Effect of different levels of sodium sesquicarbonate on *in vitro* rumen fermentation parameters

Hunny Sharma¹, Veena Mani¹, Sachin Kumar¹, Srobana Sarkar² and Hujaz Tariq³

Received: 05 February 2020 / Accepted: 31 March 2020 / Published online: 12 July 2020
© Indian Dairy Association (India) 2020

Abstract: This experiment was conducted to study the effects of sodium sesquicarbonate on *in vitro* rumen fermentation parameters. Sodium sesquicarbonate (NaSc) was added at different levels (0.5, 1, 1.5, 2, 2.5 and 3%) to the substrates having concentrate: roughage ratio (concentrate: sugargraze fodder) of 50:50 and 60:40. There was no significant effect of addition of sodium sesquicarbonate (upto 3%) on *in vitro* DM digestibility and organic matter digestibility in different treatments. Average value of NH₃-N, IVFA, MBP, PF, molar proportion of acetate, propionate and butyrate also remained unaffected due to NaSc supplementation.

Keywords: Buffer, *In vitro*, Rumen fermentation, Sodium sesquicarbonate

Introduction

There is decline in milk yield in many dairy herds which appear to be temporarily increased by overfeeding of grains (Kmicikewycz and Heinrichs, 2014; Abdela, 2016) and such diets may potentially exacerbate subacute acidosis which represents one of the most important metabolic disorders that affects rumen fermentation resulting in decrease in DMI, milk yield, and milk fat content, animal welfare and farm profitability (Morgante et al. 2007; Mao et al. 2017). Such rations also tend to support less rumination which reduces the production of salivary bicarbonate. Subacute ruminal acidosis is characterized by low ruminal pH (5.8-5.0) and

an alteration in ruminal biohydrogenation of dietary polyunsaturated fatty acids (Bauman and Grinari, 2003; Plaizier et al. 2008).

Different approaches have been made for improving the ruminant production by searching the alternatives for stabilizing the rumen pH to minimize the occurrence of rumen acidosis and related disorders (Owens and Basalan, 2016). Rumen buffer could be one such alternative considering facts that addition of dietary buffers to control rumen pH can be justified if bunk management and nutritional factors cause low pH (Kang and Wanapat, 2013). Buffers can neutralize the excessive acidity due to increased production of volatile fatty acids but the effects depend upon type of buffer, dose and type of animal in which it is supplemented (Sen et al. 2006). Bicarbonates are commonly used as exogenous buffer as their dissociation constant ($pK_a=6.25$) is close to normal rumen pH thus possessing high acid consuming capacity (Marden et al. 2008) and thus prevent further depression in pH (Humer et al. 2018). Sodium bicarbonate increases rumen pH, produces a more desirable rumen fermentation and increases rumen fluid outflow. Dietary supplementation of sodium sesquicarbonate could be one of the alternative as it is a mixture of sodium bicarbonate and sodium carbonate and is naturally occurring an alkalizing agent. The pH of a one percent sodium sesquicarbonate solution is 9.9 as compared to sodium bicarbonate which is 8.4 thus expected to have better potential for buffering action besides being cost effective. Therefore, the present experiment was conducted to study the effects of sodium sesquicarbonate on *in vitro* rumen fermentation parameters.

Materials and Methods

Substrate composition, treatments and parameters estimated

Sugargraze (moderately draught tolerant sweet sorghum hybrid) fodder and concentrate mixture were dried in hot air oven at 60°C for 48 h and ground using a hammer mill to pass through 1 mm sieve. The substrate was prepared by mixing concentrate mixture and sugargraze fodder in the ratio of 50:50 and 60:40. Sodium sesquicarbonate was added in treatment groups at different levels viz. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% of substrate whereas the control group comprised of only substrate. Proximate principles and fibre

¹ Animal Nutrition Division, National Dairy Research Institute, Karnal, 132 001, Haryana, India

² Animal Nutrition Division, Central Sheep and Wool Research Institute, Avikanagar, 304 501, Rajasthan, India

³ De Heus India, Rajpura, 140 401, Punjab, India

Hunny Sharma (✉)
Animal Nutrition Division
National Dairy Research Institute, Karnal, 132 001, Haryana, India
Email: vet657@gmail.com; Tel: +91-9149964783

fractions of the concentrate mixture and the substrate were determined according to AOAC (2005) and Van Soest et al. (1991). Rumen fluid and particulate matter were collected from the goats (n=4) with the help of stomach tube in the morning before feeding (sugargraze and concentrate in 70:30 ratio) and watering of animals, in a pre-warmed thermo flask. The incubations were carried out as described by Menke and Steingass (1988) to study *in vitro* gas production. Three sets of *in vitro* trials in triplicates were conducted to estimate the effect of addition of different levels of sodium sesquicarbonate in diet/substrate on the various parameters such as IVGPT, partition factor, pH and microbial protein synthesis along with *in vitro* DM/OM degradability to obtain a complete picture for rumen fermentation pattern.

Analytical procedure

The substrate (sugargraze and concentrate; 200 mg) was weighed and placed into the bottom of the 100 mL graduated glass syringes without sticking it to the sides of syringe. The medium mixture solution and rumen liquor were mixed in the ratio 2:1 and immediately after thorough mixing, 30 mL of this incubation medium was injected to glass syringes (Haberle, Germany) using an auto dispenser. The level of piston was recorded (initial reading) and the syringes were placed in the incubator preadjusted at $39 \pm 0.5^\circ\text{C}$ for 24 h. After the completion of incubation period, total gas production was calculated after correcting corresponding blank values and pH of syringe contents was estimated with the help of digital pH meter (Model: pH Spear, Eutech Instruments, Malaysia). The contents of the syringe were then emptied into centrifuge tubes and centrifuged at 3000 rpm for 15 min till clear supernatant was obtained which was then preserved at -20°C for the estimation of individual volatile fatty acids (Erwin et al. 1961) using gas chromatograph (Nucon 5700, Nucon Engineers, New Delhi) equipped with flame ionization detector and stainless steel column packed with Chromosorb 101 mesh 80 – 100 (length 1.5 m; o.d. 3.175 mm; i.d. 2 mm). An aliquot of supernatant was acidified with equal volume of 0.5 M HCl and kept at -20°C for estimation of ammonia nitrogen by Kjeldahl method. True DM and OM digestibility of samples were estimated as per method described by Van Soest et al. (1991). The pellet left after centrifugation was refluxed in beakers with neutral detergent solution for 1 h and thereafter, contents were filtered through the sintered glass crucible (G-1) washed with hot water and kept in hot air oven at 100°C for drying. *In vitro* true dry matter digestibility IVTDMD was calculated as the difference between the weights of the DM incubated and the DM residue left. The residue in each crucible was ashed in muffle furnace at 550°C for 2 h to determine the *in vitro* true organic matter digestibility (IVTOMD). The partitioning factor (PF) and microbial biomass production (MBP) were calculated with the help of equations based on parameters like IVTDMD, IVTOMD, total gas and net gas volume (Blummel et al. 1997; Blummel and Lebzién, 2001).

Equations

$$\text{PF} = \frac{\text{In vitro true DM digested (mg)}}{\text{Total gas produced (mL)}}$$

$$\text{MBP (mg)} = \text{TDOM (mg)} - (\text{Net gas volume} \times 2.20)$$

Statistical analysis

The statistical analysis of the data was done using one-way analysis of variance (ANOVA) by SPSS, 2010 version 16.

Results and Discussion

Detailed chemical composition of the feed ingredients and the substrate prepared from them has been given in Table 1. The same substrate was used in both the control and treatment groups. The effect of different levels of sodium sesquicarbonate in 50C:50R and 60C:40R ration *in vitro* digestibility, pH, net gas production, PF and MBP is presented in Table 2 and IVFA and NH_3 in Table 3. In the present study, the mean value of pH in 60C:40R was found to be significantly ($P < 0.001$) higher than 50C:50R and there was no effect of buffer addition on pH value. Similarly, net gas production, IVDMD and IVOMD were also higher in the diet with 60C:40R composition. Diet with 50C:50R had higher values of MBP, PF and higher proportion of acetate; and lower in propionate. However, there was no difference in the levels of NH_3 and butyrate concentrations between the two diets. Hence, supplementation of sodium sesquicarbonate (0.5 upto 3%) and the interaction between diet and treatment had no effect on rumen fermentation parameters. The findings of the present study are consistent with those of Xu et al. (1994) who reported that supplementation of rumen buffers in lactating Holstein cows (1.5% and 2.2% of DMI) and observed no change in rumen fluid

Table 1 Chemical composition (% DM basis) of the substrate

Attribute	Sugargraze fodder	Concentrate mixture
DM	28.00	89.56
CP	10.20	19.53
EE	1.29	3.74
Ash	8.64	11.22
ADF	37.29	12.38
NDF	58.65	26.37
NDICP	5.30	1.94
ADICP	1.14	0.69
Hemicellulose	21.40	13.99
Cellulose	21.00	6.40
ADL	7.75	2.32
CHO	79.87	65.51
td NFC	26.00	40.26
td CP	9.70	19.25
td FA	0.30	2.74
td NDF	24.8	13.13
TDN	54.10	71.81

Table 2 Effect of different levels of sodium sesquicarbonate on in vitro digestibility, pH, net gas production, IVFA, PF and MBP

Parameter	Substrate	Level of supplementation (%)							Mean	SEM	P value		
		0	0.5	1	1.5	2	2.5	3			S	T	S*T
pH	50 R:50 C	6.64	6.58	6.54	6.60	6.56	6.56	6.59	6.58 ^a	0.02	<0.001	0.548	0.629
	40 R:60 C	6.84	6.83	6.87	6.82	6.79	6.83	7.08	6.86 ^b	0.04			
	Mean	6.74	6.71	6.71	6.72	6.67	6.69	6.83					
Net gas (mL/24h)	50 R:50 C	35.00	34.00	35.33	34.66	35.33	35.00	35.33	34.95 ^a	0.21	<0.001	0.985	0.773
	40 R:60 C	39.33	40.00	39.33	39.67	39.67	39.00	39.50	39.50 ^b	0.26			
	Mean	37.17	37.00	37.33	37.17	37.50	37.00	37.42					
IVDMD (%)	50 R:50 C	61.48	62.19	61.90	61.21	61.29	61.19	61.23	61.50 ^a	0.29	<0.001	0.804	0.966
	40 R:60 C	65.07	65.81	66.80	66.10	65.49	65.18	65.84	65.76 ^b	0.27			
	Mean	63.27	64.00	64.35	63.65	63.39	63.19	63.54					
IVOMD (%)	50 R:50 C	62.02	62.96	62.57	62.36	61.82	61.91	62.05	62.24 ^a	0.29	<0.001	0.888	0.991
	40 R:60 C	65.93	66.71	67.38	66.89	66.81	66.40	66.45	66.65 ^b	0.28			
	Mean	63.97	64.84	64.98	64.62	64.32	64.15	64.25					
PF	50 R:50 C	3.36	3.49	3.37	3.41	3.31	3.34	3.33	3.37 ^b	0.02	<0.001	0.728	0.528
	40 R:60 C	3.16	3.12	3.20	3.21	3.15	3.21	3.13	3.17 ^a	0.02			
	Mean	3.27	3.31	3.28	3.31	3.23	3.28	3.23					
MBP (mg)	50 R:50 C	38.98	42.42	39.48	40.11	37.56	38.28	38.29	39.31 ^b	0.63	0.005	0.766	0.696
	40 R:60 C	35.91	34.98	37.46	37.70	35.61	37.32	34.78	36.25 ^a	0.68			
	Mean	37.45	38.70	38.47	38.91	36.58	37.80	36.54					

C: Concentrate mixture, R: Roughage, S: Substrate, T: Treatmnet

Table 3 Effect of different levels of sodium sesquicarbonate on in vitro ammonia and volatile fatty acid concentration

Parameter	Substrate	Level of supplementation (%)							Mean	SEM	P value		
		0	0.5	1	1.5	2	2.5	3			S	T	S*T
NH ₃ -N (mg/dL)	50 R:50 C	9.71	9.89	9.52	9.24	9.52	9.52	9.33	9.53	0.12	0.404	0.833	0.658
	40 R:60 C	9.15	9.80	9.05	10.08	9.98	10.55	9.80	9.77	0.23			
	Mean	9.43	9.85	9.29	9.66	9.75	10.03	9.57					
Acetate (mM)	50 R:50 C	38.53	39.47	39.24	37.53	38.32	37.68	38.17	38.42 ^b	0.29	<0.001	0.704	0.467
	40 R:60 C	34.22	33.33	35.88	34.86	36.47	35.81	33.36	34.85 ^a	0.52			
	Mean	36.38	36.40	37.56	36.19	37.39	36.74	35.76					
Propionate (mM)	50 R:50 C	10.84	11.36	10.80	10.13	11.72	10.80	10.13	10.83 ^a	0.21	<0.001	0.883	0.696
	40 R:60 C	16.02	15.70	16.58	17.13	16.78	17.44	16.35	16.57 ^b	0.35			
	Mean	13.43	13.53	13.69	13.63	14.25	14.12	13.24					
Butyrate (mM)	50 R:50 C	7.51	7.04	6.97	7.64	8.09	6.97	7.29	7.36	0.16	0.091	0.753	0.857
	40 R:60 C	7.01	6.68	7.12	6.41	7.20	6.82	7.00	6.89	0.18			
	Mean	7.26	6.86	7.04	7.02	7.65	6.89	7.15					

C: Concentrate mixture, R: Roughage, S: Substrate, T: Treatmnet

pH and VFA molar percentage. The results of this study are in accordance with those of Umucalilar and Seker (2000) who carried out an *in vitro* experiment with different ratios of NaHCO₃ (0, 0.5, 1.0, 1.5%) and MgO (0, 0.25, 0.5 and 1). They found that NaHCO₃ supplementation had no effect on pH, buffering capacity, TVFA and gas production but increased the levels of NH₃ whereas MgO supplementation increased the values of pH, buffering capacity, TVFA and gas production. However, results are in disagreement with Patra and Yu (2013) who reported an decrease in molar percentage of acetate and acetate-to-propionate ratio, whereas

the molar percentage of propionate increased quadratically with increasing bicarbonate concentration. This difference could possibly be due to change in pH in the above study which varied from 6.0 to 6.38 in the above study which was not found in our study because of difference in both dose and source of bicarbonate added. Furthermore, bicarbonate is regularly now a days being supplemented to dairy cattle rations to reduce incidences of acidosis and to counteract milk fat depression by increasing molar percentage of acetate and decreasing molar percentage of propionate (Cruywagen et al. 2015). Therefore, there has been a

wide discrepancy in results of addition of buffers on comparison of *in vitro* and *in vivo* studies indicating that factors other than bicarbonate concentrations in media might also affect these results. In this study there was significant effect of incubation time on fermentation parameters. Our findings were also similar with those of Pereira and Armenanto (2000), Dschaak et al. (2010) and Bougouin et al. (2018) who observed no effect on digestibility supplementing NaHCO_3 in the diet. Grant and Mertens (1992) studied the effect of buffer pH (5.2, 6.2 and 6.8) on *in vitro* digestion kinetics and observed that low pH decreased fiber digestion. Mao et al. (2017) found that sodium bicarbonate supplementation (7% of substrate) under *in vitro* condition increased the final pH levels and the concentration of total volatile fatty acids and the proportions of acetate, propionate and total branched chain VFA were also affected ($p < 0.001$) by incubation time ($p < 0.001$) and interaction between incubation time and bicarbonate supplementation. They also found that total gas production was higher in the bicarbonate group but the concentration of $\text{NH}_3\text{-N}$ was almost similar among the control and bicarbonate supplemented group.

Conclusion

Addition of sodium sesquicarbonate upto 3 % of substrate did not show any significant effect on *in vitro* rumen fermentation parameters.

Acknowledgements

The authors thanks the Meera enterprises (Gujarat) and Director National Dairy Research Institute (Karnal) for the financial support.

References

- Abdela N (2016) Sub-acute ruminal acidosis (SARA) and its consequence in dairy cattle: A review of past and recent research at global prospective. *Achiev. Life Sci* 10: 187-196
- AOAC (2005) Official Methods of Analysis, 18th edition. Association of Official Analytical Chemists, Arlington, Virginia
- Bauman DE, Griinari JM (2003) Nutritional regulation of milk fat synthesis. *Annu Rev Nutr* 23: 203-227
- Blummel M, Lebzien P (2001) Predicting ruminal microbial efficiencies of dairy rations by *in-vitro* techniques. *Livest Prod Sci* 68: 107-117
- Blummel M, Makkar HPS, Becker K (1997) *In-vitro* gas production: a technique revisited. *J Anim Physiol Anim Nutr* 77: 24-34
- Bougouin A, Ferlay A, Doreau M, Martin C (2018) Effects of carbohydrate type or bicarbonate addition to grass silage-based diets on enteric methane emissions and milk fatty acid composition in dairy cows. *J Dairy Sci* 101: 6085-6097
- Cruywagen CW, Taylor S, Beya MM, Calitz T (2015) The effect of buffering dairy cow diets with limestone, calcareous marine algae, or sodium bicarbonate on ruminal pH profiles, production responses, and rumen fermentation. *J Dairy Sci* 98: 5506-5514
- Dschaak CM, Eun JS, Young AJ, Stott RD, Peterson S (2010) Effects of supplementation of natural zeolite on intake, digestion, ruminal fermentation, and lactational performance of dairy cows. *Professional Animal Scientist* 26: 647-654
- Erwin ES, Marco GJ, Emery EM (1961) Volatile fatty acid analyses of blood and rumen fluid by gas chromatography *J Dairy Sci* 44: 1768-1771
- Goering HK, VanSoest PJ (1970) Forage Fiber Analyses (apparatus, reagents, procedures and some applications). Agriculture Handbook No 379. ARS, USDA Washington DC
- Grant RH, Mertens DR (1992) Influence of buffer pH and raw corn starch addition on *in vitro* fiber digestion kinetics. *J Dairy Sci* 75: 2762-2768
- Humer E, Petri RM, Aschenbach JR, Bradford BJ, Penner GB, Tafaj M, Zebeli Q (2018) Invited review: Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. *J Dairy Sci* 101: 872-888
- Kang S, Wanapat M (2013) Using plant source as a buffering agent to manipulating rumen fermentation in an *in vitro* gas production system. *Asian-Australas J Anim Sci* 26: 1424
- Kmicikewycz AD, Heinrichs AJ (2014) Feeding lactating dairy cattle long hay separate from the total mixed ration can maintain dry matter intake during incidents of low rumen pH. *J Dairy Sci* 97: 7175-7184
- Mao S, Huo W, Liu J, Zhang R, Zhu W (2017) *In-vitro* effects of sodium bicarbonate buffer on rumen fermentation, levels of lipopolysaccharide and biogenic amine, and composition of rumen microbiota. *J Sci Food Agric* 97: 1276-1285
- Marden JP, Julien C, Monteils V, Auclair E, Moncoulon R, Bayourthe C (2008) How does live yeast differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cows?. *J Dairy Sci* 91: 3528-3535
- Menke KH, Steingass H (1988) Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Anim Res Dev* 28: 7-55
- Morgante M, Stelletta C, Berzaghi P, Ganesella M, Andrighetto I (2007) Subacute rumen acidosis in lactating cows: an investigation in intensive Italian dairy herds. *J Anim Physiol Anim Nutr* 91: 226-234
- Owens FN, Basalan M (2016) Ruminal fermentation. In *Rumenology*: 63-102
- Patra AK, Yu Z (2013) Effects of gas composition in headspace and bicarbonate concentrations in media on gas and methane production, degradability, and rumen fermentation using *in vitro* gas production techniques. *J Dairy Sci* 96: 4592-4600
- Pereira MN, Armentano LE (2000) Partial replacement of forage with nonforage fiber sources in lactating cow diets. II. Digestion and rumen function. *J Dairy Sci* 83: 2876-2887
- Springer Cham, Sen, AR, Santra A, Karim SA (2006) Effect of dietary sodium bicarbonate supplementation on carcass and meat quality of high concentrate fed lambs. *Small Rumin Res* 65: 122-127
- Umucalilar HD, Seker E (2000) Effects of sodium bicarbonate and magnesium oxide as buffers on *in vitro* digestibility of grains. *Veteriner Bilimleri Dergisi* 16: 129-135
- VanSoest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J Dairy Sci* 74: 3583-3597
- Xu S, Harrison JH, Riley RE, Loney KA (1994) Effect of buffer addition to high grain total mixed rations on rumen pH, feed intake, milk production, and milk composition. *J Dairy Sci* 77: 782-788