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Research Article

Effect of Various Oil Sources on *in vitro* Gas Production, Digestibility and Fermentation Metabolites in Thai Friesian Crossbred

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Abstract

This investigation aims to study the effect of various oil sources on gas production, digestibility and fermentation metabolites in Thai Friesian crossbred by using *in vitro* gas production technique. The oil treatments included saturated fatty acid (SFA) contained tallow (TA), palm oil (PO) and polyunsaturated fatty acid (PUFA) included soybean oil (SBO), sunflower oil (SFO) which added at 4 %DM in the diet. The diet contained concentrate mixed with rice straw at the ratio 50:50. Gas productions (GP) and *in vitro* fermentation metabolites were recorded and calculated after incubation. At 24 and 48 hours of incubation time, the 5 bottles of inoculum were stopped and determined for *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD). The results revealed that IVDMD and IVOMD were not different among treatments ($P>0.05$). However, the rate of gas production (c) in SFO was lower than other treatments ($P<0.05$). Furthermore, the effective gas production potentials (EP) and cumulative gas production at 96 hours (GP96) of SFA were higher than PUFA treatments ($P<0.01$). Supplementation of PO was higher *in vitro* fermentation metabolites such as GP and short chain fatty acid (SCFA) than other treatments ($P<0.05$), while ammonia nitrogen (NH_3-N) concentration was higher by supplementation SFO in the diet. Results indicated that PUFA i.e. SFO inhibited cumulative gas production, the rate of gas production and *in vitro* fermentation metabolites. Supplementation of SFA i.e. PO seems more effective to increase cumulative gas production and *in vitro* rumen fermentation metabolites than other treatments.

Keywords: Oil, Gas production, Digestibility, Fermentation metabolites and *in vitro* technique

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ผลของแหล่งน้ำมันต่อการผลิตแก๊สในหลอดทดลอง การย่อยได้ และผลผลิตจากกระบวนการหมักในโคนมลูกผสมไทยฟรีเชี่ยน

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บทคัดย่อ

การวิจัยในครั้งนี้มีวัตถุประสงค์เพื่อศึกษาแหล่งของน้ำมันที่มีผลต่อการผลิตแก๊ส การย่อยได้และผลผลิตจากกระบวนการหมักในโคนมลูกผสมไทยฟรีเชี่ยนโดยใช้เทคนิคการวัดผลิตแก๊สในหลอดทดลอง โดยน้ำมันที่ใช้ในการศึกษาได้แก่กรดไขมันอิ่มตัว (SFA) ประกอบด้วยไขมันสัตว์ (TA) น้ำมันปาล์ม (PO) และกรดไขมันไม่อิ่มตัว (PUFA) ประกอบด้วยน้ำมันถั่วเหลือง (SBO) น้ำมันทานตะวัน (SFO) ซึ่งเสริมที่ระดับ 4 เปอร์เซ็นต์วัตถุแห้งในอาหาร โดยอาหารทดลองมีสัดส่วนอาหารข้นผสมกับฟางข้าวในอัตราส่วน 50:50 ค่าการผลิตแก๊ส (GP) และผลผลิตจากกระบวนการหมักในหลอดทดลองจะถูกบันทึกและนำไปคำนวณหลังจากทำการบ่ม เมื่อเวลาในการบ่มที่ 24 และ 48 ชั่วโมง สารตั้งต้นหัวเชื้อจำนวน 5 ขวดจะถูกหยุดและนำไปคำนวณหาค่าการย่อยได้ของวัตถุแห้งในหลอดทดลอง (IVDMD) และการย่อยได้ของอินทรียวัตถุในหลอดทดลอง (IVOMD) ผลการศึกษาพบว่าค่าการย่อยได้ของวัตถุแห้งและการย่อยได้ของอินทรียวัตถุไม่แตกต่างกันระหว่างกลุ่มทดลอง ($P>0.05$) อย่างไรก็ตามอัตราการผลิตแก๊ส (c) ในน้ำมันทานตะวันมีค่าต่ำกว่าน้ำมันชนิดอื่นๆ ($P<0.05$) นอกจากนี้ค่าประสิทธิภาพการผลิตแก๊ส (EP) และการผลิตแก๊สสะสมที่ 96 ชั่วโมง (GP96) ของกรดไขมันอิ่มตัวมีค่าสูงกว่ากรดไขมันไม่อิ่มตัว ($P<0.01$) การเสริมน้ำมันปาล์มทำให้ผลผลิตแก๊สในหลอดทดลอง (GP) และกรดไขมันสายสั้น (SCFA) มีค่ามากกว่าน้ำมันชนิดอื่น ๆ ($P<0.05$) ในขณะที่การเสริมน้ำมันทานตะวันในอาหารทำให้ความเข้มข้นของแอมโมเนียไนโตรเจน (NH_3-N) มีค่าสูงที่สุด ผลการทดลองชี้ให้เห็นว่ากรดไขมันไม่อิ่มตัวได้แก่น้ำมันทานตะวันยับยั้งการผลิตแก๊สสะสม ลดอัตราการผลิตแก๊สและลดผลผลิตจากกระบวนการหมักในหลอดทดลอง การเสริมกรดไขมันอิ่มตัวได้แก่น้ำมันปาล์มช่วยเพิ่มประสิทธิภาพการผลิตแก๊สสะสมและผลผลิตจากกระบวนการหมักในหลอดทดลองมากกว่าน้ำมันชนิดอื่น ๆ

คำสำคัญ: น้ำมัน การผลิตแก๊ส การย่อยได้ ผลผลิตจากกระบวนการหมัก และ เทคนิคในหลอดทดลอง

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Introduction

Lipid was very important to supply dietary energy to ruminants and they can modify the fatty acid composition in ruminant products (Chilliard *et al.*, 2000). Normally, lipid supplementation in the ruminant diet includes saturated fatty acid (SFA) such as tallow (TA), palm oil (PO); and polyunsaturated fatty acids (PUFA) such as soybean oil (SBO), sunflower oil (SFO). Jenkins (1993); NRC (2001) who suggested that total dietary lipids could not exceed 6 to 7 %dry matter basis (DM) in the total diet. In addition, high intake of dietary lipids were affected to reduce rumen fermentation efficiency and fiber digestibility (Palmquist, 1988). Consistent with, Van Soest (1994) who reported that high proportions of PUFA can manipulate rumen fermentation by inhibiting activity and the growth of rumen microorganism. Correspondingly, Wanapat *et al.* (2011) who reported that high level of lipids at 6% DM in the ruminant diet was an adverse effect to decrease cellulolytic bacteria in the rumen.Moreover, the different source of oil supplementation in ruminant diets showed the effect on rumen fermentation (Wachirapakorn, 1988). Supplementation of SBO at 4.3% DM in the diet was decreased dry matter intake (DMI) (Piamphon, 2007). Similarly, Van Cleef *et al.* (2016) who reported that supplemented SBO at 6% DM in the total mixed ration diet (TMR) resulting in a lower fiber digestibility. In addition, Zhao *et al.* (2016) suggested that added of SFO at 40 g/kg DM in the diet decreased feed intake, total volatile fatty acid (TVFAs) and inhibited fiber degradation. Never the less, Abubakr *et al.* (2013) found that supplementation of PO at 5% DM in diet enhanced feed intake and ether extract digestibility (EED). Moreover, Hollmann and Beede (2012) supplemented animal fat blend at 5% DM infeed reduced DMI and fiber digestibility. Therefore, the effects of lipids on rumen fermentation and fiber digestion are related to the lipid's chemicals and the levels of lipid in the diet (Jenkins and McGuire, 2006). However, there is a limited study of comparative effects of various oil sources supplementation in dairy cows.

Hence, the objective of this experiment was to investigate the effect of the SFA i.e. TA and PO and the PUFA i.e. SBO and SFO supplementation on gas production, *in vitro* digestibility and rumen fermentation metabolites in Thai Friesian crossbred by using *in vitro* gas production technique.

Materials and Methods

1. *In vitro* gas production technique

The experiment was conducted using an *in vitro* gas production technique at various incubation time intervals. The experimental design was a completely randomized design (CRD) with five replications per treatment. The saturated fatty acids (SFA) i.e. tallow (TA), palm oil (PO) and the PUFA i.e. soybean oil (SBO), sunflower oil (SFO) were supplemented at 4% DM in the diets. The substrates consisted of 0.1 g rice straw (R) and 0.1 g concentrate (C) on the dry matter basis (R:C ration 50:50). The concentrate diet contained 12% crude protein (CP) and 2.9 Mcal ME/kg DM, meanwhile rice straw contained 1.28 %CP and 1.70 Mcal/kg DM. Feed samples were dried at 60 °C and ground to pass through the 1-mm screen (Cyclotech Mill, Tecator, Sweden) for chemical analysis. The substrate (200 mg) was added to 50 ml bottles and supplemented with different oil

sources. Feed samples were chemically analyzed DM, OM, CP, EE and Ash use the standard methods (AOAC, 1990), NDF, ADF and ADL according to Van Soest *et al.* (1991). Chemical compositions were shown in Table 1.

In vitro gas production technique was carried out according to the procedure of Makkar *et al.* (1995). Two rumen fistulated crossbred Holstein Friesian cows (Thai Friesian) were used as rumen fluid donors. Rumen fluid (1,000 ml) was collected from cows fed with basal diet (14.0% CP and 2.4 Mcal/kg, dry matter basis). The rumen fluid was filtered through four layers of cheese cloth into pre-warmed thermos flasks and added with artificial saliva was prepared according to Menke and Steingass (1988). The fluid was made by the ratio of artificial saliva: rumen fluid at 2:1. The bottles with the mixture of substrate treatments were pre-warmed in a hot air oven at 39 °C for 24 h before filling with 30 ml of rumen inoculums mixture. During the incubation, the gas production was recorded after incubating for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, 21, 24, 30, 36, 42, 48, 60, 72 and 96 hours. Cumulative gas production data were fitted into the model of Orskov and McDonal (1979) as follows:

$$y = a + b\,(1 - e^{(-ct)})$$

Where a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), t = incubation time, (a+b) = the potential extent of gas production and y = gas produced at a time “t”.

2. The determination of *In vitro* digestibility

The 5 bottles inoculum from 24 and 48 h of incubation were collected from the fermentation condition. The content of the bottle was transferred and filtered through pre-weighed Gooch's crucibles. The DM of the residue was weighed then measured ash before calculating for *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) according to Tilley and Terry (1963).

3. Statistical analysis

Data were analyzed according to Completely Randomized Design (CRD) by using the General Linear Models (GLM) procedures (SAS, 1996). The means of all data were compared by using Duncan's New Multiple Range Test (DMRT) according to Steel and Torrie (1980).

Results and Discussion

1. Chemical composition of the diets

The chemical composition of the concentrate diet consisted of 90.16% dry matter (DM), 11.94% crude protein (CP), 2.00% ether extract (EE), 19.55% neutral detergent fiber (NDF)and 10.96% acid detergent fiber (ADF). Moreover, rice straw contained 94.50% DM, 2.67% CP, 1.28% EE, 75.00% NDF and 49.17%ADF (Table 1). The results of the roughage chemical analysis were showed that rice straw contained 2.67% CP dry matter basis. This result corresponded to Napasirth *et al.* (2012) who reported the untreated rice straw contained CP 3.8% dry matter basis. Furthermore, forage fiber analysis of this experiment found that the rice straw contained 75.0% NDF. Correspond with Togtokhbayar *et al.*

(2015) who found that wheat straw had more than 70% NDF on a dry matter basis.

2. Kinetics of cumulative gas production

Cumulative gas production was shown in Fig. 1 and Table 2. Cumulative gas production adding SFA (PO) at 4% DM in the diet was significantly higher than other groups (P<0.01). The rate of gas production (c) supplemented with PUFA (SFO) was lower than those SFA (TA and PO) treatments (P<0.05). Furthermore, effective gas production potentials (EP) of the SFA were significantly higher than PUFA group (P<0.05) (Table 2). This kinetics of cumulative gas production might due to SBO and SFO contain a high level of PUFA when compared with TA and PO (Bauman *et al*, 2003). In accordance with Palmquist and Jenkins (1980) who reported that the level of PUFA had more effect on rumen fermentation than SFA. In addition, Van Soest (1994) reported that PUFA could affect the permeability of the microbial membrane and inhibited the growth of microorganisms.

Table 1 Chemical composition analysis of the concentrate diet and roughage used *in vitro* substrate

Items	Concentrate	Rice Straw	Concentrate			
			TA	PO	SBO	SFO
Dry matter (DM, %)	90.16	94.50	95.50	95.50	95.50	95.50
Chemical composition (% DM basis)						
OM	91.75	88.75	92.75	92.25	92.75	92.25
CP	11.94	2.67	11.57	11.47	11.47	11.53
EE	2.00	1.28	6.00	6.09	6.87	6.07
NDF	19.55	75.00	23.00	23.33	24.16	22.5
ADF	10.96	49.17	11.00	10.83	11.67	11.67
ADL	5.00	5.00	4.53	4.17	5.00	4.33
Ash	8.25	11.25	7.55	7.75	7.25	7.75
TDN [1] (%)	79.93	47.10	79.88	80.07	79.13	79.13
ME [1] (Mcal/Kg)	2.89	1.70	2.88	2.89	2.86	2.86

[1] NRC (2001), [2] Harris et al., (1972)

TA= Tallow, PO = palm oil, SBO = soybean oil, SFO = sunflower oil, OM = organic matter, CP = crude protein, EE = ether extract,

NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin, TDN = total digestible nutrient and ME = metabolizable energy

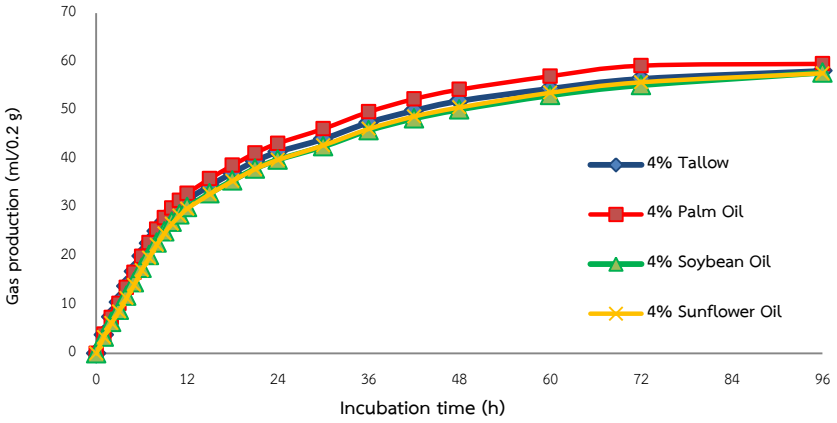

Fig.1 Effect of various oil sources on cumulative gas production

3. *In vitro* digestibility (IVDMD and IVOMD)

The effects of various oil supplementations on IVDMD and IVOMD were shown in Table 3. Supplemented various oils at 4% DM in the diet had no negative effect on the coefficient of IVDMD, IVOMD, metabolizable energy (ME) and total digestible nutrients (TDN) (P>0.05). Wanapat (1990) and Wachira pakorn (1998) who reported that the optimum of oil to the diet ranged from 2 to 5% DM. In accordance with Jenkins (1993) and NRC (2001) who suggested that whole dietary lipids could not exceed 6 to 7% DM of the diet. In addition, Shingfield *et al.* (2011) who reported that adding oil at 3% DM in the diet had no effect on the coefficient of digestibility. However, when adding oil higher than 5% in the concentrate diet decreased dry matter digestibility (DMD) and organic matter digestibility (OMD). Furthermore, supplementation oil at 4.3% DM in the diet slightly decreased dry matter intake (DMI) (Piamphon, 2007). Adding oil higher than 4% might decrease the coefficient of DMD and OMD.

4. *In vitro* rumen fermentation metabolites

The results in Table 4 were showed that at 48 h after incubation, the concentration of NH_3-N by adding SFO was significantly higher than other treatments (P<0.01). In the present study, higher rumen NH_3-N concentrations in SFO than other treatments due to the toxicity of PUFA on bacteria, which bacteria use less NH_3-N for growing in the rumen. Preston and Leng (1987) who revealed that the appropriate concentration of rumen NH_3-N for conveying the growth of microorganisms ranging from 5 to 25 mg%. Similarly, Veen (1986) who suggested that the concentration of NH_3-N in the rumen fluid reduced since the production of maximum microbial protein synthesis. However, the average concentrations of GP and SCFA from SFA (PO and TA) was significantly higher than PUFA (SBO and SFO) (P<0.05). Particularly, the GP and SCFA of PO treatment were higher than TA, SFO and SBO treatments respectively (P<0.05). High GP and SCFA concentrations in the PO and TA (SFA) due to the PO and TA contained a low proportion of PUFA (Bauman *et al.*, 2003). Polyunsaturated fatty acid was more toxic to rumen bacteria than SFA (Palmquist and Jenkins, 1980; Van Soest, 1994). Moreover, PUFA could inhibit activity and growth of bacteria, protozoa and fungi in the rumen (Nagaraja *et al.*, 1997). Additionally, in SFA group, the PO was greater than TA since PO more enriched with linoleic acid (C18:2, LA) than TA (Bauman *et al.*, 2003). The LA contained glycerol-3-phosphate (G3P), this G3P used by bacteria for the source of the glycerol backbone to produce phospholipid (cardiolipin); the bacteria used cardiolipin for producing lipid cell membrane, dividing cell and growing (Moser *et al.*, 2014).

Conclusion

The results could be summarized that the addition of different oil sources at 4% DM in the diet of an *in vitro* study had no effect on IVDMD, IVOMD, ME, TDN, PFand MCP. Supplementation of PO was showed high cumulative total gas production (GP96), EP, GPand SCFA. However, supplementation of SFO had a low rate of gas production (c) meanwhile rumen concentrations of NH_3-N was high. The utilization of oil containing high SFA such as PO and TA has a positive impact on rumen fermentation rather than oil containing high PUFA such as SBO and SFO. The use of PO in the diet has a more positive effect on rumen fermentation than other oil sources.

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Table 2 Effect of various oil sources on gas production kinetics in Thai Friesian crossbred

Items	SFA	PUFA	SEM	P-Value	SFA		PUFA		SEM	P-Value
					TA	PO	SBO	SFO		
Fermentation kinetic values										
a	1.97	1.37	0.11	0.09	2.24	1.95	1.42	1.32	0.40	0.34
b	53.23	53.30	0.51	0.95	51.93	54.31	53.09	53.46	0.79	0.23
c	0.064	0.060	0.003	0.42	0.065[a]	0.063[a]	0.061[ab]	0.059[b]	0.001	0.02
d (a+b)	55.20	54.61	0.40	0.54	54.17	56.26	54.51	54.78	0.72	0.15
Cumulative gas production (ml/0.2g)										
CG 96 h	58.87[a]	57.71[b]	0.24	0.02	58.13[b]	59.59[a]	57.77[b]	57.65[b]	0.26	<0.01
EP	32.01[a]	30.24[b]	0.10	0.01	31.92[a]	32.10[a]	30.41[b]	30.07[b]	0.15	<0.01

[ab] Means in the same row with different superscript differ (P<0.05), SEM = standard error of the mean, SFA= saturated fatty acid, PUFA = polyunsaturated fatty acid, TA = tallow, PO = palm oil, SBO = soybean oil, SFO = sunflower oil, a = the soluble fraction, b = the fermentation of the insoluble, c = rate of gas production, d = the potential extent of gas production, CG96 h= Cumulative gas production 96 hour and EP = effective gas production potential

Effective gas production potential (EP) = a + [bc/(c+k)] where k = the rumen outflow rate of 0.05 per hour [Orskov and McDonald, 1979]

Table 3 Effect of various oil sources on *in vitro* digestibility in Thai Friesian crossbred

Items	SFA	PUFA	SEM	P-Value	SFA		PUFA		SEM	P-Value
					TA	PO	SBO	SFO		
In vitro dry matter digestibility (%)										
24 h	58.62	55.91	1.16	0.15	58.56	58.69	57.61	54.18	1.58	0.20
48 h	62.77	64.25	1.39	0.25	66.85	66.69	66.38	62.13	1.88	0.56
In vitro organic matter digestibility (%)										
24 h	58.35	58.10	0.14	0.27	58.23	58.48	58.15	58.06	0.23	0.65
48 h	64.21	63.64	0.42	0.38	63.72	64.71	63.69	63.60	0.64	0.61
Metabolizable energy (MJ/kg DM)										
24 h	2.24	2.24	0.004	1.00	2.23	2.24	2.23	2.24	0.01	0.56
48 h	2.24	2.24	0.003	0.42	2.24	2.24	2.24	2.23	0.01	0.54
Total digestible nutrient (%)										
24 h	60.30	60.30	0.02	1.00	60.27	60.32	60.28	60.31	0.01	0.66
48 h	60.32	60.29	0.001	0.17	60.33	60.31	60.30	60.28	0.04	0.61

[ab] Means in the same row with different superscript differ (P<0.05), SEM = standard error of the mean, SFA= saturated fatty acid, PUFA = polyunsaturated fatty acid, TA = tallow, PO = palm oil, SBO = soybean oil, SFO = sunflower oil, Metabolizable energy (ME) =2.20+0.136GP (mL/0.2g DM) +0.057CP (g/kg DM) [Menke *et al.*, 1979], Total digestible nutrient (TDN, %) = (ME+0.45)/0.0445309 [NRC, 2001]

Table 4 Effect of various oil sources supplementation on rumen fermentation metabolites in Thai Friesian crossbred

Items	SFA	PUFA	SEM	P-Value	SFA		PUFA		SEM	P-Value
					TA	PO	SBO	SFO		
Ammonia nitrogen (NH_3-N), mg%										
24 h	12.46	13.98	0.69	0.17	12.16	12.76	13.37	14.59	1.10	0.52
48 h	16.41[a]	20.66[b]	1.24	0.05	17.02[b]	15.80[b]	18.23[b]	23.09[a]	1.22	0.05
The partitioning factor (PF), mg DMD/ mL Gas										
24 h	1.95[a]	1.89[b]	0.04	0.05	1.95	1.96	1.92	1.87	0.03	0.21
48 h	2.12	2.09	0.05	0.18	2.12	2.11	2.10	2.09	0.02	0.56
Gas production (GP), mL gas/ g DMD										
24 h	388.94	376.22	8.21	0.11	383.31	394.56	371.02	381.43	6.36	0.22
48 h	448.21[a]	430.00[b]	10.19	0.04	437.73[b]	458.69[a]	426.42[b]	433.59[b]	3.49	0.01
Short chain fatty acid concentrations (SCFA), mmol/g DM										
24 h	4.32	4.17	0.05	0.11	4.25	4.38	4.12	4.23	0.07	0.21
48 h	4.97[a]	4.77[b]	0.05	0.04	4.86[b]	5.09[a]	4.74[b]	4.81[b]	0.04	0.01
Microbial crude protein biomass production (MCP), mg / g DM										
24 h	147.59	145.43	2.41	0.55	149.25	145.94	148.71	142.16	3.28	0.49
48 h	161.39	163.99	1.71	0.32	164.04	158.74	165.32	162.67	2.10	0.28

[ab] Means in the same row with different superscript differ (P<0.05), SEM=standard of the mean, SFA=saturated fatty acid, PUFA=polyunsaturated fatty acid, TA=tallow, PO=palm oil, SBO=soybean oil, SFO=sunflower oil. The partitioning factor (PF) =IVDMD (mg)/GP72 (ml) [Blümmel *et al.*, 1997], Gas production (GP) =Gas (mL/g DM)/DMD (g) [Salem *et al.*, 2015]

Short chain fatty acid concentrations (SCFA, mmol/ 200 mg DM) =0.0222GP–0.00425 [Getachew *et al.*, 2002]

Microbial crude protein biomass production (MCP, mg/g DM) =DMD (mg)–Gas (mL) x 2.2 (mg/mL) [Blümmel *et al.*, 1997]

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