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Influence of Sulfur on the Fermentation Characteristics of Corn Distiller's Dried Grains with Solubles in *In Vitro* Culture

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ABSTRACT

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The effects of sulfur on the fermentation characteristics of corn distiller's dried grains with solubles (DDGS) in *in vitro* culture were investigated. Samples (DDGS) were analyzed for nutrient values and then two independent *in vitro* experiments were conducted to study the effects of various sulfur sources (Na_2S, $Na_2S_2O_4$, Na_2SO_3, and Na_2SO_4) and different sulfur levels (0.346, 0.692, and 1.038%) on the fermentation characteristics of DDGS. Based on sampling and chemical composition analysis, there existed a great variation in the concentrations of sulfur and proximate nutrients of DDGS. In Experiment 1, sulfur source showed a significant ($P < 0.01$) effect on the gas production parameters (asymptotic gas production (b) and gas production rate (c)) and gas production of DDGS – sulfur from Na_2SO_4 and Na_2S produced more ($P < 0.01$) gas within 48 h with a faster gas production rate as well as higher digestibilities (dry matter degradability and organic matter digestibility) and more energy supplies (metabolizable energy), net energy for maintenance and gain, and net energy for gain than sulfur from Na_2SO_3 and $Na_2S_2O_4$. Neither ammonia-nitrogen (NH_3-N) concentration nor volatile fatty acids (VFA) profile (total VFA and individual VFA proportion) were affected by sulfur source ($P > 0.05$). In Experiment 2, no significant ($P > 0.05$) effect on the fermentation characteristics of DDGS with increasing sulfur content was found. The collective findings suggest that regular chemical analyses are necessary to make full use of DDGS, and that the valence state of sulfur in DDGS exerts an effect on its *in vitro* fermentation characteristics and there appears no dose-related effect of sulfur on the fermentation of DDGS in a short-term *in vitro* culture.

Keywords: DDGS; rumen fermentation; byproduct; feeding value

List of abbreviations: DDGS = distiller's dried grains with solubles, GP = gas production, DM = dry matter, EE = ether extract, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin, Ca = calcium, P = phosphorus, CP = crude protein, NDICP = neutral detergent insoluble crude protein, ADICP = acid detergent insoluble crude protein, VFA = volatile fatty acids, MCP = microbial crude protein production, SCFA = short chain fatty acid, DDG = distiller's dried grains, DDS = distiller's dried solubles, SRB = sulfate-reducing bacteria, OM = organic matter, OMD = organic matter digestibility, DMD = dry matter degradability, NEm = net energy for maintenance, NEg = net energy for gain, ME = metabolizable energy, PF = partitioning factor

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In pace with the expansion of bio-ethanol industry around the world, the output of its byproduct, distiller's dried grains with solubles (DDGS), is swiftly increasing (Wu et al. 2015), and DDGS is becoming a common feedstuff for livestock, e.g. feedlot cattle (Smith et al. 2013). However, high sulfur level in DDGS has become a potential limiting factor to its inclusion in cattle rations (Kwiatkowski et al. 2006; Klopfenstein et al. 2007) given that high sulfur content intake would exert a detrimental effect on animal performance and carcass quality (Richter et al. 2012; Pogge and Hansen 2013), even the health status (Felix et al. 2011; Pogge and Hansen 2013).

In general, many nutrients or components in DDGS are concentrated roughly threefold because approximately two-thirds of the grain is starch, which is fermented into alcohol. This applies in particular to sulfur which originates, apart from native contents in the basal substrates (cereals, such as corn), largely from sulfurous and sulfuric acids added to the production process in order to control the technological processes (Felix and Loerch 2011). Consequently, sulfur content of corn ranges from 0.1 to 0.15% of dry matter (DM), whereas sulfur content of DDGS is usually greater than 0.6%, even greater than 1% (Felix et al. 2011). Moreover, the investigation of Buckner et al. (2011) showed that variation in sulfur content was the largest for all nutrients tested in DDGS as coefficients of variation within days and across days (within the same ethanol plants) ranged from 3 to 13%. It is noteworthy that the dietary sulfur level for beef cattle recommended by the National Research Council (NRC 2000) is only 0.15% and its maximum tolerable concentration is generally estimated at 0.40%.

As an important functional component of sulfur-containing compounds, such as amino acids, hormones, enzymes, there are several valence states of sulfur existing in the organism, the bio-availability of which could be quite different. Some investigations seem to show that the dietary sulfur source could affect rumen hydrogen sulfide production and animal productivity (Uwituze et al. 2011b), while most are essentially explaining the influence of dietary pH value or buffering capacity rather than sulfur source on sulfur metabolism (Felix et al. 2014; Wu et al. 2015). There is rare literature concerning the effects of sulfur source (valence state) on the nutritional value of DDGS. The objective of this study was to investigate the sulfur content of corn DDGS in feeding practice and determine the effects of sulfur source (valence state) and sulfur level on the feeding value of DDGS based on the data of proximate compositions, fermentation characteristics, and model predicted indicators in *in vitro* rumen culture.

MATERIAL AND METHODS

Sample collection and experimental design. In order to make clear the nutrient values (especially sulfur content) of corn DDGS in feeding practice, a survey was conducted around the country and finally 10 DDGS samples from different ethanol plants were collected and then analyzed for the concentrations of sulfur and proximate nutrients. Based on the chemical analysis, the DDGS sample with the minimum sulfur content (0.346% sulfur on dry matter basis) was selected as a basal fermentation substrate for *in vitro* culture.

Experiment 1. In order to investigate the effects of sulfur source (different valence states of sulfur) on the fermentation characteristics of DDGS in *in vitro* culture, with the consideration of setting an intermediate sulfur level, the sulfur concentration of the basal substrate was increased up to 0.692% with the addition of sodium sulfate (Na_2SO_4), sodium sulfite (Na_2SO_3), sodium hydrosulfite ($Na_2S_2O_4$) or sodium sulfide (Na_2S), respectively, on the assumption that the sulfur in the basal substrate existed in SO_4^{2-}.

Experiment 2. In another independent *in vitro* study, Na_2SO_4 was added into the basal substrate at different levels (0, 0.346, and 0.692%) in order to determine the effects of sulfur level (0.346, 0.692, and 1.038%) on the fermentation characteristics of DDGS.

Chemical analysis. Samples were analyzed in duplication for dry matter (DM), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin (ADL), ash, calcium (Ca), phosphorus (P), and crude protein (CP), neutral detergent insoluble crude protein (NDICP), acid detergent insoluble crude protein (ADICP) according to the AOAC (2000) procedures. Specifically, EE was extracted with an Extraction System (ANKOM Technology Corp., USA). The analyses of NDF, ADF, and ADL were done using an A220 Fiber Analyzer (ANKOM Technology Corp.). Crude protein was measured using the combustion nitrogen analysis

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(FP-528; Leco, USA). Calcium was determined by an atomic absorption spectrophotometer WFX-320 (BRAIC, China) and phosphorus was determined by an UV-VIS 8500 spectrophotometer (Tianmei Scientific Instrument Co. Ltd., China). Sulfur was analyzed using the Magnesium nitrate method described in GB/T 17776–1999 (National Standards of the People's Republic of China).

In vitro incubation procedure. *In vitro* incubation was carried out according to the procedures of Menke et al. (1979). Before the morning feeding, rumen fluid was collected from 3 Simmental × Limousin crossbred steers (approximately 600 kg body weight) fitted with permanent rumen fistula and fed twice a total mixed ration consisting of 50% hay and 50% concentrates (25% corn, 23% brewer's grain, 2% premix), then strained through four layers of cheesecloth into a vacuum bottle, made gently upside-down blending and transported immediately to the Laboratory of Beef Cattle Research Center of China Agricultural University. The rumen fluid was mixed with the buffer solution in a 1 : 2 (v/v) proportion under a continuous flux of CO_2. The buffer solution and rumen inoculum were prepared according to the method of Menke and Steingass (1988). All the procedures with animals were approved by the Animal Care and Use Committee of China Agricultural University in accord with Regulations for the Administration of Affairs Concerning Experimental Animals by the State Scientific and Technological Commission of China 2017.

Samples of each treatment (including different sulfur sources/levels) were prepared and weighed (220 mg air dry matter) into 100 ml glass syringes in triplicate and kept at 39°C cultivator in advance, simultaneously setting three syringes as blank control (i.e. without fermentation substrate). Each syringe was injected in 30 ml incubation fluid with a varispenser (Eppendorf, Germany) and then incubated at 39°C for 72 h. During the incubation, the volume of cumulative gas production (GP) was recorded manually at the time points of 0, 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60, and 72 h. In the end, the fermentation mixture was sampled and then centrifuged at 8000 *g* and 4°C for 15 min to obtain the supernatant designated for the determination of volatile fatty acids (VFA) and ammonia-nitrogen (NH_3-N). The VFA profile was determined with a GC 3420 gas chromatograph (6890N; Agilent Technologies, USA) fitted with HP-INNO wax capillary column (30 m × 0.32 mm) as stated by Erwin et al. (1961), and NH_3-N concentration was colorimetrically (UV-VIS 8500; Tianmei Scientific Instrument Co. Ltd.) measured based on the method described by Broderick and Kang (1980).

Simutaneously, *in vitro* dry matter degradability (DMD) in 24 h was measured based on the method of Oba et al. (2005) with some modifications as follows: (1) seal samples (approximately 0.5 g) in special nylon bags (8 mm × 3.5 mm, pore size 38 μm); (2) immerse in glass tubes (three bags in each tube) filled with 80 ml incubation fluid as used in GP; (3) eject air with a flux of CO_2 and cap the tube with Bunsen valve, then incubate in shaking water bath at 39°C for 24 h; (4) take out and clean the nylon bags with distilled water, dry at 105°C overnight to measure the residue. Then DMD is calculated as:

$$DMD\ (\%) = (m_{sample} - m_{residue}) \times 100/m_{sample}$$

where:

m = weight expressed on dry matter basis.

Calculations. To estimate kinetic parameters of GP, all the results of GP were fitted using the NLIN Procedure of the SAS software (Version 9.0, 2007) according to France et al. (2000) as:

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

where:

a = volume (ml) of gas production per 0.2 g DM substrate at time t

b = asymptotic gas production (ml) of 0.2 g DM substrate

c = rate of gas production per hour

Organic matter digestibility (OMD; g/kg DM) and metabolizable energy (ME; MJ/kg DM) were estimated according to the models stated by Menke and Steingass (1988), and net energy for maintenance (NEm; MJ/kg DM) and net energy for gain (NEg; MJ/kg DM) were calculated according to NRC (2000):

$$OMD = 148.8 + 8.893GP + 0.448CP + 0.651Ash$$

$$ME = 2.20 + 0.1357GP + 0.0057CP + 0.0002859CP^2$$

$$NEm = (1.37ME\ (Mcal/kg) - 0.138ME\ (Mcal/kg)^2 + 0.0105ME\ (Mcal/kg)^3 - 1.12) \times 4.184$$

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Table 1. Sulfur and proximate nutrients contents in DDGS used in feeding practice

Item	Chemical composition (% of DM)											
	DM	S	CP	NDICP	ADICP	EE	Ash	NDF	ADF	ADL	Ca	P
Maximum	95.6	1.0	27.8	13.4	5.5	14.4	5.6	43.8	18.1	15.2	0.09	1.4
Minimum	92.6	0.4	22.7	3.9	0.9	6.0	4.8	31.4	10.8	4.7	0.02	1.2
Average (n = 10)	94.1	0.7	26.0	8.1	1.9	9.6	5.2	37.9	13.9	9.7	0.03	1.3
SD	1.4	0.3	2.2	3.3	1.7	2.9	0.3	4.1	2.4	3.8	0.03	0.1

DDGS = distiller's dried grains with solubles, DM = dry matter, S = sulfur, CP = crude protein, NDICP = neutral detergent insoluble crude protein, ADICP = acid detergent insoluble crude protein, EE = ether extract, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin, Ca = calcium, P = phosphorus, SD = standard deviation

$$NEg = (1.42ME\ (Mcal/kg) - 0.174ME\ (Mcal/kg)^2 + 0.122ME\ (Mcal/kg)^3 - 1.65) \times 4.184$$

where:

GP = net cumulative gas production (ml) of 0.2 g DM sample after 24 h of incubation

CP = crude protein (g/kg DM)

Ash = ash of the feed (g/kg DM)

The partitioning factor at 24 h of incubation (PF_{24}), a measure of fermentation efficiency, was calculated as the ratio of DM degradability *in vitro* (DMD; mg/g) to the volume (ml/g) of GP at 24 h (i.e. DMD/total gas production (GP_{24})) according to Blummel et al. (1997).

Microbial crude protein production (MCP) was calculated according to Blummel et al. (1997) as:

$$MCP\ (mg/g\ DM) = DMD\ (mg/g) - (ml/g\ gas \times 2.2\ mg/ml)$$

where 2.2 mg/ml is a stoichiometric factor which expresses mg of C, H, and O required for the production of short chain fatty acid (SCFA) gas associated with production of 1 ml of gas.

Statistical analysis. The experimental design for the *in vitro* rumen GP and fermentation parameters analysis was a completely randomized design separately considering sulfur source (Na_2SO_4, Na_2SO_3, $Na_2S_2O_4$ and Na_2S; Experiment 1) or sulfur level (0.346, 0.692, and 1.038%; Experiment 2) as fixed factor in the linear model. The statistical model was:

$$Y_{ij} = \mu + S_i + e_{ij}$$

where:

Y_{ij} = each observation of the i^{th} sulfur source or sulfur level

μ = general mean

S_i = effect of sulfur source (i = 1–4) or sulfur level (i = 1–3)

e_{ij} = experimental random residual error

In order to examine the responses of different sulfur source or sulfur level, data were subjected to the GLM Procedure of the SAS software (Version 9.0, 2007), and TDIFF option was used to compare the differences between the treatments with difference declared significant when P-value < 0.05.

RESULTS

Chemical compositions of DDGS in feeding practice. Based on the sampling in a survey, the contents of sulfur and proximate nutrients (DM, CP, NDICP, ADICP, EE, Ash, NDF, ADF, ADL, Ca, and P) of DDGS used in feeding practice (Table 1) showed a large variation, and DDGS proved to be a high-quality feedstuff, e.g. 26.0% protein and

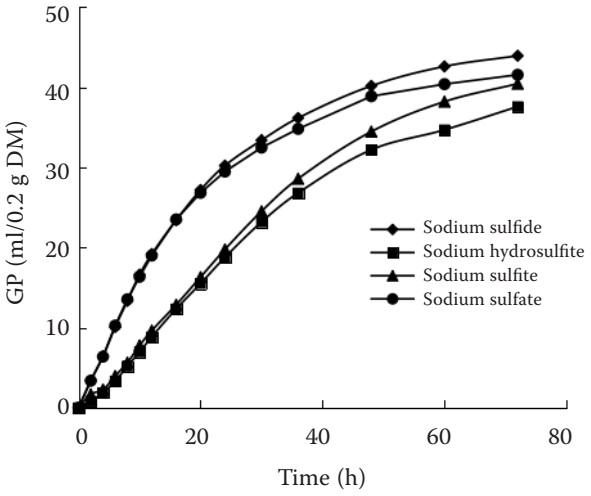

Figure 1. Gas production (GP) (ml gas/0.2 g DM) curves of distiller's dried grains with solubles with different sulfur sources in *in vitro* culture (average standard deviations are 1.6, 1.9, 1.7, and 2.5 ml gas/0.2 g DM for sulfur source of sulfide, hydrosulfite, sulfite, and sulfate, respectively)

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Table 2. Experiment 1 – effects of sulfur source on the gas production of DDGS in *in vitro* culture

Item	Sulfur source				SEM	*P*-value
	sodium sulfide	sodium hydrosulfite	sodium sulfite	sodium sulfate		
Gas production parameters						
b (ml/0.2g DM)	49.1[b]	49.0[b]	60.2[a]	42.8[c]	1.3	< 0.01
c (per h)	0.045[a]	0.021[b]	0.018[b]	0.049[a]	0.003	< 0.01
Gas production (ml/0.2g DM)						
GP_{12}	19.2[a]	8.8[b]	10.3[b]	19.0[a]	1.4	< 0.01
GP_{24}	30.2[a]	18.7[b]	21.1[b]	29.4[a]	1.5	< 0.01
GP_{48}	40.2	32.1	35.8	38.9	1.9	0.09
GP_{72}	43.9	37.5	41.3	41.5	1.5	0.14

DDGS = distiller's dried grains with solubles, b = asymptotic gas production, c = rate of gas production, GP_t = accumulative gas production at time t, DM = dry matter

[a–c]different letters in the same row denote significant ($P < 0.05$) differences between treatments

9.6% fat on DM basis. It is noteworthy that the sulfur concentration ranged from 0.35 to 1.04%.

Experiment 1

Effects of sulfur source on in vitro gas production kinetics and cumulative gas production. GP parameters (b and c) of DDGS were significantly influenced ($P < 0.01$) by its sulfur source. Sulfur from Na_2SO_3 developed the most asymptotic GP (b), intermediate GP was from $Na_2S_2O_4$ and Na_2S, and the least from Na_2SO_4 (Table 2). Moreover, sulfur from Na_2SO_4 and Na_2S produced higher ($P < 0.01$) GP within 48 h with faster rates than that from Na_2SO_3 and $Na_2S_2O_4$, along with an apparent gap between the GP curves (Figure 1).

Effects of sulfur source on in vitro fermentation parameters and fermentation profile. Neither the NH_3-N concentration nor the VFA profile (total VFA and individual VFA proportion) were affected ($P > 0.05$) by the sulfur source of DDGS (Table 3). DDGS with sulfur from $Na_2S_2O_4$ and Na_2SO_3 had poorer ($P < 0.01$) digestibilities (DMD and OMD) and less energy supplies (ME, NEm, and NEg) and higher ($P < 0.05$) PF_{24} than those with sulfur from Na_2SO_4 and Na_2S along with similar MCP_{24} values (Table 4).

Experiment 2

Effects of sulfur level on in vitro gas production kinetics and cumulative gas production. GP (b and c) of DDGS showed no difference ($P > 0.05$) when its sulfur content increased in *in vitro* culture (Table 5), and their GP curves nearly overlapped (Figure 2).

Effects of sulfur level on in vitro fermentation parameters and fermentation profile. The fermentation parameters (NH_3-N and VFA profile) of DDGS did not exhibit significant changes ($P > 0.05$) with increasing sulfur levels (Table 6). The rumen

Table 3. Experiment 1 – effects of sulfur source on the NH_3-N concentration and VFA profile of DDGS in *in vitro* culture

Item	Sulfur source				SEM	*P*-value
	sodium sulfide	sodium hydrosulfite	sodium sulfite	sodium sulfate		
NH_3-N (mg/100ml)	39.57	38.29	35.16	34.10	2.13	0.22
TVFA (mmol/l)	37.34	39.10	37.98	29.10	2.13	0.09
Acetate (% TVFA)	60.66	60.80	60.17	59.98	0.36	0.41
Propionate (% TVFA)	24.57	23.91	24.23	24.73	0.81	0.89
Isobutyrate (% TVFA)	1.46	1.50	1.62	1.62	0.22	0.93
Butyrate (% TVFA)	7.87	8.07	8.44	7.45	0.40	0.47
Isovalerate (% TVFA)	3.81	4.04	3.70	4.43	0.30	0.43
Valerate (% TVFA)	1.64	1.71	1.87	1.80	0.18	0.82
A/P	2.47	2.55	2.48	2.43	0.08	0.74

DDGS = distiller's dried grains with solubles, TVFA = total volatile fatty acids, A/P = acetic acid/ propionic acid ratio

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Table 4. Experiment 1 – effects of sulfur source on the fermentation profile of DDGS in *in vitro* culture

Item (on DM basis)	Sulfur source				SEM	*P*-value
	sodium sulfide	sodium hydrosulfite	sodium sulfite	sodium sulfate		
DMD_{24} (%)	50.93[a]	42.90[b]	43.12[b]	48.21[a]	1.41	< 0.01
ME (MJ/kg)	10.52[a]	8.72[b]	8.87[b]	10.39[a]	0.27	< 0.01
OMD (%)	55.44[a]	45.26[b]	46.10[b]	54.74[a]	1.51	< 0.01
NEm (MJ/kg)	6.77[a]	5.15[b]	5.29[b]	6.66[a]	0.24	< 0.01
NEg (MJ/kg)	4.24[a]	2.78[b]	2.90[b]	4.14[a]	0.22	< 0.01
MCP_{24} (mg/g)	180	223	214	158	21	0.14
PF_{24} (mg/ml)	3.39[b]	4.60[a]	4.41[a]	3.29[b]	0.30	0.03

DDGS = distiller's dried grains with solubles, DM = dry matter, DMD_{24} = dry matter degradability at 24 h, ME = metabolizable energy, OMD = organic matter digestibility, NEm = net energy for maintenance, NEg = net energy for growth, MCP_{24} = microbial crude protein production at 24 h, PF_{24} = partitioning factor at 24 h

ME and OMD were estimated according to Menke and Steingass (1988), NE (for maintenance and growth) was calculated according to NRC (2000), PF and MCP were calculated according to Blummel et al. (1997)

[a,b]different letters in the same row denote significant ($P < 0.05$) differences between treatments

Table 5. Experiment 2 – effects of sulfur level on the gas production of DDGS in *in vitro* culture

Item	Sulfur level			SEM	*P*-value
	0.346%	0.692%	1.038%		
Gas production parameters					
b (ml/0.2 g DM)	49.1	43.0	43.0	1.7	0.07
c (per h)	0.051	0.048	0.050	0.002	0.43
Gas production (ml/0.2 g DM)					
GP_{12}	21.6	19.0	20.1	1.2	0.39
GP_{24}	32.8	29.4	31.1	1.5	0.34
GP_{48}	42.8	38.9	39.7	1.9	0.35
GP_{72}	46.6	41.5	42.1	1.9	0.19

DDGS = distiller's dried grains with solubles, b = asymptotic gas production, c = rate of gas production, GP_t = accumulative gas production at time t, DM = dry matter

fermentation profile (DMD_{24}, ME, NEm, NEg, OMD, MCP_{24}, and PF_{24}) showed no difference in different sulfur levels yet (Table 7).

DISCUSSION

Chemical compositions of DDGS used in feeding practice. As is well known, DDGS is an abundantly available feedstuff with high nutrient values. Salim et al. (2010) showed that the CP content of DDGS in 395 samples ranged from 25.9 to 30.4% and Tjardes and Wright (2002) reported that the CP content of DDGS could vary from 20 to 30%. The levels of CP and EE in the present study were comparable with those reported previously (Felix et al. 2011; Kerr et al. 2013), being almost three times

Table 6. Experiment 2 – effects of sulfur level on the NH_3-N concentration and VFA profile of DDGS in *in vitro* culture

Item	Sulfur level			SEM	*P*-value
	0.346%	0.692%	1.038%		
NH_3-N (mg/100 ml)	33.94	34.30	37.05	1.56	0.28
TVFA (mmol/l)	36.91	29.10	36.17	2.48	0.19
Acetate (% TVFA)	61.40	59.98	59.67	0.91	0.46
Propionate (% TVFA)	24.16	24.73	24.97	0.60	0.66
Isobutyrate (% TVFA)	1.65	1.62	1.58	0.27	0.98
Butyrate (% TVFA)	7.30	7.45	7.72	0.37	0.74
Isovalerate (% TVFA)	3.83	4.43	4.26	0.30	0.44
Valerate (% TVFA)	1.68	1.80	1.82	0.22	0.89
A/P	2.54	2.43	2.39	0.07	0.41

DDGS = distiller's dried grains with solubles, TVFA = total volatile fatty acids, A/P = acetic acid/propionic acid ratio

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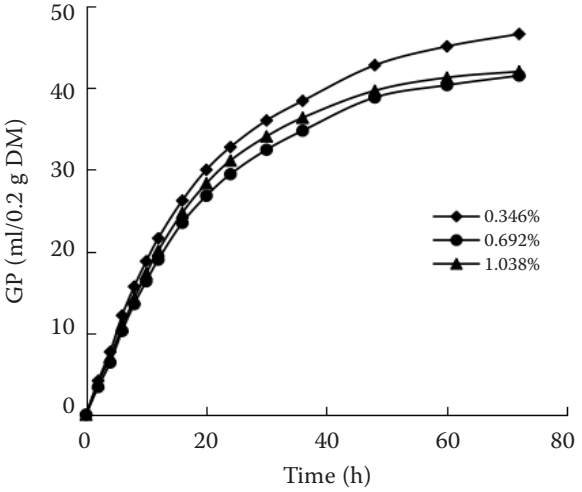

Figure 2. Gas production (GP) (ml gas/0.2 g DM) curves of distiller's dried grains with solubles with different sulfur levels in *in vitro* culture (average standard deviations are 2.8, 2.5, and 1.4 ml gas/0.2 g DM for sulfur level of 0.346, 0.692, and 1.038%, respectively)

as high as the values of feedstuff corn (NRC 2001). The sulfur content varied from 0.35 to 1.04%, well in line with the result reported by Felix et al. (2011). Based on several published articles summarized, Benton (2010) reported that the average nutrient composition for corn distiller's dried solubles (DDS) is approximately 31.5% CP, 10.5% EE, 6% starch, 43.2% NDF, 0.80% P, and 0.71% S, varying across ethanol plants and production batches. The nutrient values varied in a wide range, inferring that DDGS from different ethanol plants showed quite different nutrient profiles, consequently impairing formulation of accurate rations when using standard feedstuff tables. There were several factors likely contributing to the variance, e.g. the feedstuff, manufacturing process, the proportion of distiller's dried grains (DDG) and DDS. Cromwell et al. (1993) suggested that differences in processing procedure could be responsible for a substantial amount of variability in the nutritional value of DDGS.

Experiment 1

Effects of sulfur source on the fermentation characteristics of DDGS in in vitro culture. In general, the *in vitro* GP method is a common way to evaluate the nutritional values of feedstuff for ruminants, in which GP appeared to be related to the compositions of substrate and fermentation efficiency. In the present study, DDGS with different sulfur sources produced various GP with different GP rate, indicating that its sulfur source exerted a significant effect on the *in vitro* fermentation efficiency, especially in the initial stage of fermentation. Consistently, Felix et al. (2014) reported that the source of sulfur affected rumen sulfur metabolism and that sulfur from DDGS was more readily reduced than sulfur from Na_2SO_4. As one of the most important rumen metabolic pathway of sulfur, sulfate-reducing bacteria (SRB) play an important role in the reduction of sulfate to sulfide (Beauchamp et al. 2008) and its reduction ability would be cut down if sulfate concentration is excessive (Coleman 1960).

Generally, VFA is the major source of energy supply for ruminants and NH_3-N is a common indicator in nitrogen metabolism. In the present study, sulfur source had no influence on the endpoint VFA and NH_3-N profiles of DDGS. It almost coincided with the comparison of GP, which showed no difference in the end. It might be explained by the declining inhibition effect with microbial flora acclimation over time. Consistently, the study results of Wu et al. (2015) showed that the VFA profile of *in vitro* fermentation did not differ significantly among the substrates added with H_2SO_4, Na_2SO_4 or not, suggesting little effect of the added sulfur (at about 0.3% level) on feed fermentation.

Typically, the *in vitro* GP at 24 h of ruminant feed is highly correlated with its digestibility (Menke

Table 7. Experiment 2 – effects of sulfur level on the fermentation profile of DDGS in *in vitro* culture

Item (on DM basis)	Sulfur level			SEM	*P*-value
	0.346%	0.692%	1.038%		
DMD_{24} (%)	46.98	48.21	48.51	0.57	0.21
ME (MJ/kg)	10.94	10.39	10.66	0.23	0.33
OMD (%)	57.82	54.74	56.22	1.32	0.33
NEm (MJ/kg)	7.13	6.66	6.89	0.21	0.33
NEg (MJ/kg)	4.56	4.14	4.35	0.18	0.33
MCP_{24} (mg/g)	108	158	143	20	0.26
PF_{24} (mg/ml)	2.87	3.29	3.13	0.17	0.29

DDGS = distiller's dried grains with solubles, DM = dry matter, DMD_{24} = dry matter degradability at 24 h, ME = metabolizable energy, OMD = organic matter digestibility, NEm = net energy for maintenance, NEg = net energy for growth, MCP_{24} = microbial crude protein production at 24 h, PF_{24} = partitioning factor at 24 h

ME and OMD were estimated according to Menke and Steingass (1988), NE (for maintenance and gain) was calculated according to NRC (2000), PF and MCP were calculated according to Blummel et al. (1997)

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et al. 1979) and the energy content of feedstuff is highly correlated with digestibility of its DM or OM (Rittenhouse et al. 1971; Yan and Agnew 2011). In the present study, DDGS with sulfur from $Na_2S_2O_4$ and Na_2SO_3 had lower digestibilities (DMD_{24} and OMD) and less energy supplies (ME, NEm, and NEg) than those from Na_2S and Na_2SO_4, which could explain the difference of GP, inferring that the valence state of sulfur in DDGS exerted a prominent effect on its fermentation profile and feeding value. Similarly, Wu et al. (2015) reported that DM degradation was different among the diets added with various sulfur sources. However, it is worth noting that the energy content might be underestimated with such an *in vitro* system as the inhibition effect of $Na_2S_2O_4$ and Na_2SO_3 would be diluted by the other feed components in feeding practice.

Experiment 2

Effects of sulfur level on the fermentation characteristics of DDGS in in vitro culture. There are numerous studies dealing with the effects of dietary sulfur level with DDGS inclusion, most results of which infer that sulfur exerts a significant dose-related effect on rumen fermentation and animal performance (Smith et al. 2013; Amat et al. 2014). Based on these previous studies, it was hypothesized that the GP of DDGS would decline with increasing sulfur concentration. While, sulfur dose-related effect on the fermentation of DDGS did not appear in the present study. It could be explained as that the negative effect of increasing sulfur level is essentially end-product inhibition, i.e. the effect would not appear until the product accumulates to a certain level. Drewnoski et al. (2012) demonstrated that it took at least 29 days for the SRB to achieve peak rumen H_2S production after abrupt exposure to diets containing a readily available sulfur source from Na_2SO_4. Maybe that is why it would present damage on animal performance when feeding high dietary sulfur for a long time in feeding practice.

Controversially, the previously reported results concerning the effects of dietary sulfur level on nutrient digestibility were confusing, being either promotion (Uwituze et al. 2011a) or inhibition (Felix et al. 2011). There was no dose-related effect found in the present study. The variation in different studies could be ascribed to various sulfur levels and different fermentation duration. The functional mode of sulfur in *in vitro* culture might be quite different to that in *in vivo* and a short-term fermentation study would likely result in a wrong assessment of the effect of sulfur level. *In vivo* study should be conducted further.

CONCLUSION

The present survey showed that there exists a great variation in the concentrations of sulfur and proximate nutrients of DDGS used in feeding practice; the valence state of sulfur in DDGS made a big difference on its *in vitro* DMD and OMD, consequently resulting in different GP and energy supplies (ME, NEm, and NEg), ultimately initiating different feeding values; however, the sulfur level showed no effect on the fermentation characteristics of DDGS in *in vitro* rumen culture. The collective findings suggest that regular chemical analyses are necessary to make full use of DDGS, and the valence state of sulfur in DDGS exerts an effect on its *in vitro* fermentation characteristics while there appears no dose-related effect of sulfur on the fermentation of DDGS in a short-term *in vitro* culture.

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