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

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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 NH_3-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 Griinari, 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 (pka = 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
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±0.5°C for 24 h. After the completion of incubation period, total gas production was calculate 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 1h 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 IVTMD 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 IVTMD, IVTOMD, total gas and net gas volume (Blummel et al. 1997; Blummel and Lebzien, 2001).

**Equations**

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

\[
\text{MBP (mg)} = \text{TDOM (mg)} – (\text{Net gas volume} × 2.20)
\]

**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 NH3 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 NH3 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 pH.

**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               |

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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 a 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

| Parameter         | Substrate | Level of supplementation (%) | Mean | SEM | P value |
|-------------------|-----------|------------------------------|------|-----|---------|
| pH                | 0 0.5 1 1.5 2 2.5 3 | S T S*T |
| 50 R:50 C         | 6.64 6.58 6.54 6.60 6.56 6.56 6.59 6.58<0.02 0.02 0.629 |
| 40 R:60 C         | 6.84 6.83 6.87 6.82 6.79 6.83 7.08 6.86<0.04 |
| Mean              | 6.74 6.71 6.71 6.72 6.67 6.69 6.83 |
| Net gas (mL/24h)  | 35.00 34.00 35.33 34.66 35.33 35.00 35.33 34.95<0.21 0.01 0.773 |
| 40 R:60 C         | 39.33 40.00 39.33 39.67 39.67 39.00 39.50 39.50<0.26 |
| Mean              | 37.17 37.00 37.33 37.17 37.50 37.00 37.42 |
| IVDMD (%)         | 61.48 62.19 61.90 61.21 61.29 61.19 61.23 61.50<0.29 0.01 0.966 |
| 40 R:60 C         | 65.07 65.81 66.80 66.10 65.49 65.18 65.84 65.76<0.27 |
| Mean              | 63.27 64.00 64.35 63.65 63.39 63.19 63.54 |
| IVOMD (%)         | 62.02 62.96 62.57 62.36 61.82 61.91 62.05 62.24<0.29 0.01 0.991 |
| 40 R:60 C         | 65.93 66.71 67.38 68.89 68.61 66.40 66.45 66.65<0.28 |
| Mean              | 63.97 64.84 64.98 64.62 64.32 64.15 64.25 |
| PF                | 3.36 3.49 3.37 3.41 3.31 3.34 3.33 3.37<0.02 0.01 0.528 |
| 40 R:60 C         | 3.16 3.12 3.20 3.21 3.15 3.21 3.13 3.17<0.02 |
| Mean              | 3.27 3.31 3.28 3.31 3.23 3.28 3.23 |
| MBP (mg)          | 38.98 42.42 39.48 40.11 37.56 38.28 38.29 39.31<0.63 0.05 0.696 |
| 40 R:60 C         | 35.91 34.98 37.46 37.70 35.61 37.32 37.48 36.25<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: Treatment

| Parameter         | Substrate | Level of supplementation (%) | Mean | SEM | P value |
|-------------------|-----------|------------------------------|------|-----|---------|
| NH₃-N (mg/dL)     | 0.5 1.5 2 2.5 3 | S T S*T |
| 50 R:50 C         | 9.71 9.89 9.52 9.24 9.52 9.52 9.33 9.53<0.12 0.12 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)      | 38.53 39.47 39.24 37.53 38.32 37.68 38.17 38.42<0.29 0.01 0.467 |
| 40 R:60 C         | 34.22 33.33 35.88 34.86 36.47 35.81 33.36 34.85<0.52 |
| Mean              | 36.38 36.40 37.56 36.19 37.39 36.74 35.76 |
| Propionate (mM)   | 10.84 11.36 10.80 10.13 11.72 10.80 10.13 10.83<0.21 0.01 0.696 |
| 40 R:60 C         | 16.02 15.70 16.58 17.13 16.78 17.44 16.35 16.57<0.35 |
| Mean              | 13.43 13.53 13.69 13.63 14.25 14.12 13.24 |
| Butyrate (mM)     | 7.51 7.04 6.97 7.64 8.09 6.97 7.29 7.36<0.16 0.09 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: Treatment

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

Table 3 Effect of different levels of sodium sesquicarbonate on in vitro ammonia and volatile fatty acid concentration
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 Armamento (2000), Dschaak et al. (2010) and Bougouin et al. (2018) who observed no effect on digestibility supplementing NaHCO₃ 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 NH₃-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.

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