PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 8 (9): 1411-1414, 2009
ISSN 1680-5194

Effect of Microbial Degradation Lignin on Fermentation Characteristic of Distillers Grain *In vitro*

B.G. Zhao[1,2], Z.S. Wang*[1,2], A.G. Zhou[1,2], L.Z. Wang[1,2] and Q.L. Yu[1,2]
[1]Institute of Animal Nutrition, Sichuan Agricultural University, Ya'an, 625014, China
[2]Engineering Research Center of Animal Disease, Resistance Nutrition of China, Ministry of Education, Sichuan Agricultural University, Ya'an, 625014, China

Abstract: The experiment was conducted to determine the Gas Production (GP) of untreated and degraded distillers grain (namely distillers grain's lignin by microorganism fermentation degeneration) *in vitro* at 2, 4, 6, 8, 12, 24 and 48 h. And determine the pH, Ammonium condition nitrogen (NH_3-N), Volatile Fatty Acid (VFA) and Microbial Crude Protein (MCP) after 48 h. Thus inspected the effect of degraded lignin to digestion and utilization of distillers grain. There were two treatments and each had six replicates. The blank fermented liquid came from the treatment that did not adding sample of distillers grain. The results showed that: the GP of each treatment raised by the time extend. The GP of the degraded distillers grain were significantly higher than the untreated distillers grain at corresponding time ($p<0.05$). The highest GP of the degraded distillers grain was 21.45 ml. It was increased by 28.83% compared with the untreated distillers grain after 48 h, the pH of degraded distillers grain was 6.72. It was significantly lower than the blank group and the untreated distillers grain ($p<0.05$). The NH_3-N, MCP, VFA were 35.23 mg/100 ml, 57.40 mg/100 ml, 56.33 mmol/L respectively. It was all significantly higher than the blank group and the untreated distillers grain ($p<0.05$). The proportion of acetic was increased, but the proportion of propionate and butyrate were lower than the blank group and the untreated distillers grain. These results indicated that degraded lignin can raise the digestibility and availability of distillers grain in rumen.

Key words: Gas production, distillers grain, lignin

INTRODUCTION

Lignin which connected with cell wall polysaccharides restricts the degradation of cell wall polysaccharide. Roughage such as straw has poor feed effect for a long time and the degradation rate was only 30% ~50% (Cheason *et al*., 1993). Only increased the content of lignin does not affect the activity of cellulase. It was only slightly affected at high concentrations (Akin and Benner, 1988). The existence of lignin lead poor palatability and reduce the rumen degradation rate of feed. A large number of lignin in ruminant coarse fodder affect nutrient digestion and utilization and makes limited use of natural straw. Some could be used as the coarse fodder industrial slag, such as distillers grain, the bagasse, the alcohol fuel and so on, were also facing the same difficult position. If the problem of lignin can be solved, it will alleviate the strictly lack of foodstuff for people and livestock and also enhance the breeding efficiency and ecological benefits to a certain extent. At present, in domestic and foreign concentrates to the industry bad dregs's was used to product enzymes preparation, protein, or to fed directly with simply disposed. The degradation of lignin is still at the stage of research. The report on the biological treatment of lignin for industrial slag has not be reported. This study purpose on the GP *in vitro* and fermentation characteristics of untreated and degraded distillers grain, thus provides the essential data for the distillers grain's effective use.

MATERIALS AND METHODS

The distillers grain was provided by the Ya'an Shaping Town spring scene white liquor factory. (Brewing raw materials were mainly for corn, thick rice polishings, rice husk and rice husk which accounted for about 50%). Using phanerochaete chrysosporium fermented distillers grain 20 d at 38°C confined to degrade lignin, fermentation moisture was 65%, inoculum size was 10%.

Nutrient analysis: Untreated and degraded distillers grain's Dry Material (DM), Crude Protein (CP), crude fat (EE) and crude ash (Ash) determined by reference to AOAC (1990) and Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL) and Acid Insoluble Ash (AIA) was determined by the method of Van Soest *et al.* (1991). Part of nutrient composition is shown in Table 1. The ADL of degraded distillers grain compared untreated to reduce 22.47%. The levels of CP, NDF and ADF declined slightly.

Animals and feeding: Three *nan-jiang goats* with the weight of 23±2 kg which installated of permanent rumen fistula were rumen fluid donor animals. The ratio of concentration and roughages is 3:7. And coarse fodder for perennial ryegrass and alfalfa mixed 1:1. The feeding amount was 1.5% of body weight.

Table 1 the nutritional ingredient and the ratio of AIA about distillers grain*

	CP	NDF	ADF	ADL	AIA (%)
Untreated distillers grains	2.63	11.18	7.38	3.07	5.89
Degraded distillers grains	3.07	8.82	6.09	2.32	6.98

*The total quantity of various nutrient exception AIA changed in the fermentative process, therefore making the indicator by AIA, the content of other nutrients was in the ratio with the AIA in order to form a true reflection of changes in nutrients

Equipment: The fermentor is 150 ml glass syringe and the minimum scale is 1 ml. There was about 5 cm soft silicone rubber tube onto the front of glass syringe and fixed end clip to prevent the leakage. Washing and drying the syringe before using, coating evenly with a small amount of vaseline around the piston to prevent leakage and reduce the gases when the piston was movementing. Precise temperature control with automatic constant temperature water bath shaker simulated rumen environment. The temperature was 39°C.

Experimental design and determination: The design was single-factor. Untreated and degraded distillers grain fermented respectively *in vitro*. There were treatments and each had six replicates. And there were two blank group that did not adding any sample but fermentation fluid.

GP: Taking 200 mg dry sample put into the syringe at the bottom of the glass syringe and added 30 ml fermentation fluid, then wabbled 3 min (substrate and culture medium fully mixed) before wabbling in water bath at 39°C, evaluate the GP of at 2, 4, 6, 8, 12, 24 and 48 h respectively. The preparation of culture medium and process of experiment operation were refer to *ruminant nutrition* (Feng, 2004). The GP at the certain time was equivalent to the training period of GP minus the average of three control group s' GP of the corresponding period.

Fermentation parameters: Evaluate the pH, NH_3-N,VFA and MCP of nutrient fluid after 48 h.

After the GP of 48 h, rapidly discharged the fermentation broth into 50 ml centrifuge tube and immediately measured the pH, centrifugation (8000 x g, 5 min), took out 5 ml supernatant, added 0.5 ml 25% solution of metaphosphoric acid. After mixed the test liquid, stored at -20°C for more than 24 h. After thawing 10000 x g centrifugation for 15 min, collected the supernatant for determination of VFA (determined by CP-3800 gas chromatograph-type). Part of the supernatant was taken for determination of NH_3-N (Bu, 2006). All test liquid were stored at -20°C. Take 10 ml fermentation broth for 4000 r/min 15 min. Take the supernatant for 6000 x g 30 min. The precipitation was collected and washed 2 times with normal saline. Then diverted the precipitation into the digestion flask to determination MCP (Ling *et al*., 2007; Michael and James, 1982).

Statistical analysis: Statistical analysis was performed using SPSS14.0. Data was analyzed by one-way ANOVA. The form of results was expressed by the mean± standard deviation. Duncan multiple comparisons were used to test the differences between treatments, which were denoted by different letter superscripts. Statistical significance was accepted at $p<0.05$.

RESULTS

GP: The results showed that: the GP of each treatment raised by the time extend ($p<0.05$) as shown in Fig. 1. The GP of the degraded distillers grain were significantly higher than the untreated distillers grain at the corresponding time ($p<0.05$) as shown in Table 2 and achieved 21.45 ml and 16.65 ml respectively at 48 h. The degradation of lignin elevated the rapidly GP of distillers grains (a), the slowly part of GP (b) and the parameter of GP (c) ($p>0.05$). This meant that more carbohydrate of degraded distillers grain were degraded faster.

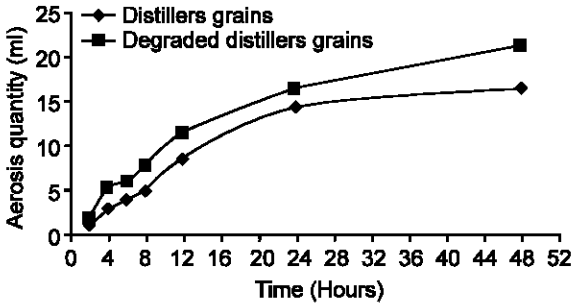

Fig. 1: Effects of fermentation on aerosis quantity of distillers grains

Table 2: The GP of different times and different treatments*

		GP (ml)	
Fermentation time (h)		Distillers grains	Degraded distillers grains
2		1.00±0.01^a	1.83±0.32^h
4		3.00±0.01^b	5.41±0.51^i
6		4.00±0.01^c	6.07±0.43^g
8		5.00±0.01^d	7.98±1.51^k
12		8.59±0.21^e	11.47±1.0^l
24		14.58±0.41^h	16.46±1.0^m
48		16.65±0.45^i	21.45±1.21^n
Parameter	a (ml)	-0.33±0.01	0.20±0.01
	b (ml)	16.98±0.13	21.25±0.52
	c	0.054±0.004	0.070±0.007

*It means having a different superscript at the same line is not significantly different ($p<0.05$), the contrary is not significant

Table 3: Content of PH, NH_3-N, MCP and VFA of culture medium supernate fluid at the end of fermentation*

	Treatments		
Items	The Blank Group	Untreated Distillers Grains	Degraded Distillers Grains
pH	6.98±0.02[a]	6.86±0.04[b]	6.72±0.03[c]
NH_3-N (mg/100ml)	5.03±0.31[a]	29.31±0.53[b]	35.23±0.45[c]
MCP (mg/100ml)	30.23±1.06[a]	50.60±1.21[b]	57.40±1.40[c]
Total VFA (mmol/l)	20.12±0.57[a]	45.30±1.21[b]	56.33±1.01[c]
Acetate: Total VFA	60.23±0.31[a]	67.01±0.52[b]	71.78±0.31[c]
Propionate: Total VFA	22.25±0.22[a]	19.23±0.34[b]	17.18±0.35[c]
Butyrate: Total VFA	17.52±0.19[a]	13.76±0.26[b]	11.03±0.17[c]

*It means having a different superscript at the same line is not significantly different ($p<0.05$), the contrary is not significant

Parameters of fermentation: As shown in Table 3, compared to the blank group, the treatments of degraded distillers grains and untreated distillers grains were significantly reduced PH of fermentation broth ($p<0.05$). The PH of degraded distillers grain was the lowest ($p<0.05$). It was 6.72. The depress of PH with the content of VFA in the fermentation broth were negative correlation. It meant that fermentation distillers grain was conducive to microbial degrade carbohydrates.

After fermenting 48 h *in vitro*, the density of NH_3-N of blank group, degraded distillers grain and untreated distillers grain were 5.03±0.31 mg/100 ml, 29.31±0.53 mg/100 ml and 35.23±0.45 mg/100 ml respective as shown in Table 3. The density of NH_3-N of blank group was the lowest ($p<0.05$) and the degraded distillers grain's was the highest ($p<0.05$). It meant that dealing distillers grain with microbial could enhance the degradation rate of CP.

As shown in Table 3, the treatments of degraded distillers grain and untreated distillers grain had improved the total of VFA ($p<0.05$) *in vitro*. And degraded distillers grain's total VFA was the highest ($p<0.05$). It meant that degraded distillers grain was in favor of the degradation of fermentable carbohydrates in rumen. The acetate to total VFA of degraded distillers grain is 71.78%. It was significantly higher than the untreated distillers grain ($p<0.05$). This result was concordant with the decrease of PH in fermentation broth. It reflected the carbohydrates of degraded distillers grain were degraded more sufficiently.

As shown in Table 3, the treatments of degraded distillers grains and untreated distillers grains were significantly higher than the blank group ($p<0.05$). The amount of MCP of degraded distillers grains is 57.40±1.40 mg/100 ml. It was significantly higher than the untreated distillers grains 50.60±1.21 mg/100 ml ($p<0.05$). The amount of MCP reflect the utilization of nutrients by rumen microbes. The increase of MCP showed that rumen micro-organisms can utilize the nutrients of degraded distillers grains to synthesis the microbial protein preferably.

DISCUSSION

In this experiment, the GP of degraded distillers grain at 48 h was 21.45 ml. It was 28.83% higher than untreated distillers grain. The result of this trial indicated that using phanerochaete chrysosporium fermented distillers grain of lignin can significantly improve the GP of distillers grain *in vitro* fermentation ($p<0.05$). Degraded the lignin may be released the cell wall polysaccharides which closely integrated with the lignin and produced more easy to ferment organic matter. Gamble *et al*. (1994) reported that the total GP of Bermuda grass which had decomposed of lignin improved 21% compared with not decomposed. Bu (2006) reported that carbohydrates was the main material for aerogenesis and it directly impact on GP *in vitro* fermentation. Blummel *et al*. (1997) found the GP *in vitro* fermentation had a high positive correlation with the apparent degradation rate of carbohydrates in vitro fermentation ($p<0.001$). Armenante *et al*. (1994) indicated that through biological treatment, the lignin of the plant cell wall can be destroyed and the contents were released. Exposed these available materials can make the rumen microbes easier and more comprehensive to utilize. So it increased the utilization efficiency of these available materials. The increase of GP showed that using phanerochaete chrysosporium fermented lignin can improve the apparent digestibility of nutrients of distillers grain *in vitro*.

The results of fermentation parameyers after 48 h *in vitro* showed that PH of the fermentation broth for degraded distillers grain significantly degrade ($p<0.05$) and the content of NH_3-N, VFA and MCP significantly increased ($p<0.05$), all were higher than untreated distillers grain. Gao and Qing (2003) indicated that degradation rate of feeding increased can lead to pH of the fermentation broth decreased and the concentration of NH3-N, VFA increased. Shi (2006) reported that the DM, CP, NDF and ADF degradation rate of every treatment increased, pH gradually become smaller. But the acetate, propionate and butyrate gradually increased. The test results were similar with previous studies. Hiltner and Dehority (1983) also reported that the degradation rate of carbohydrate increased can lead the reduced of PH. The degraded distillers grain's PH was 6.72. Depeter and Bath (1986) thought the rumen maintain's PH between 6.6-6.8 can guarantee the eligible circumstances to digestion fiber. The density of NH_3-N of degraded distillers grain was 35.23 mg/100 ml. And Satter and

Slyter (1974) thought the density of NH_3-N in fermentation broth maintain between 20-50 mg/100 ml can guarantee the rapid growth of microbes. The test result showed that the digestibility and utilization of distillers grain's nutrients improved after fermenting and degrading lignin by microbe.

Conclusion: The distillers grain's nutrients were more easily degested and utilized by ruminants after fermenting and degrading lignin by microbe.

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