Indian J. Anim. Nutr. 2022. 39 (4): 406-412
doi: 10.5958/2231-6744.2022.00051.2
Indian Journal of Animal Nutrition

Influence of Silage Additives on *In Vitro* Rumen Fermentation Pattern of Wheat Straw and Green Maize Silage

S.Y. Belim*, H. H. Savsani, M. R. Chavda, M. D. Odedra, D. D. Garg, V.K. Karangiya, Y. G. Kansagara, K. R. Makwana and P. H. Agravat
Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh-362001, Gujarat
*Correspondence: belimsaman1998@gmail.com

ABSTRACT

The current investigation was conducted to study various combinations of biological and chemical additives on *in vitro* rumen fermentation parameters of wheat straw and green maize silage. Different silages were prepared using green maize fodder and wheat straw in the proportion of 10:0 & 7:3 ratio in plastic jar of 3 kg capacity (3 replication in each) by adding common salt @ 0.5%, urea @1% and molasses @ 1.5% in each silages with seven different treatments *viz.* Control (only green maize), WS (green maize and wheat straw in 7:3 ratio), X (WS added with Xylanase), LP (WS added with *L. plantarum*), LF (WS added with *L. fermentum*), LPLF (WS added with both bacterial inoculants) and XLPLF (WS added with Xylanase and both bacterial inoculants). Xylanase, *L. plantarum* and *L. fermentum* were used @ 1500 IU/g, 1 x 10^6cfu/g and 2 x 10^6cfu/g, respectively. All silages were used for *in vitro* study after 45 days of ensiling. The result showed that significantly (P<0.05) higher TVFA content was noticed in Xylanase inoculated silage during *in vitro* rumen fermentation. Values of *in vitro* rumen ammonia nitrogen (NH_3-N) and total nitrogen (Total N) were found significantly (P<0.01) higher in *L. plantarum* and combination of *L. plantarum* and *L. Fermentum* inoculated silage. IVDMD was significantly (P<0.05) higher in Xylanase inoculated silage. Also, IVOMD and total gas production were significantly (P<0.01) higher in Xylanase treated silage. Partitioning factor (PF) was observed significantly (P<0.01) lower in Xylanase inoculated silage. Thus, it is inferred that Xylanase can be used as additive when maize and wheat straw are the principal fodder for silage production in the ratio of 7:3 for maximum nutrient utilisation.

KEYWORDS: *In vitro* rumen fermentation, *L. Fermentum, L. plantarum,* Wheat straw, Xylanase

Article received: 29 November 2022; Article accepted: 25 December 2022

INTRODUCTION

Silage is a fermented feed resulting from the storage of high moisture crop, usually green forages, under controlled anaerobic condition in closed air tight structure called silo (Banerjee, 2019). Quality of silage can be improved through stimulation of the ensiling process by adding different types of chemical and biological additives. The main aim of using silage additives is to promote the growth of lactic acid producing bacteria during the fermentation cycle, as well as minimize losses and improve the quality of the silage to avoid other fermentation (Clostridial fermentation) products (Chauhan et al., 2021). Biological additives like bacterial inoculants have been used to increase the rate of acidification of ensiled forages (Weinberg and Muck, 1996). Adding *Lactobacillus plantarum* and *Lactobacillus fermentum* as an inoculant during ensiling ensures rapid and robust fermentation resulting in better-quality forage preservation.

Chemical additives like enzymes may improve their digestibility via number of mechanisms that include direct hydrolysis of sugar, improvement in palatability, change in gut viscosity and change in the site of digestion (Kung Jr, 2010).The main function of the exogenous fibrolytic enzymes is to release maximum amount of nutrients from the digestible, potentially digestible and indigestible fractions of the cell wall (Mocherla et al., 2017). Considering huge availability of wheat straw at cheapest price in India, and role of biological and chemical additives in silage production, the present study was envisaged to study their effects on *in vitro* rumen fermentation pattern of wheat straw and green maize based silage.

MATERIALS AND METHODS

The present study was conducted at Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat.

Preparation of silage

Different silages were prepared using green maize fodder and wheat straw in the proportion of 10:0 & 7:3 ratio in plastic jar of 3 kg capacity (3 replication in each) by adding common salt @ 0.5%, urea @1% and molasses @ 1.5% in each silages with seven different treatments *viz.* Control (only green maize), WS (green maize and wheat straw in 7:3 ratio), X (WS added with Xylanase), LP (WS added with *L. plantarum*), LF (WS added with *L. fermentum*), LPLF (WS added with both bacterial inoculants) and XLPLF (WS added with Xylanase and both bacterial inoculants). Xylanase, *L. plantarum* and *L. fermentum* were used @ 1500 IU/g, 1 x 10^6cfu/g and 2 x 10^6cfu/g, respectively. All silages were evaluated for *in vitro* rumen fermentation pattern after 45 days of ensiling.

Estimation of *in vitro* rumen fermentation pattern

After 45 days of ensiling, sampling of silage was done. Silage samples were oven dried at 100 ± 5 °C for overnight. The dried sample were ground to pass through a 1 mm screen and used as substrate for determining the *in vitro* dry matter degradability (IVDMD), *in vitro* organic matter degradability (IVOMD) and total gas production. IVDMD and IVOMD were analyzed as per the method described by Tilley and Terry (1963). Approximately 0.5 g finely ground silage sample was taken with 40 ml CO_2 saturated phosphate carbonate buffer solution and 10 ml strained rumen liquor in erlynmayer flask and incubated at 39°C for 48 hours in CO_2 incubator with periodic shaking. After 48 hours of incubation, contents were filtered through sintered crucible and dried at 100°C overnight and weighed. Dry residues were ashed at 550ºC.

Total gas production was determined by method of Menke and Steingass (1988). About 200 mg sample was taken into glass syringes with 30 ml buffer solution and rumen fluid. Then glass syringes were placed into incubator at 39! for 24 hr. After 24 hours, total gas production was measured and suitable aliquot was taken from glass syringe for determination of rumen pH, total volatile fatty acids (TVFA), ammonia nitrogen (NH_3-N) and total nitrogen (N). The rumen pH was measured by pentype pH meter. TVFA and NH_3-N were analyzed as per the methods given by Barnett and Reid (1957) and Conway's micro-diffusion method (Conway, 1957), respectively. The total N content was measured as per the Kjeldahl method (AOAC, 2005).

Statistical analysis and design of experiment

The data were analysed for descriptive statistics (mean and standard error). Treatment effects on different parameters were analyzed by one way analysis of variance according to Snedecor and Cochran (1994). Pair wise mean difference between groups were compared by Duncan's New Multiple Range Testas modified by Kramer (1957) for the significance at $p<0.05$.

RESULTS AND DISCUSSION

Chemical composition of green maize, wheat straw and mixture of green maize and wheat straw used for ensiling is given in Table 1.

Table 1. Chemical composition and cell wall constituents of green maize wheat straw and mixture of green maize and wheat straw used for ensiling (% DM basis)

Parameter	Green maize	Wheat straw	GM:WS-7:3
DM	33.2±0.38	90.8±0.18	42.0±1.45
OM	90.9±0.20	86.9±0.15	88.7±0.19
CP	9.10±0.55	4.22±0.05	6.33±0.83
EE	1.64±0.05	1.04±0.05	1.35±0.07
CF	32.9±1.02	40.2±0.70	38.7±0.42
TA	9.10±0.20	13.0±0.15	11.3±0.19
NFE	47.2±1.42	41.4±0.86	42.3±0.67
NDF	68.0±0.27	79.0±0.14	71.7±0.59
ADF	41.9±1.05	56.8±0.64	48.1±0.09
Cellulose	33.9±1.54	40.3±1.20	36.6±0.35
Hemicellulose	26.1±1.32	22.2±0.79	23.6±0.50
ADL	5.35±0.17	11.1±0.45	8.74±0.30

DM- dry matter; OM- organic matter; CP- crude protein; EE- ether extract; CF- crude fibre; NFE- nitrogen free extract; TA- total ash; NDF- neutral detergent fibre; ADF- acid detergent fibre; ADL- acid detergent lignin

Influence of silage additives on *in vitro* rumen fermentation pattern are presented in Table 2 and 3, respectively. Analysis of variance revealed that pH value of rumen liquor for different experimental silage was observed significantly (P<0.05) higher in LP as compared to X, WS and control. pH values of rumen liquor for different experimental silages were comparable with control. In accordance with present result, Lee et al. (2020) noticed same trend for *in vitro* rumen pH in different additives inoculated silage and Marbun et al. (2020), also observed numerically higher pH in *Lactobacillus plantarum* treated rice straw silage, which was significantly higher in present study.

The findings of present investigation revealed that significantly (P<0.05) higher TVFA content was observed in X (9.54±0.34) and XLPLF silage as compared to control and WS silage, which were statistically similar with each other. The results of the current study wasconcurred well with the findings of Gang et al. (2020), Huo et al. (2021) and Oskoueian et al. (2021), they recorded significantly higher TVFA content in all additives inoculated silage as compared to control silage. While, in contrary to present investigation Ce et al. (2016) observed non significant reduction in TVFA content of all additives inoculated silage.

Ammonia nitrogen content was found to be significantly (P<0.01) higher in LP (*Lactobacillus plantarum*) and LPLF as compared to all other experimental silage. However, significantly (P<0.01) higher ammonia nitrogen content was also observed in XLPLF as compared to WS. In corresponding to present investigation, Lee et al. (2020) and Marbun et al. (2020) observed significantly higher ammonia nitrogen content in all additives treated experimental silage as compared to control silage of Italian Ryegrass silage and maize silage, respectively. Compared to control and WS silage, significantly (P<0.01) higher total nitrogen was observed in LP (91.1±1.37), LPLF (90.6±2.04), XLPLF (86.3±1.28) and LF (85.8±1.40) among them LP and LPLF did not differ from each other. While total nitrogen content of X (Xylanase) inoculated experimental silage was numerically lower as compared to control but significantly (P<0.01) higher than WS silage.Higher total nitrogen content in LP, LPLF, XLPLF and LF was might be due to higher crude protein content with high degradability.

Statistical analysis of the data revealed that significantly (P<0.05) higher IVDMD was observed in Xylanase (57.67±1.64) inoculated silageas compared to WS silage and remains statistically similar with control and all other experimental silage, which also did not differ from each other and remains at par with WS silage. Higher IVDMD values in xylanase treated silage as compared to other experimental silage might be due to effects of xylanase on lignocellulose bond which resulted in effective utilisation of CF. In corresponding to current results, Dakore (2018), Yadav (2018) Gang et al. (2020), Huo et al. (2021) and Oskoueian et al. (2021) observed significantly higher IVDMD in all additives inoculated silage as compared to control silage but, in present study significantly higher IVDMD was found in Xylanase treated silage, whereas, in other additives silage it was numerically higher.

Significantly (P<0.01) higher IVOMD values were obtained in X (Xylanase) silage as compared to all other experimental silages, however, as compared to control and WS higher IVOMD was also observed in XLPLF, LPLF, LP and LF among which, LP silage had significantly (P<0.01) lower IVOMD values as compared to LF, XLPLF and LPLF, which also did not differ from each other. While, figures of IVOMD (%) for control and WS revealed non-significant effect. Higher IVOMD (%) values in Xylanase and different additives inoculated silage are might be due to its effect on rumen fermentation. The findings of present study were supported by the findings of Dakore (2018) and Yadav (2018) as they recorded significantly higher IVOMD in all additives inoculated silages as compared to control silage. However, Khota et al. (2017) also supported the current findings but they noted numerically higher values of IVOMD in all additives inoculated silage as compared to control silage.

Statistical analysis of the data revealed that significantly (P<0.01) higher gas production was noted in all additives inoculated experimental silage as compared to WS silage among which Xylanase inoculated silage shows significantly highest gas volume followed by XLPLF and LPLF and was statistically differ from control. Similar findings were observed by many workers, Dakore (2018), Yadav (2018) and Huo et al. (2021) observed significantly higher total gas production in all additives inoculated experimental silage as compared to control silage. As, gas production is positively correlated with DM digestibility, higher the gas production more will be IVDMD. Partitioning factor was significantly (P<0.01) lower in X followed by XLPLF, LPLF, LP and LF as compared to control and WS silage. Lower PF values in X inoculated silage might be due to higher gas production.

Table 2. Influence of silage additives on *in vitro* rumen fermentation pattern for different experimental silage

Treatments	Parameters			
	pH*	TVFA**	NH_3-N**	Total N**
C	$6.70^{a}\pm0.02$	$6.95^{ab}\pm0.15$	$41.6^{ab}\pm2.10$	$83.1^{bc}\pm0.90$
WS	$6.68^{a}\pm0.03$	$5.88^{a}\pm0.68$	$39.1^{a}\pm1.53$	$74.6^{a}\pm1.60$
X	$6.71^{a}\pm0.03$	$9.54^{c}\pm0.34$	$42.5^{ab}\pm1.11$	$81.0^{b}\pm1.26$
LP	$6.86^{b}\pm0.02$	$8.57^{bc}\pm0.60$	$53.3^{c}\pm1.05$	$91.1^{d}\pm1.37$
LF	$6.76^{ab}\pm0.03$	$7.79^{abc}\pm0.66$	$43.3^{ab}\pm1.05$	$85.8^{c}\pm1.40$
LPLF	$6.80^{ab}\pm0.04$	$8.69^{bc}\pm1.16$	$51.6^{c}\pm1.05$	$90.6^{d}\pm2.04$
XLPLF	$6.78^{ab}\pm0.06$	$9.18^{c}\pm0.22$	$45.8^{b}\pm1.53$	$86.3^{c}\pm1.28$
P value	0.018	0.003	<0.001	<0.001

TVFA – Total volatile fatty acids; NH_3-N- Ammonia nitrogen; Total N- Total Nitrogen

Note: [abcde]Means with different superscript within a column differ significantly (**P<0.01, *P<0.05)

Table 3. Influence of silage additives on*in vitro* rumen fermentation pattern for different experimental silage

Treatments	Parameters			
	IVDMD* (%)	IVOMD** (%)	Total Gas Production** (ml/200mg)	PF** (mg/ml)
C	52.8^{ab}±3.17	53.4^{ab} ±1.31	20.4^{b} ±0.95	4.33^{cd} ±0.06
WS	50.8^{a} ±0.50	51.0^{a} ±0.46	17.0^{a} ±0.51	4.42^{d} ±0.02
X	57.6^{b} ±1.64	59.5^{d} ±0.95	25.2^{e} ±0.51	3.81^{a} ±0.07
LP	53.7^{ab} ±0.97	54.8^{bc} ±0.74	22.0^{bc} ±0.62	4.15^{bc} ±0.10
LF	53.4^{ab} ±2.32	54.2^{bc} ±0.23	21.3^{b} ±0.46	4.23^{cd} ±0.04
LPLF	54.7^{ab} ±2.46	55.8^{bc} ±0.78	23.1^{cd} ±0.39	4.02^{b}±0.05
XLPLF	56.7^{ab} ±1.83	57.3^{cd} ±0.32	24.28^{de}±0.28	3.95^{ab} ±0.06
P value	0.028	<0.001	<0.001	<0.001

IVDMD- *in vitro* dry matter degradability; IVOMD- *in vitro* organic matter degradability; PF- partitioning factor
Note: ^{abcde}Means with different superscript within a column differ significantly (**P<0.01, *P<0.05)

CONCLUSIONS

Xylanase significantly improves TVFA, total gas production, IVDMD, IVOMD and PF.*Lactobacillus plantarum* and combination of both bacterial inoculants (LPLF) significantly increase *in vitro* rumen ammonia nitrogen and total nitrogen content.Thus, it is concluded that xylanase can be used as additive when maize and wheat straw are principal fodder in the ratio of 7:3 for maximum nutrient utilisation.

ACKNOWLEDGEMENT

The authors thank Principle & Dean, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh for necessary facilities and cooperation extended for this research work.

REFERENCES

Aboudi, K., Alvarez-Gallego, C. J. and Romero-García, L. I. 2016. Biomethanization of sugar beet byproduct by semi-continuous single digestion and co-digestion with cow manure. Bioresource Technology. 200: 311-319.

AOAC. 2005. Official methods of analysis of the association of official analytical chemists. 18th Edn. Horwitz William Publication, Washington DC, USA. P: 71

Banerjee, G. C. 2019. Conservation of forage crops. In: Principles of Animal Nutrition and Feeds. 2nd Edn. Oxford & IBH Publishing Co. Pvt. Ltd. Delhi. pp: 35-51.

Barnett, A. J. G. and Reid, R. L. 1957. Studies on the production of VFA from grass by rumen liquor in artificial rumen. Journal of Agricultural Science. 48: 315-321.

Ce, L. I. U., Lai, Y. J., Lu, X. N., Guo, P. T. and Luo, H. L. 2016. Effect of lactic acid bacteria inoculants on alfalfa (*Medicago sativa L.*) silage quality: assessment of degradation (in situ) and gas production (*in vitro*). Journal of Integrative Agriculture. 15(12): 2834-2841.

Chauhan, N., Singh, D., Kumari, N. and Tyagi, N. 2021. Impact of lactic acid bacteria and enzymes on the fermentation processes of sugarcane tops silage at a particular time interval.The Pharma Innovation Journal. 10(2): 332-336.

Conway, E. J. 1957. Micro-Diffusion Analysis and Volumetric Error, 4th Edn. Crossby Lockwood and Sons Ltd., University press, Glasgow.

Dakore, S. 2018. Effect of bacterial inoculants and exogenous enzymes on the ensiling characteristic of sugargraze silage and nutrient utilization in crossbred calves. M.V.Sc. Thesis, ICAR-National Dairy Reserch Institute, Karnal, Haryana, India.

Gang, G. U. O., Chen, S. H. E. N., Qiang, L. I. U., Zhang, S. L., Tao, S. H. A. O., Cong, W. A. N. G., Yong-xin W. A. N. G., Qing fang X.U. and Huo, W. J. 2020. The effect of lactic acid bacteria inoculums on *in vitro* rumen fermentation, methane production, ruminal cellulolytic bacteria populations and cellulase activities of corn stover silage. Journal of Integrative Agriculture. 19(3): 838-847.

Hemdane, S., Jacobs, P. J., Dornez, E., Verspreet, J., Delcour, J. A. and Courtin, C. M. 2016. Wheat (*Triticum aestivum L.*) bran in bread making: A critical review. Comprehensive Reviews in Food Science and Food Safety. 15(1): 28-42.

Huo, W., Wang, X., Wei, Z., Zhang, H., Liu, Q., Zhang, S., Wang, C., Chen, L., Xu, Q. and Guo, G. 2021. Effect of lactic acid bacteria on the ensiling characteristics and *in vitro* ruminal fermentation parameters of alfalfa silage. Italian Journal of Animal Science. 20(1): 623-631.

ICAR. 2013. Nutrient requirements of animal-cattle and buffalo. 3rd Edn Indian Council of Agricultural Research, New Delhi.

Khota, W., Pholsen, S., Higgs, D. and Cai, Y. 2017. Fermentation quality and *in vitro* methane production of sorghum silage prepared with cellulase and lactic acid bacteria. Asian-Australian Journal of Animal Sciences. 30(11): 1568-1574.

Kramer, C. Y. 1957. Extension of multiple range tests to group correlated adjusted means. Biometrics. 13(1):13-18.

Kung Jr., L. 2010. A Review on Silage Additives and Enzymes. Department of Animal and Food Sciences University of Delaware Newark, DE 19717-1303.

Lee, K., Marbun, T. D., Kim, S., Song, J., Kwon, C. H., Yoon, D., Kang, J., Lee, C., Cho, S. and Kim, E. J. 2020. Effect of lactic acid bacteria treatment on nutritive value and*in vitro* ruminal fermentation of italian ryegrass (*Lolium multiflorum L.*) Silage. Journal of The Korean Society of Grassland and Forage Science. 40(3): 182-189.

Marbun, T. D., Lee, K., Song, J., Kwon, C. H., Yoon, D., Lee, S. M., Kang, J. Lee, C., Cho, S. and Kim, E. J. 2020. Effect of lactic acid bacteria on the nutritive value and *in vitro* ruminal digestibility of maize and rice straw silage. Applied Sciences. 10(21): 7801.

Menke, K. H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Animal Research and Development. 28: 7-55.

Mocherla, V. A. N., Suryanarayana and Kavitha, P. 2017. Role of exogenous fibrolytic enzymes in ruminant digestion-A review. International Journal of Current Microbiology and Applied Science. 6(11):1400-1408.

Oskoueian, E., Jahromi, M. F., Jafari, S., Shakeri, M., Le, H. H. and Ebrahimi, M. 2021. Manipulation of rice straw silage fermentation with different types of lactic acid bacteria inoculant affects rumen microbial fermentation characteristics and methane production. Veterinary Sciences. 8(6): 100.

Reddy, N., and Yang, Y. 2005. Biofibers from agricultural byproducts for industrial applications. TRENDS in Biotechnology. 23(1): 22-27.

Snedecore, G. and Cochran, W. 1994. Statistical Methods. 8th Edn. Oxford and IBH. New Delhi. P: 503.

Tilley, J. M. A. and Terry, D. R. 1963. A two stage technique for the *in vitro* digestion of forage crops. Grass and Forage Science. 18(2): 104-111.

Weinberg, Z. G. and Muck, R. E. 1996. New trends and opportunities in the development and use of inoculants for silage. In: FEMS Microbiology Reviews. 19(1):53–68.

Yadav, S. K. 2018. The effect of lactic acid bacterial inoculants and exogenous enzymes on maize silage quality and nutrient utilisation in buffalo calves. M.V.Sc. Thesis, ICAR-National Dairy Research Institute, Karnal, Haryana, India.