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In vitro evaluation of graded levels of chitosan in total mixed ration

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Abstract
The health and productivity of livestock are closely related to quantum of quality forage feed provided to the animals. For ruminant animals, roughages are the main source of feed. These are rich in fibrous portion which has low digestibility. There are several methods to improve the efficiency of utilization of roughages. The present study was envisaged to study the effect of *in vitro* evaluation of graded levels of chitosan in total mixed ration. Total mixed ration was prepared by using wheat straw and concentrate in the ratio of 60:40 and was used as substrates for experiment. Chitosan was added at the level 0, 0.1, 0.2 0.5 and 1% of TMR. Statistical analysis revealed IVDMD, *In vitro* total gas production, TDOMR, total nitrogen, TVFA and ammonia nitrogen were significantly increased at 0.5% chitosan level in TMR as compared to control.

Keywords: Chitosan, concentrate, roughage, total mixed ration

1. Introduction
The health and productivity of livestock are closely related to quantum of quality forage feed provided to the animals. For ruminant animals, roughages are the main source of feed. These are rich in fibrous portion which has low digestibility. In ruminant feeding system, some of the main additives used are ionophores, organic acids, plant extracts (Calsamiglia *et al.*, 2007) [4], exogenous fibrolytic enzyme and more recently added chitosan (Dias *et al.*, 2017) [7]. Recently, Goiri *et al.* (2009c) [10] proposed the use of chitosan as an additive similar to ionophore antibiotics in ruminant nutrition.

Chitin and chitosan belongs to a family of linear polysaccharides comprising of varying amounts of b-(1-4) - linked N-acetyl-2-amino-2-deoxy-D-glucose (acetylated, A-unit) and 2-amino-2-deoxy-D-glucose (deacetylated, D-units). Chitosan is a natural biopolymer derived from the DE acetylation of chitin. It involves the need of high-temperature treatment of chitin with a strong alkali solution of sodium hydroxide (Mengistu Lemma *et al.*, 2016) [15]. Chitin, the second most important organic compound on earth next to cellulose, can be found in the exoskeletons of some insect, arthropods and shell waste of crustaceans (e.g., crab and shrimp) and the cell wall of lower plants (Kong *et al.*, 2010) [13]. Chitin is also found in jellyfish, squid bones, algae, nematodes, external structure components of insects and fungal cell walls (Gohel *et al.*, 2006) [9]. Haryati *et al.* (2019) [11] revealed that chitosan might be mechanically similar to monensin which is associated with shifts in volatile fatty acid (VFA) profiles, primarily improving propionate and reducing acetate as well as depressing CH_4 emissions. Researchers examined the effect of chitosan on ruminal fermentation and digestibility of ruminants *in vivo* (Araújo *et al.*, 2015; Dias *et al.*, 2017) [2, 7] and reported increase in the digestibility of dry matter, crude protein and neutral detergent fibre and in the ruminal propionate content. Some authors noted that chitosan increased apparent digestibility of dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF), while having no effect on nutrient intake (Araújo *et al.*, 2015; De Paiva *et al.*, 2016) [2, 6]. Thus, this study was planned to explore the effect of incorporation of chitosan in total mixed ration at different roughage levels on nutrient digestibility, total gas production and rumen fermentation parameters by conducting *in vitro* experiment.

2. Materials and Methods

2.1 Preparations of TMR

Chitosan from shrimp species *Parapenaeopsis stylifera* having degree of acetylation 85±2%, viscosity 50-80 pascal second (PS) was used. Total mixed ration was prepared by taking wheat straw and concentrate in the ratio of 60:40 was used as substrates for experiment. Chitosan was added at the levels of 0, 0.1, 0.2, 0.5 and 1% of total mixed ration.

2.2 Donner animal and rumen liquor collection

Ruminal fluid was obtained from two adult Surti goats of the same age and uniform conformation fed as per ICAR feeding standards (2013). Rumen liquor was collected at 2 hours post-feeding and strained through a four-layered muslin cloth and referred as Strained Rumen Liquor (SRL). Carbon dioxide gas was passed through the SRL for one minute and was maintained at 39±1 °C temperature for further analysis.

2.3 Estimation of chemical composition and rumen fermentation parameters: Samples of wheat straw, concentrate and chitosan were analyzed for proximate composition according to AOAC (2005) [1] and fibre fractions as per Goering and Van Soest (1970) [8]. TMR with different chitosan levels were analyzed for *in vitro* dry matter degradability by Tilley and Terry method (1963). Approximately 0.5 g finely ground TMR sample was taken with 40 ml CO_2 saturated phosphate carbonate buffer solution and 10 ml strained rumen liquor in Erlenmeyer flask and then incubated at 39ºC for 48 h in CO_2 incubator with periodic shaking. After 48 h incubation, contents were filtered through sintered crucible and dried at 100ºC overnight and weighed then, dry residues were ashed at 550ºC.

Total gas production was determined by method of Menke and Steingass (1988) [16]. For determination of total gas production, about 200mg sample was taken into glass syringe with 30 ml buffer solution and rumen fluid. Then glass syringe were placed into incubator at 39ºC for 24 hr. After 24 hr, total gas production was measured and suitable aliquot was taken from glass syringe for determination of rumen TVFA and ammonia nitrogen. Total volatile fatty acids (TVFA) and ammonia nitrogen (NH_3-N) were analyzed as per the method given by Barnett and Reid (1957) [3] using Markham's steam distillation apparatus and by Conway (1957), respectively.

2.4 Experimental design and Statistical analysis

The data were collected and statistically analyzed Significance of mean differences were tested by Duncan's New Multiple Range Test as modified by Kramer (1957) [14].

3. Results and Discussion

Table 1. Showing the chemical composition of wheat straw, concentrate and chitosan used as TMR.

Table 1: Proximate and chemical composition (% DM basis) of wheat straw, concentrate and chitosan

Proximate and chemical composition	Wheat straw	Concentrate	Chitosan
DM	92.65	91.18	93.86
CP	3.88	19.55	32.09
EE	1.09	2.65	0.40
CF	42.80	12.00	33.76
NFE	43.13	55.96	32.62
TA	9.10	9.84	1.13
NDF	79.31	37.90	94.15
ADF	50.05	19.75	1.75

Effects of chitosan on IVDMD, IVOMD, *in vitro* total gas production and TDOMR are presented in Table 2. Result shows that IVDMD was significantly ($p < 0.01$) higher in 0.5% chitosan level as compared to control and 1% chitosan level. While, it was remain at par with 0.1 and 0.2% chitosan level TMR. In earlier findings, reported numerically increased IVDMD of different forage-to-concentrate diets in chitosan added groups than the control group. Similarly, significant ($p < 0.05$) improvement in IVOMD was observed at 0.5% level chitosan as compared to control and 0.1% level and it was remain at par with 0.2% and 1% level. In earlier reports, Goiri *et al.* (2010b) [10] reported IVOMD was numerically increased in chitosan added groups than the control group.

Statistical analysis showed that IVTGP was significantly ($p < 0.01$) higher at 0.5% chitosan level and non-significantly ($p > 0.01$) higher at 0.2, 0.1 and 1% chitosan level as compared to control. In earlier reports, Haryati *et al.* (2019) [11] reported numerically increased IVTGP in chitosan added groups than the control group.

Statistical analysis of data revealed significant ($p < 0.01$) improvement in TDOMR was observed at 0.5% chitosan level followed by 0.2%, 1% and 0.1% as compared to control in TMR. In contrast with the above findings, Goiri *et al.* (2009a) [10] noticed significantly ($p < 0.01$) decreased TDOMR in chitosan added groups than the control group.

Table 2: IVDMD, IVOMD, IVTGP and TDOMR

Chitosan %	IVDMD	IVOMD	IVTGP	TDOMR
0	48.59^{A}±2.07	52.40^{A}±2.28	19.61^{A}±0.98	53.39^{A}±1.27
0.1	49.65^{AB}±1.05	52.98^{A}±1.38	20.46^{AB}±1.34	54.47^{A}±1.87
0.2	53.69^{AB}±2.45	57.19^{AB}±1.06	22.13^{AB}±1.37	60.19^{BC}±2.05
0.5	54.22^{B}±1.47	60.24^{B}±1.54	24.37^{B}±1.22	63.72^{C}±1.02
1	48.62^{A}±1.31	56.52^{AB}±2.21	21.32^{AB}±1.41	57.12^{AB}±1.81
Mean ± SE	50.95±1.01	55.86±1.13	21.58±0.69	57.78±1.38

Note: Means superscripted with different superscripts within a column (A, B, C) differ significantly from each other

IVDMD = *in vitro* dry matter degradability, IVOMD= *in vitro* organic matter degradability, IVTGP= *in vitro* total gas production and TDOMR= truly degradable organic matter

Effects of chitosan on TVFA, NH_3-N, total-N and TCA-N are presented in Table 3. Statistical analysis of data revealed significantly ($p < 0.01$) higher amount of TVFA and TCA-N production at 0.5 % chitosan level as compared to control and 0.1% level and at par with 0.2% and 1% level. In earlier findings, Goiri *et al.* (2009b) [10] reported numerically increased TVFA in chitosan added groups than the control group.

Statistical analysis of data revealed significant improvement in ammonia nitrogen at 0.5% chitosan level of TMR as compared to control, while 0.1, 0.2 and 1% chitosan level remain at par. While, total nitrogen was significantly ($p < 0.01$) higher in all chitosan added TMR as compared to control, being significantly highest in 0.5% followed by 1, 0.2 and 0.1 % chitosan level TMR. In agreement to present findings, Pereira *et al.* (2019) revealed that ammonia nitrogen was significantly ($p < 0.001$) increased in chitosan added groups as compared to control group.

Table 3: TVFA, NH_3-N, total N and TCA-N

Chitosan %	TVFA	NH_3-N	total N	TCA-N
0	5.70^{A}±0.9	25.00^{A}±2.00	38.19^{A}±1.11	24.90^{A}±1.30
0.1	7.12^{A}±1.60	26.00^{AB}±2.00	43.86^{B}±1.58	26.20^{A}±1.00
0.2	8.26^{AB}±0.74	28.00^{AB}±3.00	46.61^{BC}±1.28	28.50^{AB}±1.70
0.5	9.78^{B}±0.78	32.00^{B}±1.00	51.85^{D}±1.95	32.40^{B}±2.00
1	7.56^{AB}±0.76	31.00^{AB}±2.00	48.67^{CD}±1.13	28.40^{AB}±1.80
Mean ± SE	7.68±0.67	28.4 1.15	45.84±1.62	28.08±1.00

Note: Means superscripted with different superscripts within a column (A, B, C, D) differ significantly from each other

TVFA= total volatile fatty acid, NH_3-N = ammonia nitrogen, total N = total nitrogen and TCA-N = TCA perceptible nitrogen

4. Conclusions

The results of the present study indicated that supplementation of chitosan improved *in vitro* dry matter degradability, IVOMD, *in vitro* total gas production, TDOMR, TVFA, NH_3-N, total-N and TCA-N at 0.5% chitosan level in TMR.

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6. References

1. AOAC. Official methods of analysis of the Association of Official Analytical Chemists. 18th edition. Horwitz William Publication, Washington DC, USA; c2005. p. 71.
2. Araujo APC, Venturelli BC, Santos MCB, Gardinal R, Consolo NRB, Calomeni GD, *et al*. Chitosan affects total nutrient digestion and ruminal fermentation in Nellore steers. Animal Feed Science and Technology. 2015 Aug 1;206:114-118.
3. Barnett AJG, Reid RL. Studies on the production of VFA from grass by rumen liquor in artificial rumen. Journal of Agricultural Science. 1957;48(31):5-321.
4. Calsamiglia S, Busquet M, Cardozo PW, Castillejos L, Ferret A. Invited review: essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science. 2007 Jun 1;90(6):2580-2595.
5. Conway EJ. Micro-diffusion analysis and volumetric error, 4th ed. Crossby Lockwood and Sons Ltd., University press, Glasgow; c1957.
6. De Paiva PG, De Jesus EF, Del Valle TA, De Almeida GF, Costa AGBVB, *et al*. Effects of chitosan on ruminal fermentation, nutrient digestibility, milk yield and composition of dairy cows. Animal Production Science. 2016 Feb 18;57(2):301-307.
7. Dias AOC, Goes RHTB, Gandra JR, Takiya CS, Branco AF, Jacauna AG, *et al*. Increasing doses of chitosan to grazing beef steers: Nutrient intake and digestibility, ruminal fermentation, and nitrogen utilization. Animal Feed Science and Technology. 2017;225:73-80.
8. Goering HK, Van Soest PJ. Forage fiber analyses (apparatus, reagents, procedures and some applications) In: Agriculture Handbook No 379, Arsusda, Washington, DC; c1970.
9. Gohel V, Singh A, Vimal M, Ashwini P, Chhatpar HS. Bio prospecting and antifungal potential of chitinolytic microorganisms. African Journal of Biotechnology. 2006;5(2):54-72.
10. Goiri I, Oregui LM, Garcia-Rodriguez A. Dose–response effects of chitosans on in vitro rumen digestion and fermentation of mixtures differing in forage-to-concentrate ratios. Animal Feed Science and Technology. 2009 Jan 16;151(3-4):215-227.
11. Haryati RP, Jayanegara A, Laconi EB, Ridla M, Suptijah P. Evaluation of chitin and chitosan from insect as feed additives to mitigate ruminal methane mission. In: Proceedings of American Institute of Physics Conference. 2019 Jul 3;2120(1):040008.
12. ICAR. Nutrient requirements of animal cattle and buffalo. 3rd edition Indian council of Agricultural Research, New Delhi; c2013.
13. Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of chitosan and mode of action: a state of the art review. International Journal of Food Microbiology. 2010 Nov 15;144(1):51-63.
14. Kramer CY. Extension of multiple range tests to group correlated adjusted means. Biometrics. 1957;13(1):13-18.
15. Mengistu Lemma S, Bossard F, Rinaudo M. Preparation of pure and stable chitosan Nano fibers by electro spinning in the presence of poly ethylene oxide. International Journal of Molecular Sciences. 2016;17(11):1790.
16. Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Animal Research and Development. 1988;28:7-55.
17. Tilley JMA, Terry DR. A two stage technique for the in vitro digestion of forage crops. Grass and Forage Science. 1963 Jun;18(2):104-111.