Influence of reducing agent on microbial fermentation characteristics using an *in vitro* gas production method

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Introduction

In vitro gas production methods are used to assess nutritive value of ruminant (Theodorou *et al.*, 1994) or monogastric (Williams *et al.*, 2005) feed ingredients.

The *in vitro* incubation medium is composed of :

- a mixed organic solution, containing usually a reducing agent ;
- the microbial inoculum ;
- the test substrate.

The reducing agent, generally Na_2S or cysteine HCl, generates the required anaerobic environment.

BUT the addition of a reducing agent can disrupt the balance between bacterial species by the production of toxic metabolites in non physiological concentration.

Materials and Methods

4 Test substrates :

Soy proteins, casein, potato starch, cellulose

3 Incubation media :

One of the 3 organic solutions (containing Na_2S, cysteine HCl or without reducing agent) (Table 1)
+ pig faecal inoculum (5%)

Table 1. Composition of the three organic solutions, based on Menke and Steingass (1988).

Medium Na_2S	Medium Cysteine HCl	Medium Control
Macrominerals : Na, K, Mg		
Microminerals : Ca, Mn, Co, Fe		
Buffer : $NaHCO_3$/NH_4Cl		
Resazurin		
Na_2S (285 mg/l)	**Cysteine HCl (500 mg/l)**	**Ø**
Water		

Fermentation procedure :

- 200 mg of the test substrate + 30 ml of the incubation media (saturated with CO_2) placed in glass bottle equipped with a pressure sensor module (Fig. 1a)
- Fermentation for 72h at 39°C (Fig. 1b)

Analyses :

- Volume of gas produced from the pressure data
- SCFA production after 8, 24 and 72h of fermentation by HPLC

Figure 1a. Glass bottle equipped with a pressure sensor module (Gas Production System, Ankom RF).
Figure 1b. Fermentation during 72h at 39°C in a shaking water-bath.

References

Menke K. and Steingass H., 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev.*, 28, 7-55.
Groot J.C.J., Cone J.W., Williams B.A., Debersaques F.M.A. and Lantinga E.A., 1996. Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feeds. *Anim. Feed Sci. Technol.*, 64, 77-89.
Theodorou M.K., Williams B.A., Dhanoa M.S., McAllan A.B. and France J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim. Feed Sci. Technol.*, 48, 185-197.
Williams B.A., Bosch M.W., Boer H., Verstegen M.W.A and Tamminga S., 2005. An *in vitro* batch culture method to assess potential fermentability of feed ingredients for monogastric diets. *Anim. Feed Sci. Technol.*, 123–124, 445-462.

Results

A. Gas production

As shown on Fig. 2 and Table 2 (A), the fermentation of carbohydrate ingredients produces a higher final gas volume than the protein ingredients *(P<0.05)*.

Microbial inoculum produces quickly an important quantity of gas with the potato starch substrate, which explains the higher rate of gas production of this substrate (*P<0.05*) (Table 2, Rmax).

Use of cysteine HCl as reducing agent negatively influences the maximum rate of gas production of the carbohydrate ingredients (*P<0.05*) (Table 2, Rmax).

Fermentation of the cellulose by intestinal bacteria requires a higher incubation period than other tested substrates *(P<0.05)* (Table 2, Tmax).

Table 2. Gas fermentation parameters (A : maximum gas volume for t=∞ ; Rmax : maximum rate of gas production ; Tmax : time at which Rmax is reached) modelled according to Groot *et al.* (1996).

Substrate	Reducing agent	A (ml/gDM)	Rmax (ml/gDMxh)	Tmax (h)
Casein	Control	123.2^{b}	10.1^{c}	7.4^{cde}
	Na_2S	121.8^{b}	10.5^{c}	7.7^{cde}
	Cystéine	116.6^{b}	9.4^{cd}	5.9^{de}
Soy proteins	Control	116.8^{b}	3.6^{f}	14.2^{b}
	Na_2S	99.3^{b}	3.2^{f}	12.2^{b}
	Cystéine	109.4^{b}	3.6^{f}	5.5^{e}
Potato starch	Control	282.5^{a}	54.3^{a}	8.5^{cd}
	Na_2S	295.1^{a}	53.2^{a}	8.4^{cde}
	Cystéine	247.5^{a}	50.6^{b}	8.9^{c}
Cellulose	Control	305.1^{a}	8.2^{d}	40.6^{a}
	Na_2S	279.1^{a}	8.1^{d}	42.9^{a}
	Cystéine	343.4^{a}	6.3^{e}	43.0^{a}
Sources of variation		**P-value**		
Substrate		<0.001 ***	<0.001 ***	<0.001 ***
Reducing agent		0.917	0.001 **	0.021 *
Substrate x Red. agent		0.641	0.030 *	0.002 **

a-e : different letters within a column indicate significant (P<0.05) differences
DM, dry matter

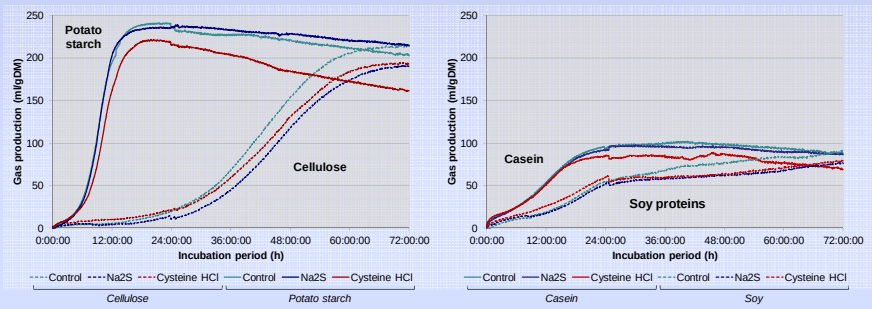

Figure 2. Gas production curves during *in vitro* fermentation of carbohydrate (potato starch, cellulose) and protein (casein, soy proteins) ingredients in three incubation media (red curve : with cysteine HCl ; blue curve : with Na_2S ; green curve : without reducing agent).
DM, dry matter

B. Short-chain fatty acid (SCFA) production

As shown on Fig. 3, production of total SCFA for cellulose increases considerably at the end of the incubation period, which confirms the gas production curve observed (Fig. 2).

Except for soy proteins, fermentation of the ingredients in the three tested incubation media is similar.

A higher total SCFA production is observed when cysteine HCl is used as reducing agent for the fermentation of soy proteins.

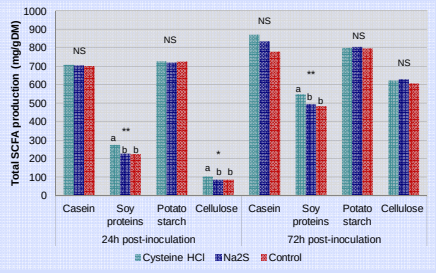

Figure 3. Short-chain fatty acid production (SCFA) after 24 and 72h of fermentation of the ingredients in three incubation media (red : with cysteine HCl ; blue : with Na_2S ; green : without reducing agent).
a, b : different letters for a given test substrate and incubation period indicate significant (P<0.05) differences
DM, dry matter ; NS, not significant.

Conclusion

The addition of a reducing agent, usually Na_2S or cysteine HCl, in the incubation medium can disrupt the balance between bacterial species by the production of toxic metabolites.

These results suggest that the simplification of the *in vitro* incubation media by omissing the use of a reducing agent doesn't alter the fermentation kinetics and the total short-chain fatty acid production after 72h of fermentation. The saturation of the incubation medium with CO_2 seems sufficient to generate the required anaerobic environment.