Journal of Middle East Applied Science and Technology (JMEAST)

ISSN (Online): 2305-0225
Issue 20(2), January 2014, pp. 57-60

In vitro effect of the adding of sodium hydroxide on rumen gas production of whole barley grain

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***Abstract*—** The purpose of this study was to examine the effect of treatment of whole barley grain (WBG) with sodium hydroxide (NaOH) on *in vitro* rumen gas production parameters. Experimental treatments included untreated whole barley grain ($B_{Control}$) and treated barley grain with NaOH+water (35 g + 220 ml/kg DM WBG) for 30 days (B_{NaOH}). *In vitro* gas production technique was used to determine the gas production parameters of the samples. The amount of produced gas at each time of incubation in each glass serum bottle was taken as the gas production parameter. Results of the *in vitro* technique revealed that alkali treatment of WBG caused an increase in the asymptotic gas volume (b) versus $B_{Control}$ (122.157 and 130.969 ml, respectively). Constant rate of gas production (c) significantly decreased by B_{NaOH} compared with $B_{Control}$ (0.0247 and 0.0351 ml/h, respectively). Furthermore, treatment of WBG caused a significant increase in the halftime of maximal gas production ($t_{1/2}$) versus $B_{Control}$ (28.05 and 19.74 h, respectively). The finding of the present study leads us to conclude that treatment of WBG with NaOH improved *in vitro* gas production parameters.

***Keywords*—** barley grain, gas production, in vitro, sodium hydroxide.

INTRODUCTION

WHOLE barley grain (WBG) is one of the most common starchy feed grains used in the diets of dairy and beef cattle, as it is a readily available source of dietary energy. Barley grain contains 80% carbohydrate on a dry matter basis, which is about 65% of the starch [1] .However, the amount of starch that can be included in the diets of dairy cows is limited as it increases incidence of acidosis, laminitis and other metabolic disorders [2]. In barley grain, endosperm is surrounded by the pericarp, which is overlain by a fibrous hull and is extremely resistant to microbial degradation in the rumen [3]. Therefore, barley grain needs to be processed in order to improve its utilization by beef and dairy cattle [3]. Processing makes the starch more accessible to rumen microbes and increases the digestive extent of starch degradation in the rumen [3]. Although processing is essential to maximize the utilization of barley grain by cattle, extensive grain processing increases ruminal starch degradation, which has a reductive effect on feed intake in ruminants [4]. Gas production technique widely used by nutritionists during the past decade has been used to study the digestion of food [5] - [6]. Treatment of barley grain with sodium hydroxide causes a decrease in rate of gas production, and an increase in the half time of maximal gas production ($t_{1/2}$) [7]. The objective of this experiment was to determine the in vitro impact of barley grain treatment with NaOH on rumen fermentation and digestion response using gas production technique.

MATERIALS AND METHOD

Chemical processing

In the present study an Iranian cultivar of barley grain (*Rihan-45*) containing 11.6% CP, 22.83% NDF and 5.98% ADF was used. The applied treatments were untreated whole barley grain ($B_{Control}$) and whole barley grain (WBG) treated with sodium hydroxide (B_{NaOH}). For chemical processing 35 g of NaOH was dissolved in 220 ml distilled water and was sprayed on 1 kg of WBG. Later, the WBG was packed in airless nylon bags. After 30 days, treated samples were

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allowed to aerate for 12 h. All samples were oven dried (65°C, 48 h), then ground through a 2-mm diameter screen [2].

In vitro gas production technique

In the current study, the gas production technique was conducted as described by Grings and Blümmel [8]. Samples (250 mg) were incubated with 20 mL incubation medium in triplicate. Substrate (250 mg) was weighed into a 120 ml glass serum bottle. Rumen content was collected from three rumen fistulated Holstein steers that were fed 5.1 kg of dry matter (DM) of alfalfa hay, 3.2 kg of DM corn silage and 2.5 kg of DM concentrate (170 g CP kg^{-1} of DM). Ruminal fluid and particulate matter, in the approximate proportion of 60:40, were collected before the morning feeding into a pre-warmed CO_2-filled thermos bottle. Rumen contents were homogenized in a blender and were subsequently strained through a nylon filter (40 µm pore size), then filtered through glass wool. The filtrate was mixed with carbonate buffer [containing ammonium bicarbonate at (4 g/l) and sodium bicarbonate (35 g/l), macromineral solution (5.7 g anhydrous Na_2HPO_4, 6.2 g anhydrous KH_2PO_4 and 0.6 g $MgSO_4 \cdot 7H_2O$ per liter), and deionized water in a ratio of 1:1:0.5:1.5]. Next, 0.1 ml micromineral solution (13.2 g $CaCl_2 \cdot 2H_2O$, 10.0 g $MnCl_2 \cdot 4H_2O$, 1 g $CoCl_2 \cdot 6H_2O$ and 8.0 g $FeCl_3 \cdot 6H_2O$ per 100 ml) was added per liter. The medium was reduced by the addition of 41.7 ml reducing agent (40 ml deionized water, 1 ml 1N NaOH and 1 g $Na_2S \cdot 9H_2O$) per liter of medium. Twenty milliliters of medium were dispensed into a 120 ml glass serum bottle containing 250 mg of each sample and placed upright in a 39◦C water bath. Blank samples (i.e., medium only, no substrate) were placed throughout the water bath and used to measure gas production from the medium alone. All handling of ruminal inoculum was under a constant stream of CO_2 and all the used containers were prewarmed and filled with CO_2.

Cumulative gas volume measurements of treated and untreated samples were recorded manually for the incubations from 3 runs in 6 replicates each after 2, 4, 6, 8, 10, 12, 14, 16, 24, 30, 36, 48, 54, 60, 72 and 96 h of incubation. After the subtraction of gas produced from blank serum bottles, the data was fitted to an exponential model [9] as:

$$y = b \times (1 - e^{-ct}) \quad (1)$$

Where 'y' is the cumulative volume of gas produced at time't' (h), 'b' is the asymptotic gas volume and 'c' is the constant rate of gas production.

Halftime of maximal gas production ($t_{1/2}$) [i.e., the time (h) when half of the asymptotic gas volume (b; ml) was produced] was calculated as:

$$t_{1/2} = \frac{\ln 2}{c} \quad (2)$$

Where 'ln 2' is about 0.693 and 'c' is the constant rate of gas production.

Calculations and statistical analysis

The data was analyzed using General Linear Models (GLM) procedures (SAS Institute Inc., Campus Drive Cary NC). The gas production procedure, repeated in 3 runs, was conducted as a complete randomized block design with the treatment as the main effect. Statistical model was $Yij=\mu+Ti+Bj+\varepsilon ij$, where Yij is the observation from treatment i, µ, the overall mean, Ti the mean of treatment, Bj, block and εij, the residual effect. Multiple comparisons among treatment means were performed by Duncan's New Multiple Range Test (DMRT).

RESULT

Cumulative gas production for each of the substrate treatments were presented as gas production curves (Figure 1). Gas production profiles for two treatment incubations were adequately described by the exponential model. As can be seen in fig. 1, treatment of whole barley grain with 3.5% NaOH leads to a decrease in gas volume production in contrast to untreated whole barley grain ($B_{Control}$).

Reference [10] expressed according to matched volume of gas produced and the parameters of *in sacco*, *in vitro* gas production technique correctly reflects substrate fermentation conditions. In addition, [11] reported that the digestibility of barley treated with sodium hydroxide with increasing levels of sodium hydroxide increased. Reference [12] concluded that treatment of barley grain with sodium hydroxide leads to slower rates of starch digestibility compared with rolled barley grain.

Values for asymptotic gas volume (b) and constant rate of gas production (c) after 96 h of *in vitro* incubation and halftime of maximal gas production ($t_{1/2}$) of $B_{Control}$ and B_{NaOH} were shown in table 1.

Asymptotic gas volume increased by B_{NaOH} in comparison to $B_{Control}$. Constant rate of gas production decreased by whole barley grain treatment with NaOH compared with $B_{Control}$. The effect of NaOH treatment of whole barley grain on constant rate of gas production between the two treatments was significant ($P<0.05$). Treatment of whole barley grain with NaOH significantly ($P=0.006$) increased the halftime of maximal gas production ($t_{1/2}$) versus $B_{Control}$.

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DISCUSSION

This research was conducted to investigate the effects of NaOH treatment of whole barley grain on in vitro rumen fermentation characteristics using gas production technique. The agreement of volume of gas with the in situ parameters indicates that in vitro gas production accurately reflects substrate fermentation [10]. The purpose of grain processing is to obtain a balance between maximizing the extent of ruminal starch fermentability and controlling the rate of starch fermentation in order to avoid digestive and metabolic disturbances [13]. Results of gas production measurement revealed that whole barley grain treated with NaOH resulted in a decrease in constant rate of gas production compared with $B_{Control}$. The reason is that slower digestion of treated barley results in a decrease in fluctuations in ruminal pH, and lower metabolic disorders of rumen such as acidosis. Reference [11] reported that the rate of digestion of NaOH-treated grain increased along with an increase in applying NaOH. Finally, [12] concluded that treating barley with 40 g NaOH/kg results in a slightly lower starch digestibility than if the grain is rolled. The major site of cereal grain starch digestion is usually the rumen. Processing increases microbial degradation of starch in the rumen and decreases amounts of starch digested post-ruminally [14]. The rate of gas production directly affects $t_{1/2}$. The effect of NaOH treatment on rate of gas production between the two treatments was significant (P<0.05) and thus time to half maximal gas production ($t_{1/2}$) of incubated barley grains significantly increased from 19.74 to 28.05 h in $B_{Control}$ and B_{NaOH} incubations, respectively.

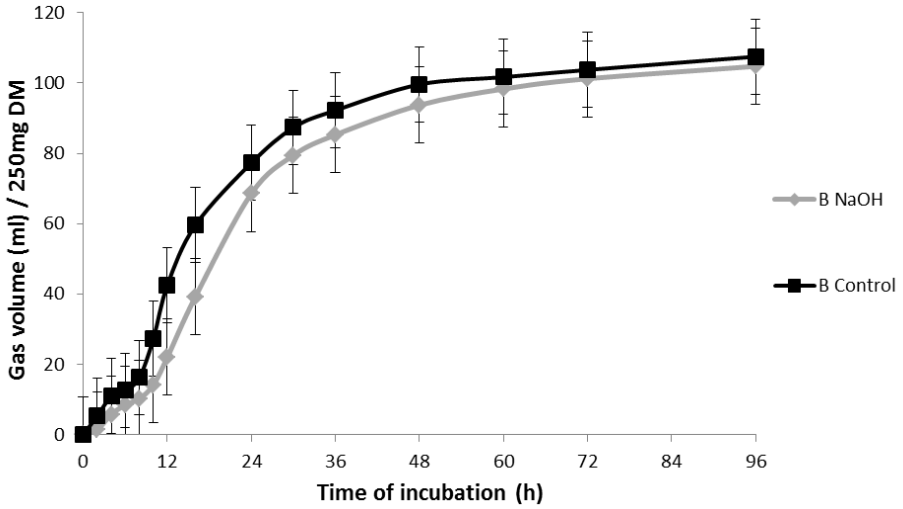
CONCLUSION

The findings of the present study lead us to conclude that treatment of whole barley grain with sodium hydroxide causes a decrease in rate of gas production, and an increase in the half time of maximal gas production ($t_{1/2}$). This might demonstrate a phenomenon under which a reduction in rumen starch digestion takes place.

ACKNOWLEDGMENT

The authors acknowledge the invaluable cooperation and support from the Deputy and Laboratory staff of the Ferdowsi University of Mashhad and the Excellence Center for Animal Science for facilitating the issue of this project.

Figure 1. In vitro gas production profiles of untreated ($B_{Control}$) and treated WBG with NaOH (B_{NaOH}), p<0.05.



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	Treatment		SEM [3]	P [4]
	Control [1]	NaOH [2]		
b (ml) [5]	122.157	130.969	4.751	0.4646
c (ml/h) [6]	0.0351 [a]	0.0247 [b]	0.003	0.0009
$t_{1/2}$ (h) [7]	19.74 [a]	28.05 [b]	2.401	0.0006

The data relating to the incubation of 250 mg of dry substrate (barley grain).
a,b Means within a row with different letters differ (P<0.05).
1 Untreated barley grain, 2 Treated WBG with NaOH (35 g + 220 ml/kg DM), 3 Standard error of mean,
4 Probability, 5 Asymptotic gas volumes, 6 Constant rate of gas production, 7 Halftime of maximal gas production [the time (h) when half of the asymptotic gas volume (b; ml) was produced] was calculated as:
$t_{1/2} = \frac{\ln 2}{c}$.

Table 1. Asymptotic gas volume (b), constant rate of gas production (c) and halftime of maximal gas production ($t_{1/2}$) of untreated and treated WBG with NaOH.

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