty acid (BCVFA). Other integrated parameters were organic matter digestibility (OMD), crude protein digestibility (CPD), neutral detergent fiber digestibility (NDFD), gas production, methane emission per unit OMD, and long-chain fatty acids concentration that related to biohydrogenation, i.e., conjugated linoleic acid (CLA, *c*9,*t*11 $C_{18:2}$), vaccenic acid (*t*11 $C_{18:1}$) and stearic acid ($C_{18:0}$).

Data were analyzed by mixed model procedure in which various studies were treated as random effects whereas experimental treatments (control vs chitosan addition) were considered as fixed effects [9]. Significance was declared when P<0.05 and tendency was stated when P<0.1. Software SPSS version 20.0 was employed to perform the statistical meta-analysis.

## 3. Results and discussion

Chitosan addition did not influence ruminal pH and ammonia concentration (Table 1). Total volatile fatty acid concentration was not altered due to chitosan, but acetate proportion was decreased (P<0.05) while propionate proportion tended to elevate in the addition of chitosan (P<0.1). Chitosan increased valerate proportion (P<0.05) and decreased acetate-to-propionate ratio (P<0.05). Proportions of butyrate and BCVFA were not influenced by the addition of chitosan. Chitosan did not influence nutrient digestibility (organic matter, crude protein and neutral detergent fiber) of substrate (Table 2). Chitosan addition reduced methane emission in comparison to control by as much as 28% (P<0.05). Proportions of vaccenic acid (P<0.01) and conjugated linoleic acid (P<0.001) were increased by the addition of chitosan but it did not influence the stearic acid proportion.

**Table 1.** Influence of chitosan on rumen fermentation under rusitec (rumen simulation technique) system.

| Parameter | Unit | Control | Chitosan | SEM | P-value |
|---|---|---|---|---|---|
| pH | | 6.24 | 6.25 | 0.078 | 0.871 |
| $NH_3$ | mg/dl | 37.7 | 27.0 | 8.07 | 0.457 |
| Total VFA | mmol/l | 122.9 | 122.8 | 5.43 | 0.987 |
| $C_2$ | % | 60.0 | 51.9 | 2.8 | 0.039 |
| $C_3$ | % | 23.6 | 34.6 | 2.92 | 0.084 |
| $C_4$ | % | 12.2 | 9.91 | 1.3 | 0.104 |
| $C_5$ | % | 4.54 | 7.22 | 0.662 | 0.010 |
| $C_2/C_3$ | | 2.56 | 1.49 | 0.279 | 0.045 |
| BCVFA | % | 3.34 | 1.98 | 0.58 | 0.163 |

The ability of chitosan to decrease methane emission is apparently related to its broad-spectrum antimicrobial activity. It is likely that there is an electrostatic interaction between chitosan and cell membrane of methanogens, causing a destabilization and thus may reduce the population and/or activity of the archaea [5]. Mitigating enteric methane emission from livestock is presently an important factor to be considered since methane is one of the major greenhouse gases that accumulate in the atmosphere and contribute to global warming [10]. Such antimicrobial activity of chitosan may also explain the alteration of individual VFA profiles. Cellulolytic bacteria such as *Fibrobacter*, *Butyrivibrio* and *Ruminococcus* are inhibited due to chitosan [6] and this condition leads to a lower acetate production since acetate is produced primarily from fiber fermentation. Inhibition of chitosan on *Butyrivibrio* may explain the increase of CLA and vaccenic acid proportions. This bacteria is responsible in various steps of biohydrogenation of unsaturated fatty acids in the rumen [11].

**Table 2.** Influence of chitosan on nutrient digestibility, methane emission and biohydrogenation under rusitec (rumen simulation technique) system.

| Parameter | Unit | Control | Chitosan | SEM | P-value |
|---|---|---|---|---|---|
| OMD | % | 72.3 | 64.0 | 3.22 | 0.368 |
| CPD | % | 72.0 | 64.7 | 4.80 | 0.479 |
| NDFD | % | 46.5 | 30.6 | 6.41 | 0.363 |
| Gas | l/d | 3.19 | 2.53 | 0.216 | 0.145 |
| $CH_4$/OMD | mmol/g | 0.57 | 0.41 | 0.091 | 0.026 |
| CLA | % | 0.20 | 0.40 | 0.058 | <0.001 |
| *t*11 $C_{18:1}$ | % | 4.55 | 19.6 | 4.33 | 0.004 |
| $C_{18:0}$ | % | 48.7 | 23.3 | 7.87 | 0.231 |

## 4. Conclusion

Chitosan provides a beneficial effect in the rumen by lowering enteric methane emission. Therefore, the compound may contribute to mitigating the global warming problem due to accumulation of greenhouse gases including methane. In addition, lower methane emission by chitosan is energetically more efficient for livestock and may enhance their production performance. Apart from its methane mitigating effect, chitosan elevates the proportions of favorable fatty acids that contribute to human health such as conjugated linoleic acid and vaccenic acid.

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