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Comparison of two buffers on *in vitro* gas production, pH and NH_3-N concentration of feedstuffs for ruminants

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ABSTRACT

The objective of the study was to compare Menke et al.'s buffer (1979) with Zhao and Lebzien's buffer (2000) on gas production, pH and NH_3-N of 16 feedstuffs for ruminants in *in vitro* incubation. The gas production was recorded after incubation at 12 and 24 h. The pH and NH_3-N concentration of incubation residues were determined after incubation for 24 h. The results showed that there was no significant difference in *in vitro* gas production, pH and NH_3-N concentration between the two buffers ($P>0.05$). It was found that *in vitro* gas production, pH or NH_3-N concentration of the two buffers were significantly correlated ($P<0.05$). It was concluded that the two buffers had similar results on *in vitro* gas production, pH and NH_3-N concentration in *in vitro* incubation. It is suggested to compare these two buffers on volatile fatty acids (VFA) concentration and rumen microorganism activities in the future.

KEY WORDS: buffer, gas production, pH, NH_3-N, *in vitro* incubation

INTRODUCTION

Menke et al.'s buffer (1979) has been widely used in the determination of *in vitro* gas production. Zhao and Lebzien's buffer (2000) has been used for the measurement of utilizable crude protein, utilizable amino acids (Zhao and Lebzien, 2002) and utilizable true protein (Li and Zhao, 2007) of feedstuffs for ruminants. Some components of the two buffers are the same whereas others are different. When *in vitro* incubation technique is used in ruminant nutrition research, it is interesting to know whether Menke et al.'s buffer (1979) and Zhao and Lebzien's buffer (2000) have similar experimental results and could replace

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each other. Gas production, pH, NH_3-N concentration, VFA concentration and rumen microorganism activities are important indices of *in vitro* incubation. The aim of the study is to compare Menke et al.'s buffer (1979) and Zhao and Lebzien's buffer (2000) on gas production, pH and NH_3-N concentration in *in vitro* incubation. If no significant difference in these indices between the two buffers was found, then the further step of the study is to compare the two buffers on VFA concentration, rumen microorganism activities and other indices for complete comparison.

MATERIAL AND METHODS

Animal and feedstuffs

An adult Yellow cattle (550 kg BW), fitted with a rumen fistula, was used as the donor of rumen fluid. The animal was fed with lucerne hay twice daily and fresh water was freely available. Sixteen air-dried feedstuffs for ruminants, grounded through 3 mm sieve, were used for incubation. The air-dried feedstuffs were lucerne hay, brewer's dried grain, Chinese rye-grass, maize grain, cottonseed meal, peanut meal, rapeseed meal, sorghum grain, soyabean meal, straw, maize stover, soyabean stalk, wheat flour middling, wheat bran and wheat grain.

Buffers

Menke et al. (1979): Buffer A:13.2 g $CaCl_2 \cdot 2H_2O$, 10.0 g $MnCl_2 \cdot 4H_2O$, 1.0 g $CoCl_2 \cdot 6H_2O$ and 8.0 g $FeCl_3 \cdot 6H_2O$ were dissolved in 100 ml distilled water. Buffer B:39.0 g $NaHCO_3$ and 2.0 g NH_4HCO_3 were dissolved in 1000 ml distilled water. Buffer C: 5.7 g Na_2HPO_4 and 0.6 g $MgSO_4 \cdot 7H_2O$ were dissolved in 1000 ml distilled water. Deoxidize fluid: 4 ml 1 N NaOH and 0.625 g $Na_2S \cdot 9H_2O$ were dissolved in 95 ml distilled water. 0.1 ml buffer A, 200 ml buffer B, 200 ml buffer C, 40 ml deoxidize fluid and 400 ml distilled water were mixed. Then 600 ml mixed buffer were mixed with 300 ml rumen fluid.

Zhao and Lebzien (2000): Buffer A: 23.5 g $Na_2HPO_4 \cdot 12H_2O$, 12.5 g $NaHCO_3$ and 11.5 g NH_4HCO_3 were dissolved in 400 ml distilled water. Buffer B: 23.5 g NaCl, 28.5 g KCl, 6.0 g $MgCl_2 \cdot 6H_2O$ and 2.63 g $CaCl_2 \cdot 2H_2O$ were dissolved in 1000 ml distilled water. 50 ml buffer B was mixed with 400 ml buffer A. An adequate amount of distilled water was added to the mixed buffer to yield a final volume of 500 ml. Then 250 ml of the mixed buffer was diluted with 1000 ml distilled water and pre-warmed to 38°C. Then 312.5 ml rumen fluid were added and continuously gassed with CO_2.

Experimental design

Syringes with volume of 100 ml were used as incubation vessels. About 0.20 g of feed sample was weighed into each syringe. Each feed sample was incubated in two duplicates. The rumen fluid was taken 2 h after morning feeding. Thirty ml of buffer-rumen fluid mixture was transferred into each syringe. Three blank syringes that only contained buffer-rumen fluid mixture were used in incubation. The syringes were kept at 38°C in a water bath and shaken occasionally to mix the liquids with the solids. The incubation time was set to 24 h. The 12 and 24 h gas production were recorded. The 24 h pH was measured immediately after incubation. Then the incubation residues were filtered through four layers of surgical cloth and then distilled for determination of NH_3-N.

Statistical analysis

The SAS 9.0 software (SAS, 2005) was used for regression analysis of gas production, pH and NH_3-N between the two buffers.

RESULTS

The obtained results indicated (Table 1) that there was no significant difference in 12 and 24 h *in vitro* gas production between the two buffers. It was found that there was a significant linear regression relationship between 12 h *in vitro* gas production of Menke et al.'s buffer (1979) (x, ml/0.2 g air-dried feed) and that of Zhao and Lebzien's (2000) (y, ml/0.2 g air-dried feed): $y=0.76x +3.33$, $r^2=0.82$, $n=16$; $P<0.05$. A similar relationship was also found between 24 h *in vitro* gas production of Menke et al.'s buffer (1979) (x, ml/0.2 g air-dried feed) and that of Zhao and Lebzien's buffer (2000) (y, ml/0.2 g air-dried feed): $y= 0.73x +8.55$, $r^2=0.89$, $n=16$; $P<0.05$.

The results also indicated that pH and NH_3-N concentration after 24 h incubation had no significant difference ($P>0.05$) between the two buffers. The 24 h pH of Menke et al.'s buffer (1979) (x) and that of Zhao and Lebzien's buffer (2000) (y) were significantly correlated: $y=0.38x +4.29$, $r^2=0.38$, $n=16$; $P<0.05$. A similar relationship was also found between 24 h NH_3-N concentration of Menke et al.'s buffer (1979) (x, mmol/g air-dried feed) and that of Zhao and Lebzien's buffer (2000) (y, mmol/g air-dried feed): $y=0.80x +0.33$, $r^2=0.57$, $n= 16$; $P<0.05$.

Table 1. Gas production, pH and NH_3-N concentration of *in vitro* incubation

Feedstuffs	12 h gas production ml/0.2 air-dried feed		24 h gas production ml/0.2 air-dried feed		24 h pH		24 h NH_3-N mmol/g air-dried feed	
	Menke et al.'s buffer (1979)	Zhao and Lebzien's buffer (2000)	Menke et al.'s buffer (1979)	Zhao and Lebzien's buffer (2000)	Menke et al.'s buffer (1979)	Zhao and Lebzien's buffer (2000)	Menke et al.'s buffer (1979)	Zhao and Lebzien's buffer (2000)
Lucerne hay	27 ± 3	24 ± 2	35 ± 2	37 ± 2	7.10 ± 0.03	6.99 ± 0.03	0.88 ± 0.02	1.57 ± 0.13
Brewer's dried grain	14 ± 2	13 ± 2	24 ± 2	25 ± 2	6.86 ± 0.02	6.81 ± 0.05	0.84 ± 0.03	0.95 ± 0.01
Chinese rye-grass	14 ± 2	12 ± 2	23 ± 3	20 ± 2	6.88 ± 0.05	6.82 ± 0.03	0.65 ± 0.01	0.71 ± 0.04
Maize grain	29 ± 1	24 ± 1	67 ± 1	59 ± 1	6.72 ± 0.02	6.97 ± 0.02	1.06 ± 0.04	0.86 ± 0.02
Cottonseed meal	19 ± 1	12 ± 2	20 ± 2	27 ± 2	7.18 ± 0.01	7.09 ± 0.02	1.48 ± 0.06	1.02 ± 0.05
Peanut meal	27 ± 1	29 ± 1	36 ± 2	42 ± 1	6.99 ± 0.04	6.98 ± 0.03	2.03 ± 0.03	2.17 ± 0.05
Rapeseed meal	29 ± 1	24 ± 1	37 ± 1	40 ± 1	6.92 ± 0.04	7.03 ± 0.03	1.34 ± 0.04	1.23 ± 0.03
Sorghum grain	21 ± 1	16 ± 1	57 ± 3	46 ± 2	6.77 ± 0.03	6.93 ± 0.04	0.68 ± 0.02	1.05 ± 0.02
Soyabean meal	39 ± 1	28 ± 1	47 ± 1	36 ± 1	6.98 ± 0.02	7.00 ± 0.02	2.41 ± 0.15	2.99 ± 0.05
Soya beans	28 ± 6	35 ± 6	33 ± 6	41 ± 8	7.20 ± 0.04	7.05 ± 0.03	2.43 ± 0.02	1.95 ± 0.05
Rice straw	7 ± 1	10 ± 1	19 ± 1	17 ± 1	7.14 ± 0.03	7.03 ± 0.02	0.65 ± 0.05	1.37 ± 0.03
Maize stover	32 ± 2	29 ± 2	62 ± 5	56 ± 2	7.11 ± 0.03	7.02 ± 0.04	0.66 ± 0.01	1.56 ± 0.03
Soyabean stalk	16 ± 1	19 ± 1	29 ± 1	25 ± 1	6.86 ± 0.02	6.96 ± 0.02	1.42 ± 0.04	1.06 ± 0.04
Wheat bran	37 ± 1	33 ± 1	47 ± 1	40 ± 1	6.81 ± 0.02	6.95 ± 0.02	0.85 ± 0.01	0.86 ± 0.02
Wheat flour middling	46 ± 3	37 ± 2	71 ± 4	60 ± 3	6.81 ± 0.02	6.78 ± 0.03	0.82 ± 0.06	0.80 ± 0.04
Wheat grain	9 ± 1	9 ± 1	16 ± 1	21 ± 2	6.95 ± 0.01	6.82 ± 0.02	0.73 ± 0.05	0.44 ± 0.18

DISCUSSION

One reason for the results that *in vitro* gas production and pH had no significant difference between the two buffers could be that both buffers, Menke et al. (1979) or Zhao and Lebzien (2000) contained $NaHCO_3$ and Na_2HPO_4 as the main buffer salts. Another reason could be that the rumen fluid used in incubations contained some of minerals and trace elements which were important for incubation. Therefore, although the components of the two buffers were not the same, the difference in *in vitro* gas production, pH and NH_3-N between the two buffers would not reach statistically significant level.

CONCLUSIONS

There was no significant difference in *in vitro* gas production, pH and NH_3-N concentration between Menke et al. buffer (1979) and Zhao and Lebzien's buffer (2000). It is suggested to compare VFA concentration and rumen microbial activities in *in vitro* incubation between the two buffers in the future.

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