

Influence of sodium hydroxide treatment of barley grain on *in vitro* rumen gas production and microbial-N yield

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ABSTRACT

The objective of this study was to investigate the effect of treatment of whole barley grain (WBG) with sodium hydroxide (NaOH) on *in vitro* rumen gas production parameters and microbial protein synthesis. 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. Furthermore, gas volume, apparent substrate degradability of barley grain (ASD), true substrate degradability of barley grain (TSD) and microbial N at $t_{1/2}$ for two treatments were measured. 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 a significant increase in the asymptotic gas volume (b) versus B_{Control} (113.71 and 162.86 ml, respectively). Constant rate of gas production (c) significantly decreased by B_{NaOH} compared with B_{Control} (0.018 and 0.034 ml/h, respectively). No effects of NaOH treatment were observed on ASD and TSD of whole barley grain. However, rumen microbial-N yield considerably ($p < 0.05$) built up (from 1.922 to 4.087 mg) from the treatment of barley with NaOH. The finding of the present study leads us to conclude that treatment of WBG with NaOH improved *in vitro* gas production parameters and microbial protein synthesis.

Keywords: barley grain, *in vitro*, gas production, microbial-N

Abbreviations: ASD, apparent substrate degradability; B_{Control} , untreated whole barley; B_{NaOH} , treated whole barley with NaOH; CP, crude protein; WBG, whole barley grain; DM, dry matter; MN, microbial N; ND, neutral detergent; NDFN, N content of truly undegraded residue; TSD, true substrate degradability; $t_{1/2}$, half time of maximal gas production.

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1. INTRODUCTION

Barley grain 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. 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.¹ 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.² Therefore, barley grain needs to be processed in order to improve its utilization by beef and dairy cattle.² Processing makes the starch more accessible to rumen microbes and increases the digestive extent of starch degradation in the rumen.² 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.³ Additionally, it may alter microbial yield in the rumen. Microbial biomass is the major source of protein for the ruminant host animal and prediction of efficiency of microbial production (EMP) can be crucial in ruminant nutrition.^{4,5} Several ruminant feeding systems^{6,7} include EMP to predict animal performance. However, lack of a practical laboratory technique to detect feed-specific differences in EMP is problematic. It has been proposed that variations in EMP can be detected by a combination of *in vitro* gas volume measurements⁸ with a concomitant measure of true substrate degradability (TSD).^{9,10} The difference between the measures is assumed to be microbial biomass. The objective of this experiment was to determine the *in vitro* impact of barley grain treatment with NaOH on rumen fermentation, digestion response, and microbial protein synthesis using gas production technique.

2. MATERIALS AND METHOD

2.1. Chemical processing

In the present study an Iranian cultivar of barley grain (*Dasht*) containing 13.2% CP, 21.05% NDF and 6.78% ADF was used.¹¹ 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 allowed to aerate for 12 h. All samples were oven dried (65°C, 48 h), then ground through a 2-mm diameter screen.¹

2.2. *In vitro* gas production technique

In the current study, the gas production technique was conducted as described by Blümmel and Lebzien¹² and Grings and Blümmel.¹³ 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 CO₂-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 Na₂HPO₄, 6.2 g anhydrous KH₂PO₄ and 0.6 g MgSO₄•7H₂O per liter), and deionized water in a ratio of 1:1:0.5:1.5]. Next, 0.1 ml micromineral solution (13.2 g CaCl₂•2H₂O, 10.0 g MnCl₂•4H₂O, 1 g CoCl₂•6H₂O and 8.0 g FeCl₃•6H₂O per 100 ml) was added per litre. The medium was reduced by the addition of 41.7 ml reducing agent (40 ml deionized water, 1 ml 1N NaOH and 1 g Na₂S•9H₂O) per litre of medium. Twenty millilitres 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 CO₂ and all the used containers were pre-warmed and filled with CO₂.

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 model¹⁴ as:

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

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

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

2.3. *In vitro* digestibility and microbial-N production

After the initial 96 h gas run, $t_{1/2}$ was calculated and a second incubation with the samples was conducted to obtain degradability measures at substrate-specific times (i.e., $t_{1/2}$ for each substrate). Collection and handling of ruminal fluid was the same as that described for the 96 h incubations. For each sample and each run ($n = 3$), 6 serum bottles were prepared, providing 3 serum bottles for apparent and 3 for true degradability measures. The incubations were terminated at $t_{1/2}$ and the volume of gas was recorded. True substrate degradability of samples⁹ at $t_{1/2}$ was measured by refluxing the incubation residue with neutral detergent (ND) solution (prepared without sodium sulphite) for 1 h with subsequent recovery of the truly undegraded substrate in sintered glass crucibles of porosity 'C' (i.e., 40–60 μm). Apparent substrate degradability was determined and calculated at $t_{1/2}$ by high-speed centrifugation of incubation residue (20000 $\times g$, 20°C, 10 min)¹² following placement into an iced water bath (14°C) to stop fermentation. Six 20 ml aliquots of media were collected, as were 4 blanks without substrate (20 ml) for each $t_{1/2}$. All blank samples were centrifuged and the supernatant was siphoned off, frozen (–20°C) and stored. Residue was weighed and used to correct apparent substrate degradability determinations for residue from the ruminal inoculum.

Microbial N production at $t_{1/2}$ was directly estimated using the N content of the apparently degraded residue remaining after centrifugation (pellet N) and N content of truly undegraded residue (NDFN) at $t_{1/2}$, using the equation¹³:

$$\text{Microbial-N production at } t_{1/2} = \text{pellet N at } t_{1/2} - \text{blank pellet N at 0h incubation} - \text{NDFN at } t_{1/2}.$$

2.4. Calculations and statistical analysis

The data was analysed using General Linear Models (GLM) procedures (SAS Inst. Inc., Ca 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 $Y_{ij} = \mu + T_i + B_j + \epsilon_{ij}$, where Y_{ij} is the observation from treatment i , μ , the overall mean, T_i the mean of treatment, B_j , block and ϵ_{ij} , the residual effect. Multiple comparisons among treatment means were performed by Duncan's New Multiple Range Test (DMRT).¹⁵

3. RESULTS

3.1. *In vitro* gas production profiles

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.

Values for asymptotic gas volume (b) and rate of gas production (c) after 96 h of *in vitro* incubation and halftime of 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 barley grain treatment with NaOH compared with B_{Control} . The effect of NaOH treatment of barley grain on constant rate of gas production between the two treatments was significant ($p < 0.05$). Treatment of barley grain with NaOH significantly ($p = 0.007$) increased the halftime of gas production ($t_{1/2}$) versus B_{Control} .

3.2. Gas volumes and apparent and true substrate degradability at $t_{1/2}$ of gas production

The data of gas volumes at $t_{1/2}$ and apparent (ASD) and true (TSD) degradability of B_{Control} and B_{NaOH} are illustrated in Table 2.

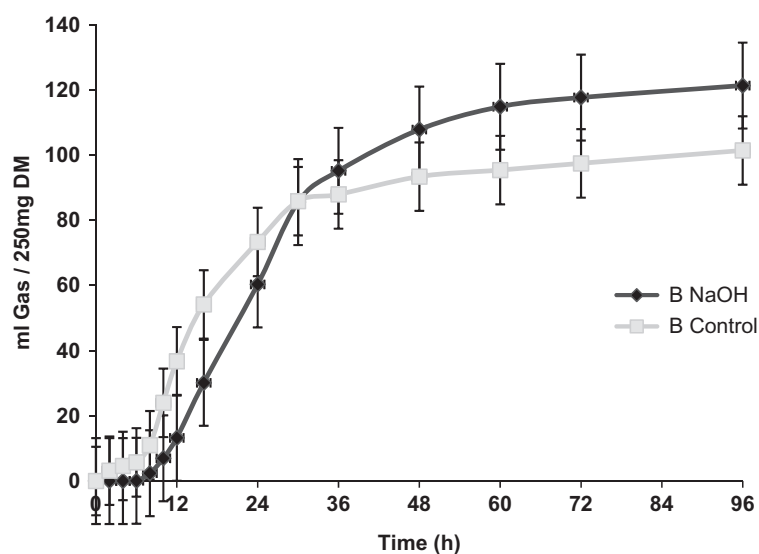


Figure 1. *In vitro* gas production profiles of untreated ($B_{Control}$) and treated WBG with NaOH (B_{NaOH}), $p < 0.05$.

Barley grain treatment with NaOH led to an increase in the gas volume, ASD and TSD in contrast to $B_{Control}$. The effect of NaOH treatment on gas volume between two treatments was significant ($p < 0.05$).

3.3. *In vitro* rumen microbial-N yield

Values for *in vitro* pellet-N, nitrogen content of truly undegraded residue (NDFN) and microbial nitrogen (MN) of $B_{Control}$ and B_{NaOH} were shown in Table 3.

Pellet nitrogen and MN increased with treatment of barley grain with NaOH compared with $B_{Control}$, however, NDFN decreased. Differences between two treatments for pellet nitrogen and MN were significant ($p < 0.05$). There was no significant difference between treatments for NDFN.

4. DISCUSSION

The present study was conducted to evaluate the influences of NaOH treatment of WBG on degradability and digestibility characteristics and microbial nitrogen yield 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.¹⁶ The objective 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.¹⁷

Results of gas production measurement revealed that WBG 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. Ørskov and Greenhalgh¹⁸ reported that the rate of digestion of NaOH-treated grain increased along with an increase in applying NaOH. Finally, Barnes and Ørskov¹⁹ concluded that

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

	Treatments		SEM ³	p-value ⁴
	$B_{Control}$ ¹	B_{NaOH} ²		
b^5 (ml)	113.717 ^a	162.863 ^b	7.429	0.042
c^6 (ml/h)	0.0346 ^a	0.0183 ^b	0.0007	0.003
$t_{1/2}$ (h) ⁷	20.03 ^a	37.92 ^b	1.064	0.007

^{a,b}Means within a row with different letters differ ($p < 0.05$).

¹Untreated barley grain, ²Treated WBG with NaOH (35 g + 220 ml/kg DM), ³Standard error of mean,

⁴Probability, ⁵Asymptotic gas volumes, ⁶Constant rate of gas production, ⁷Halftime of gas production [the time (h) when half of the asymptotic gas volume (b; ml) was produced] was calculated as: $t_{1/2} = \ln 2/c$.

Table 2. Gas volumes at $t_{1/2}$, apparent (ASD) and true (TSD) substrate degradability of untreated and treated WBG with NaOH.

	Treatments		SEM ³	p-value ⁴
	Control ¹	NaOH ²		
Gas volume (ml)	36.345 ^a	46.332 ^b	0.911	< 0.0001
ASD (mg) ⁵	171.66	188.33	7.031	0.124
TSD (mg) ⁶	220.66	233.66	3.358	0.052
ASD (%)	68.66	75.33	2.812	0.124
TSD (%)	79.73	85.20	3.242	0.299

The data relating to the incubation of 250 mg of dry substrate obtained by terminating the incubation at $t_{1/2}$.

^{a,b}Means within a row with different letters differ ($p < 0.05$).

¹Untreated barley grain, ²Treated WBG with NaOH (35 g + 220 ml/kg DM), ³Standard error of mean,

⁴Probability, ⁵Apparent substrate degradability of barley grain, ⁶True substrate degradability of barley grain.

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.²⁰

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 20.03 to 37.92 h in B_{Control} and B_{NaOH} incubations, respectively. In this study, treatment of barley grain with NaOH caused an increase in the gas volume in contrast to B_{Control} . This is because barley treatment resulted in an increase in potential gas production and a consequent rise in gas volume. Apparent substrate degradability of barley grain (ASD) and true substrate degradability of barley grain (TSD) increased with NaOH-treated barley versus B_{Control} . It may originate from reducing the resistance of seed coat by treatment. Dehghan-banadaky et al.¹ reported that NaOH treatment of whole barley disrupts the seed coat by partial hydrolysis of hemicellulose and lignin and causes a swelling of the outer starch granules, so the ruminal bacteria and enzymes gradually gain access to the starchy endosperm. This confirmed the findings of Ørskov et al.,²¹ who reported that for whole barley, the digestibility of all fractions improved by treatment with NaOH. In addition, Barnes and Ørskov²⁰ indicated that the digestibility of starch increased linearly with NaOH application. The present experiment showed a significant ($p < 0.05$) difference between the two treatments for estimating the microbial-N yield by applying *in vitro* technique. In comparison with B_{Control} , NaOH-treated barley significantly increased MN and because of MN's key role in protein synthesis, the microbial protein synthesis improved.

5. CONCLUSION

The findings of the present study lead us to conclude that treatment of WBG 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. NaOH-treatment of WBG enhanced ASD and TSD; therefore, it indicates an improvement in rumen barley availability. To sum up, treatment of WBG with NaOH causes an improvement in rumen microbial-N production and therefore, it may increase nutritive value of the grain in ruminants.

Table 3. Nitrogen of pellet, N content of truly undegraded residue (NDFN), microbial nitrogen (MN) of untreated and treated WBG with NaOH at $t_{1/2}$.

	Treatments		SEM ³	p-value ⁴
	Control ¹	NaOH ²		
Pellet N (mg) ⁵	3.239 ^a	5.219 ^b	0.403	0.025
NDFN (mg) ⁶	1.316	1.131	0.240	0.617
MN (mg) ⁷	1.922 ^a	4.087 ^b	0.266	0.004

^{a,b}Means within a row with different letters differ ($p < 0.05$).

¹Untreated barley grain, ²Treated WBG with NaOH (35 g + 220 ml/kg DM), ³Standard error of mean, ⁴Probability,

⁵N content of the apparently degraded residue remaining after centrifugation, ⁶N content of truly undegraded residue, ⁷Microbial N production at $t_{1/2}$, MN = pellet N at $t_{1/2}$ - blank pellet N at 0 h incubation - NDFN at $t_{1/2}$.

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