



# Storage conditions of rumen inoculum: impact on gas productions in mini dual flow fermenters

**V. Berthelot <sup>1</sup>, M. Charef-Mansouri <sup>1</sup>, A.M Davila <sup>2</sup>, L-P. Broudiscou <sup>1, 3</sup>**

<sup>1</sup> UMR 791 MoSAR, AgroParisTech INRAE, Université Paris-Saclay, France

<sup>2</sup> UMR 914 PNCA, AgroParisTech INRAE, Université Paris-Saclay, France

<sup>3</sup> UMR 1419 NuMeA, INRAE UPPA, France

# Context

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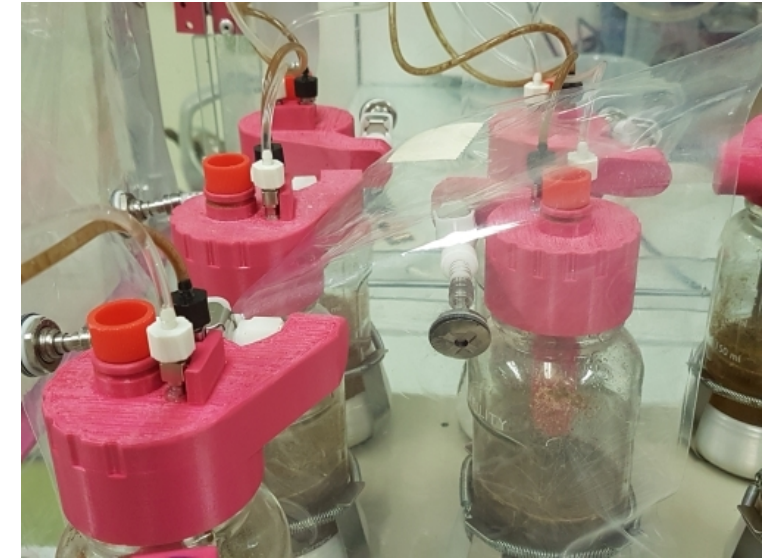
- Rumen microbiota continuous cultures allow to
  - 1) adapt microbiotas to experimental treatments
  - 2) quantify microbial activities
- In 2025, no more cannulated ruminants in INRAE experimental facilities
- Conservation of rumen fluid (Alterfi project) interesting
  - 1) for serial studies using the same inoculum as a reference
  - 2) to offset the difficulties in accessing donor animals
  - 3) to add samples of small ruminants rumen fluid to obtain a single inoculum ...



# Aim of the study

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- Optimise a method of rumen content preservation to restore *in vitro* fermentations and methanogenesis after a 5-day adaptation in mini dual flow fermenters



## test of different conditions

Cryoprotectants

Freezing storage temperatures

Duration of storage



# Experiment 1

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## test of different conditions

Cryoprotectant  
(5% DiMethylSulfOxide)

Freezing storage  
temperatures  
(-80°C vs -20°C)

Duration of storage  
(fresh vs 1 month vs 4 months)

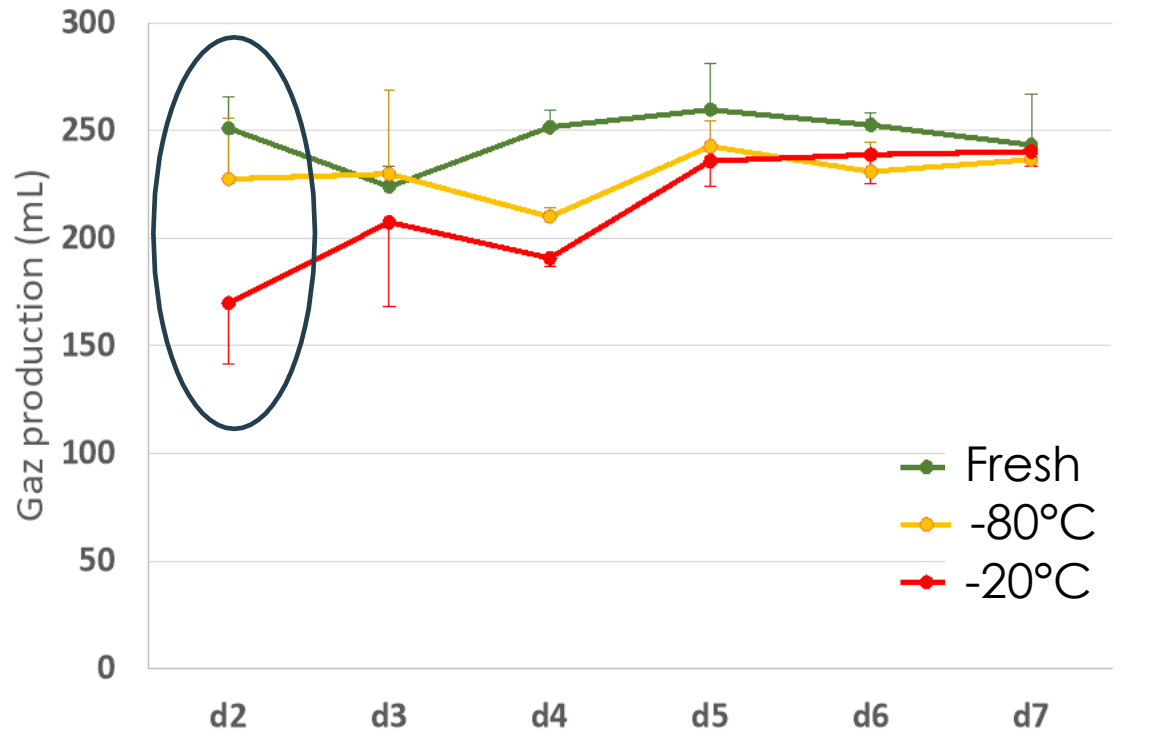
- Donors of rumen inoculum : 2 rumen cannulated dry goats
- Working volume 80 mL; liquid (7%/h) and solid (3%/h) turnover rates
- Artificial saliva at 7 mL/h
- Each condition tested in triplicate for 7 days
- Diet (hay 0.6; barley 0.2; soybean meal 0.2) given twice a day
- Measurement of daily pH, gas volume and production (CH<sub>4</sub>, H<sub>2</sub>)



# Effect of 1-month storage temperature on gas and CH<sub>4</sub> daily productions

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## Gas



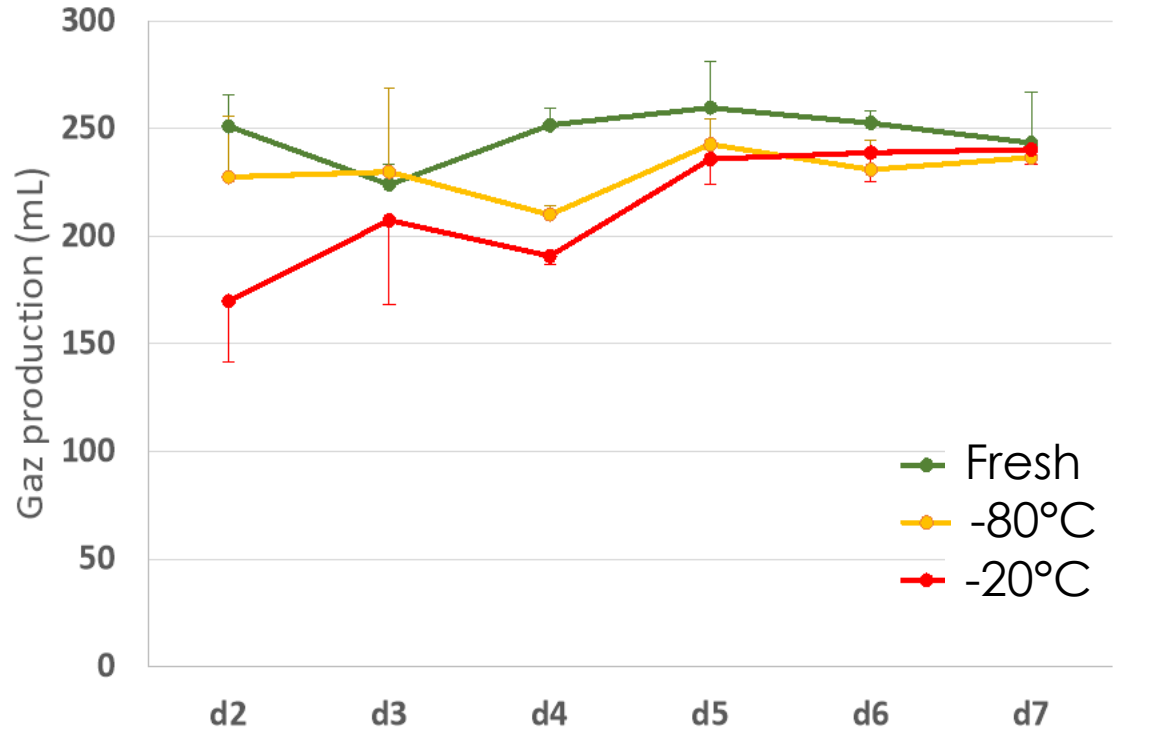
Storage at -80°C present a better start of fermentation than storage at -20°C



# Effect of 1-month storage temperature on gas and CH<sub>4</sub> daily productions

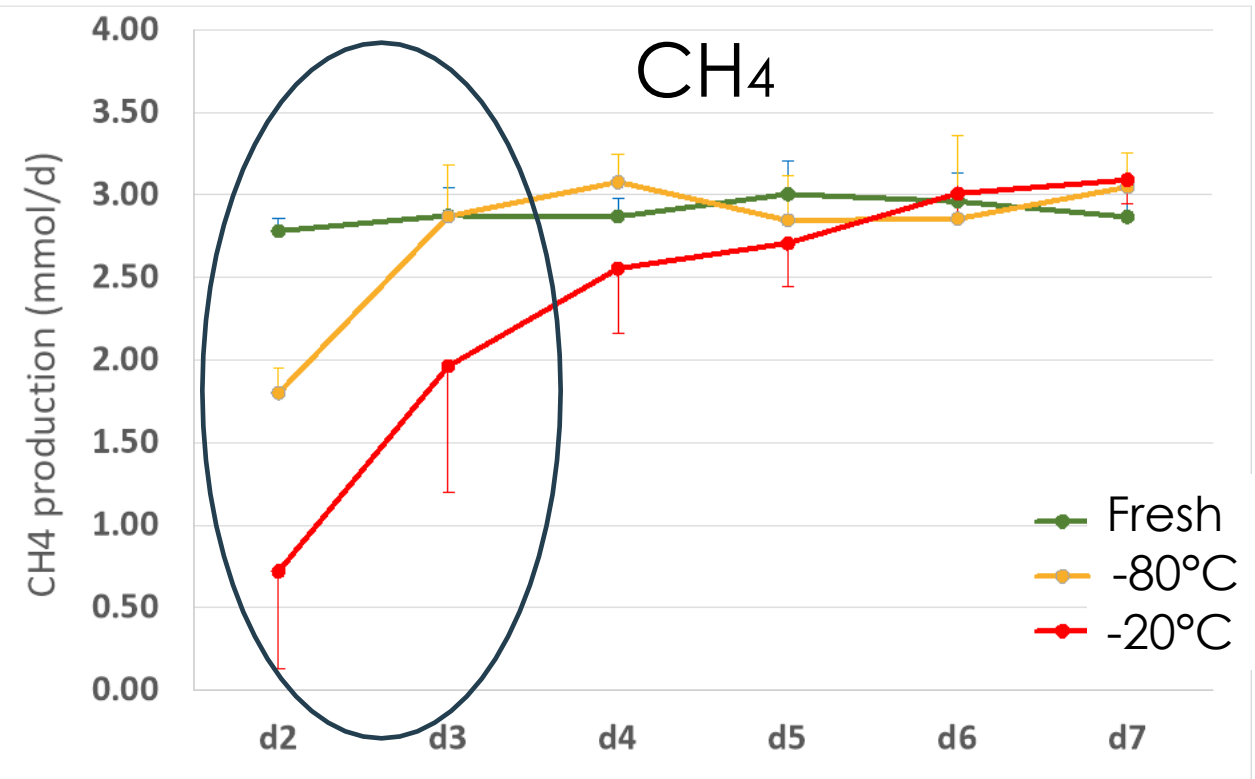
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## Gas



Storage at -80°C present a better start of fermentation than storage at -20°C

## CH<sub>4</sub>



Need 72 hours to reach reference CH<sub>4</sub> productions with storage at -20°C



# Effect of 1-month storage temperature on pH and gas production after a 5-day adaptation

d6-7	Fresh	1 month		RSD	P value	
		-20°C	-80°C		Temperature	Goat
pH	<b>6.26<sup>a</sup></b>	<b>6.26<sup>a</sup></b>	<b>6.21<sup>b</sup></b>	0.054	<b>0.04</b>	0.94
gaz volume (mL)	247	240	234	19.8	0.22	0.25
CH <sub>4</sub> (mmol/d)	2.91	3.05	2.95	0.265	0.44	0.53
H <sub>2</sub> (mmol/d)	<b>0.0097<sup>b</sup></b>	<b>0.0054<sup>a</sup></b>	<b>0.0140<sup>c</sup></b>	0.0042	<b>&lt;0.0001</b>	0.06

Means within a row with a different superscript differ (P temperature value)

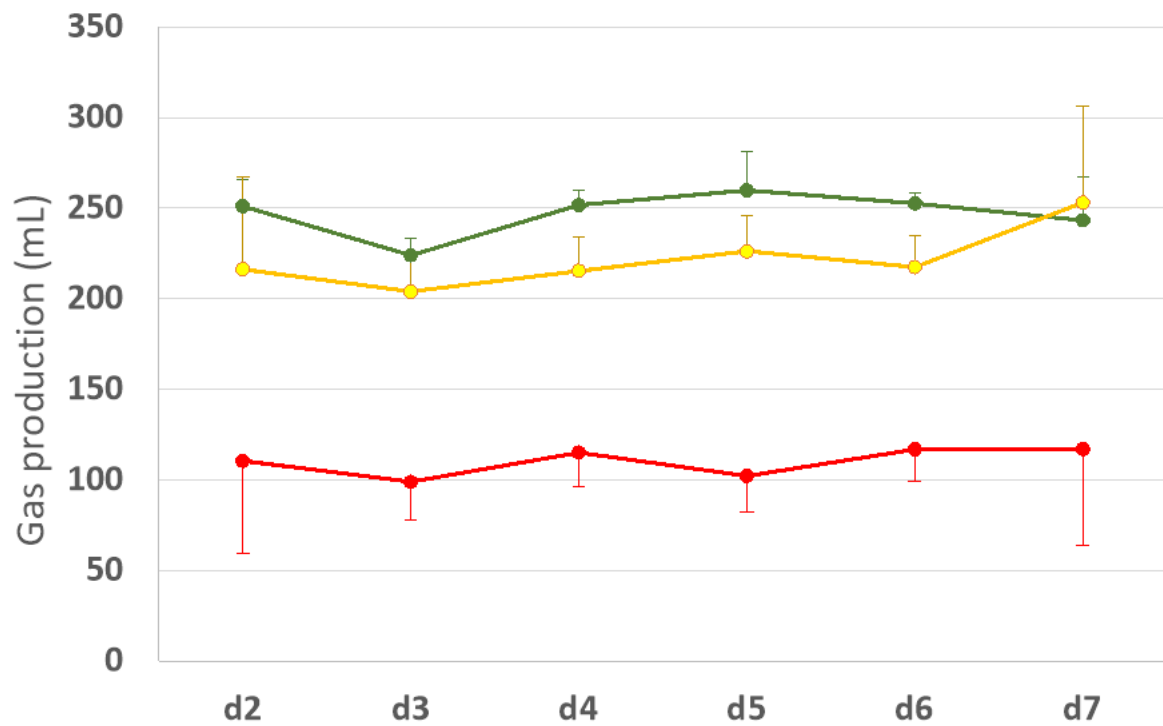
After 5 days of adaptation, storage at -20°C or -80°C is OK



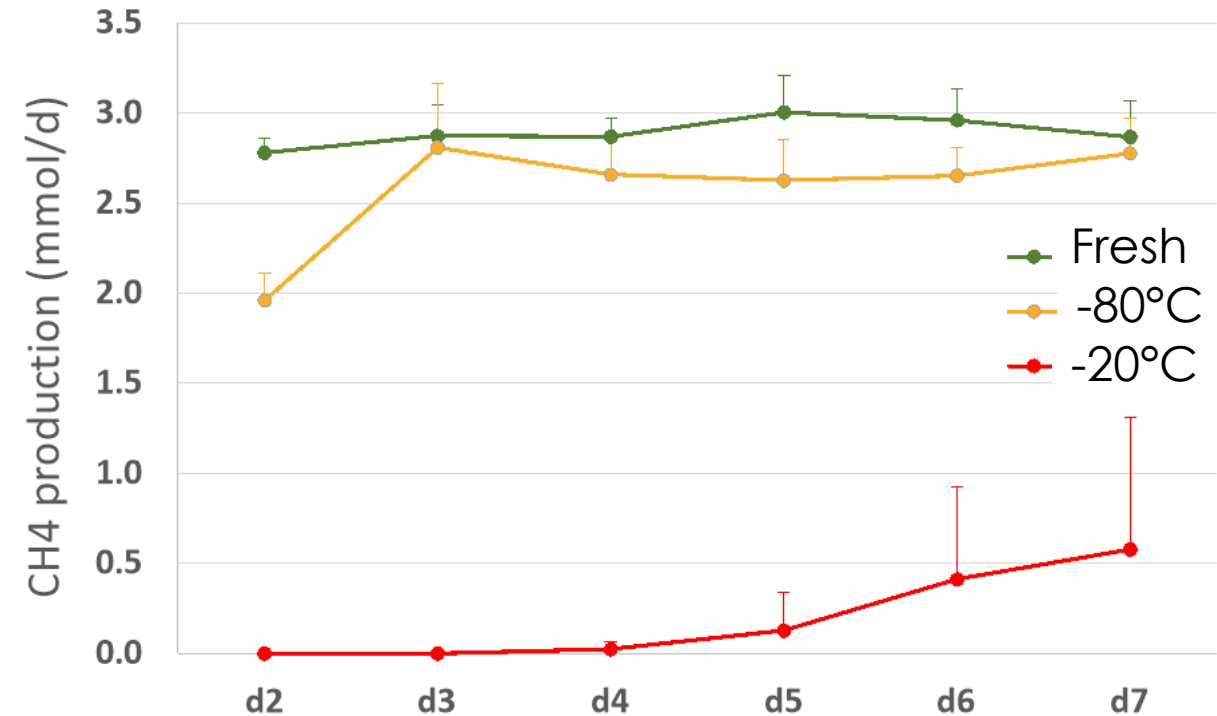
# Effect of 4-months storage temperature on gas and CH<sub>4</sub> daily productions

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## Gas



## CH<sub>4</sub>



Gas production and methanogenesis is greatly impaired by storage at -20°C



# Effect of 4-months storage temperature on pH and gas production after a 5-day adaptation

d6-7	Fresh	4 months		RSD	P value		
		-20°C	-80°C		Temperature	Goat	Temp x Goat
pH	<b>6.26<sup>a</sup></b>	<b>6.35<sup>b</sup></b>	<b>6.28<sup>a</sup></b>	0.059	<b>0.003</b>	<b>0.03</b>	<b>0.04</b>
gaz volume (mL)	<b>247<sup>a</sup></b>	<b>117<sup>b</sup></b>	<b>235<sup>a</sup></b>	26.6	<b>&lt;0.0001</b>	0.31	<b>0.03</b>
CH <sub>4</sub> (mmol/d)	<b>2.91<sup>a</sup></b>	<b>0.49<sup>b</sup></b>	<b>2.72<sup>a</sup></b>	0.367	<b>&lt;0.0001</b>	<b>0.001</b>	<b>&lt;0.0001</b>
H <sub>2</sub> (mmol/d)	<b>0.0097<sup>a</sup></b>	<b>0.117<sup>b</sup></b>	<b>0.0149<sup>a</sup></b>	0.0569	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>

Means within a row with a different superscript differ (P temperature value)

4 months later :

storage at -80°C is OK

gaz (CH<sub>4</sub>) production is greatly impaired at -20°C, in interaction with the animal donor



# Experiment 2

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## test of different conditions

Cryoprotectants  
(5% DMSO or glycerol)

Freezing storage  
temperature  
(-80°C)

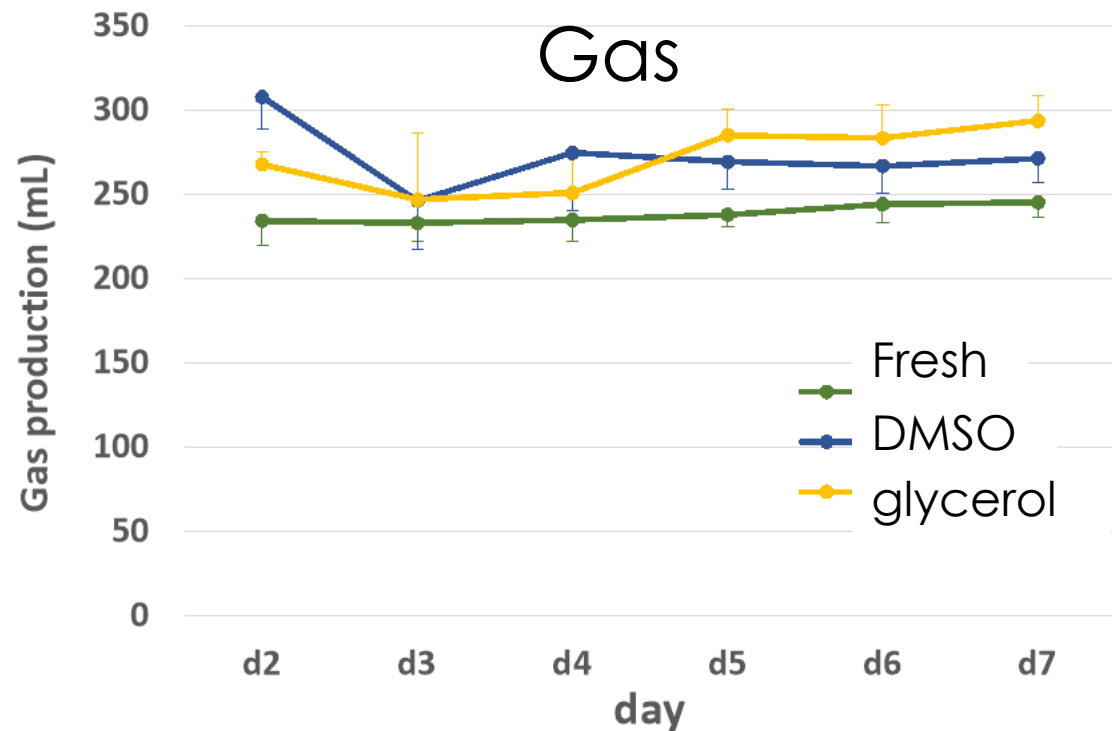
Duration of storage  
(fresh vs 2.5 months vs 12 months)

- Donors of rumen inoculum : 2 rumen cannulated dry goats
- Working volume 80 mL; liquid (7%/h) and solid (3%/h) turnover rates
- Artificial saliva at 7 mL/h
- Each condition tested in triplicate for 7 days
- Diet (hay 0.6;barley 0.2; soybean meal 0.2) given twice a diet
- Measurement of daily pH, gas volume and production (CH<sub>4</sub>, H<sub>2</sub>)

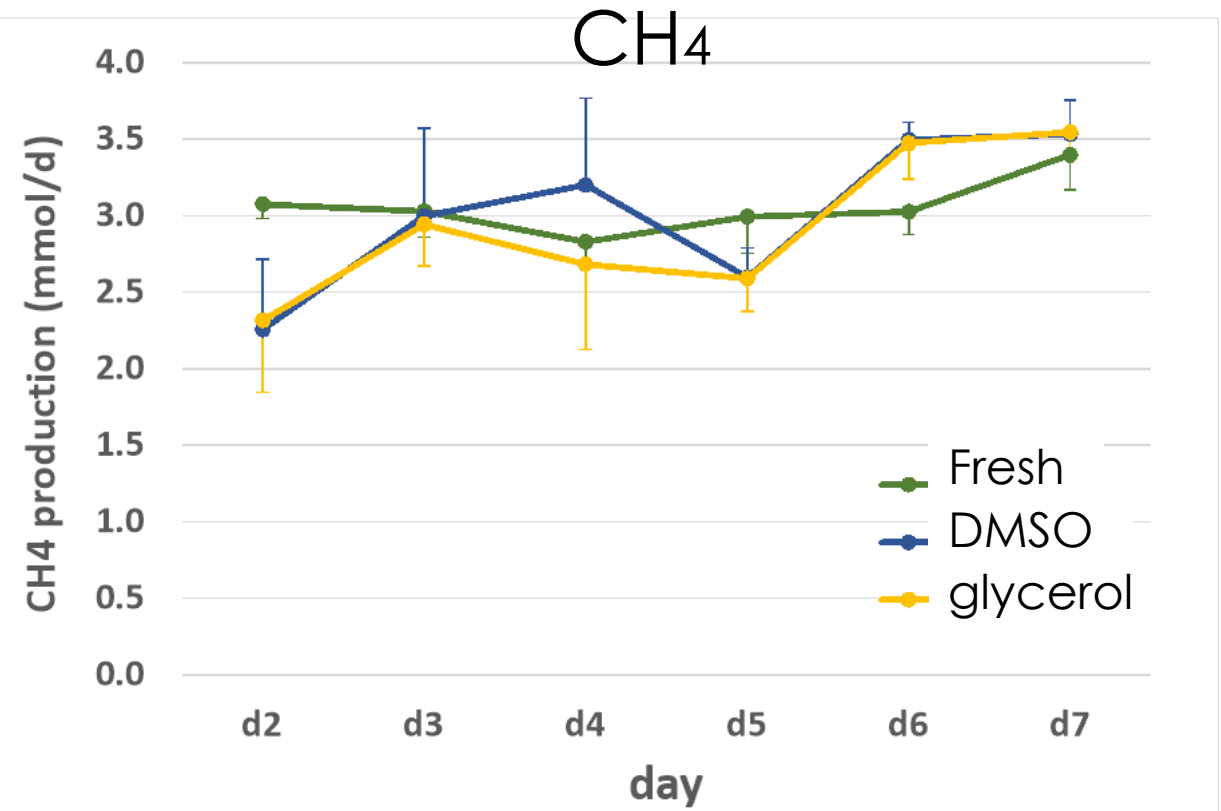
**Same procedure**



# Changes in gas and CH<sub>4</sub> productions after 2.5 months of storage according to cryoprotectants

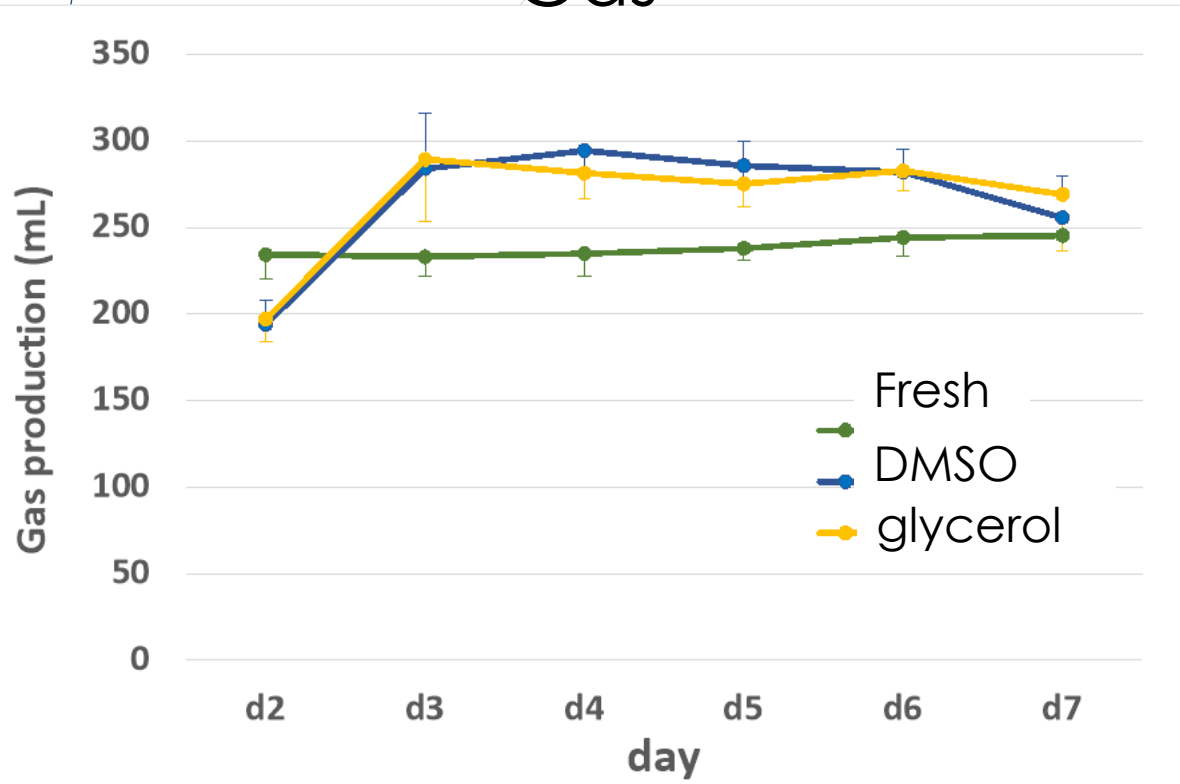


No difference in restoring CH<sub>4</sub> production for cryoprotectant tested



# Changes in gas productions after 12 months of storage according to cryoprotectants

## Gas

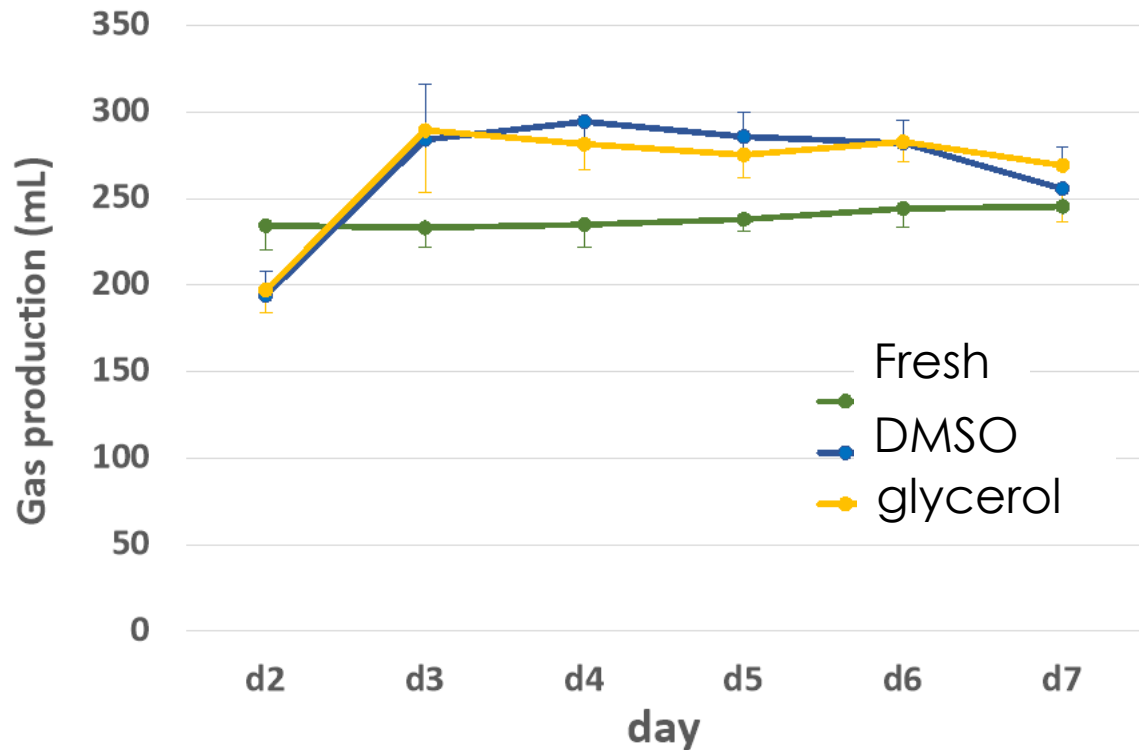


No difference in restoring gas production



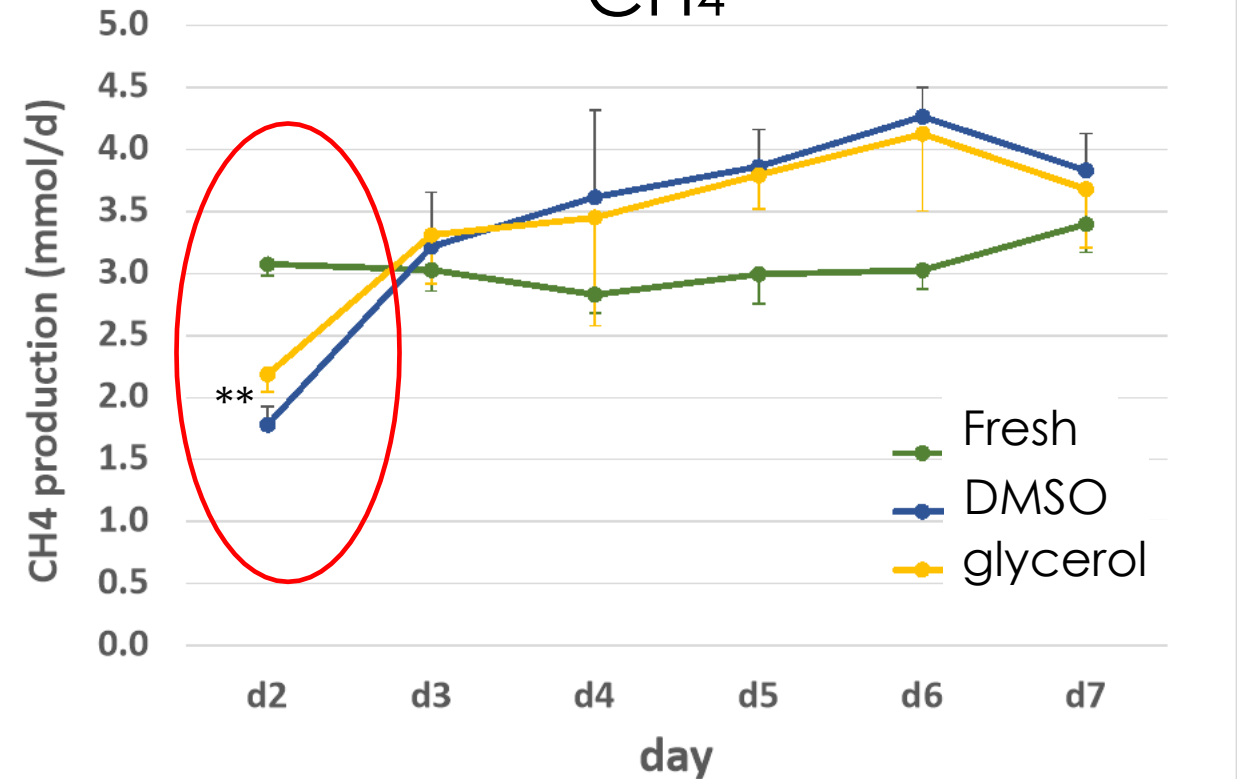
# Changes in gas and CH<sub>4</sub> productions after 12 months of storage according to cryoprotectants

## Gas



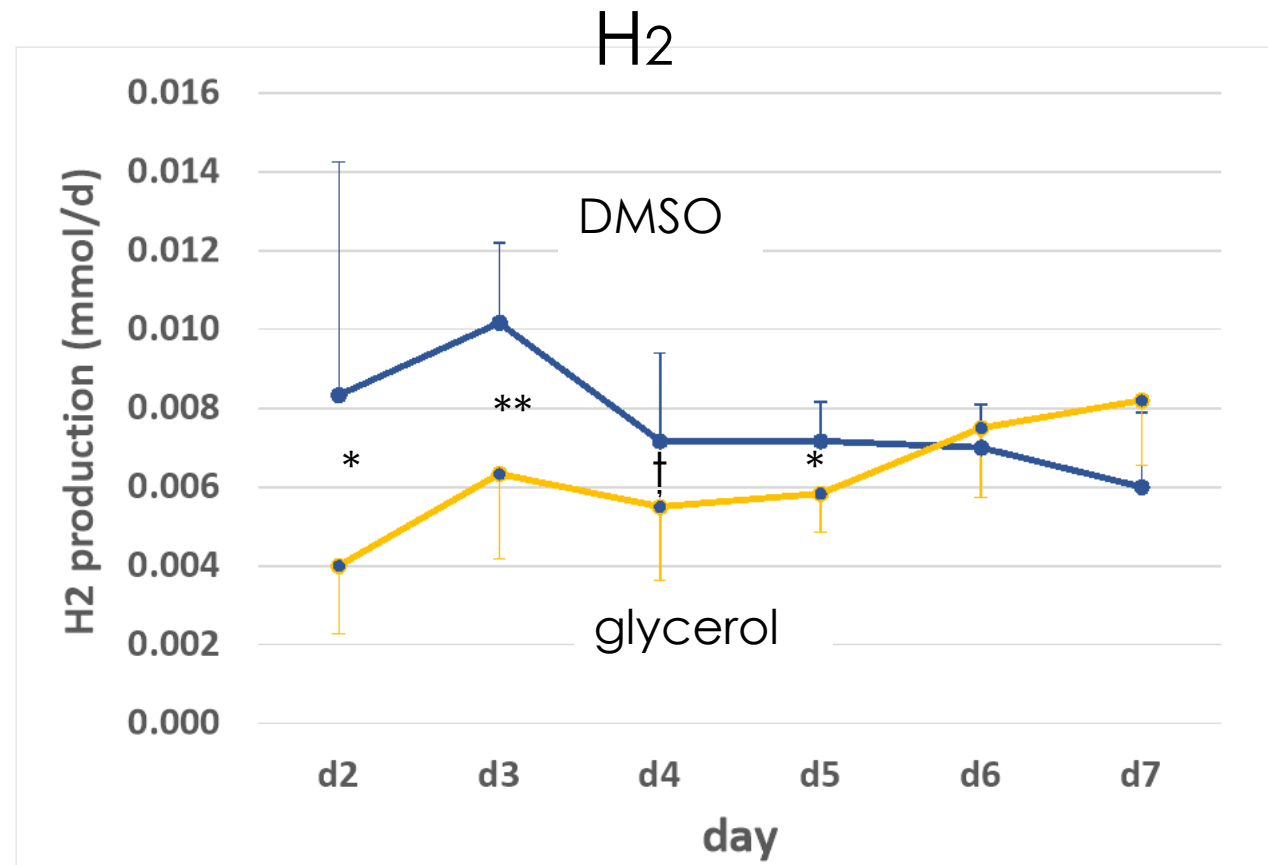
A slower restoration of CH<sub>4</sub> production for DMSO (d2)

## CH<sub>4</sub>



# Changes in H<sub>2</sub> productions after 12 months of storage according to cryoprotectants

... in line with a higher production of H<sub>2</sub> from d2 to d5



# Effect of cryoprotectants on pH and gas production after 5 days of adaptation in the device and 2.5/12 months of storage

d6-7	Fresh	2.5 months			P	12 months			P
		DMSO	Glycerol	RSD	Cryo	DMSO	Glycerol	RSD	Cryo
pH	6.35	6.27	6.28	0.034	NS	6.13	6.18	0.040	0.09
Gas production (mL)	245	269	289	15.8	0.07	262	276	13.7	NS
CH <sub>4</sub> (mmol/d)	3.21	3.51	3.51	0.181	NS	4.05	3.90	0.401	NS
H <sub>2</sub> (mmol/d)	0.0085	0.0074	0.0073	0.00045	NS	0.0065	0.0094	0.0034	NS

No impairment of pH, gas production and methanogenesis



# Conclusions

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- Freezing storage at  $-20^{\circ}\text{C}$  of rumen inoculum can be used for short duration ( $< 1$  month), otherwise it should be stored at  $-80^{\circ}\text{C}$
- Rumen inoculum can be kept for at least one year without any gas production impairment
- No clear advantage of any cryoprotectant studied at this stage
- Data on short chain fatty acid productions and microbiota compositions will soon complete this validation study

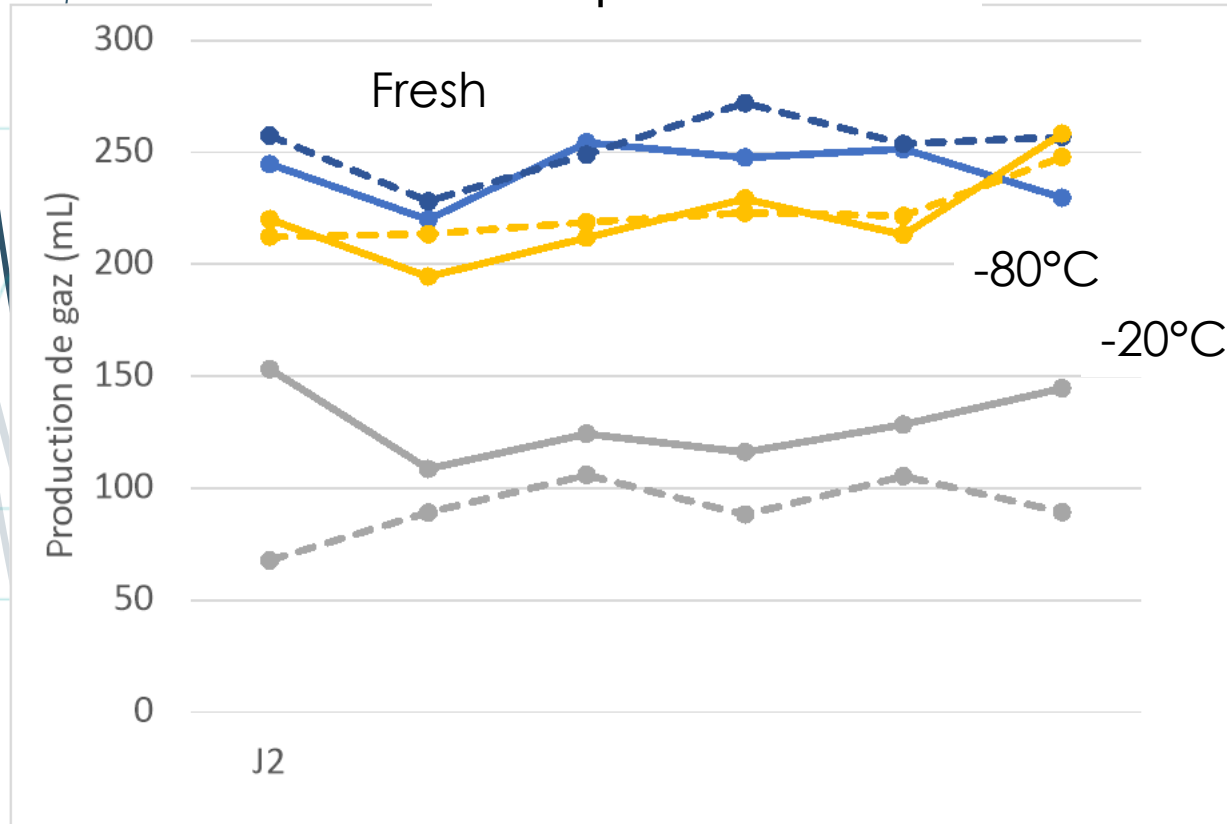


# Thank you for your attention



# Experiment 1: (4 months, goat, goat 1— ; goat 2 - - )

## Gas production



## CH4 production

