



**Ph.D. COURSE** ANIMAL & FOOD SCIENCE  
UNIVERSITY OF PADOVA

**DAFNAE**  
Department of Agronomy Food  
Natural Resources Animals Environment



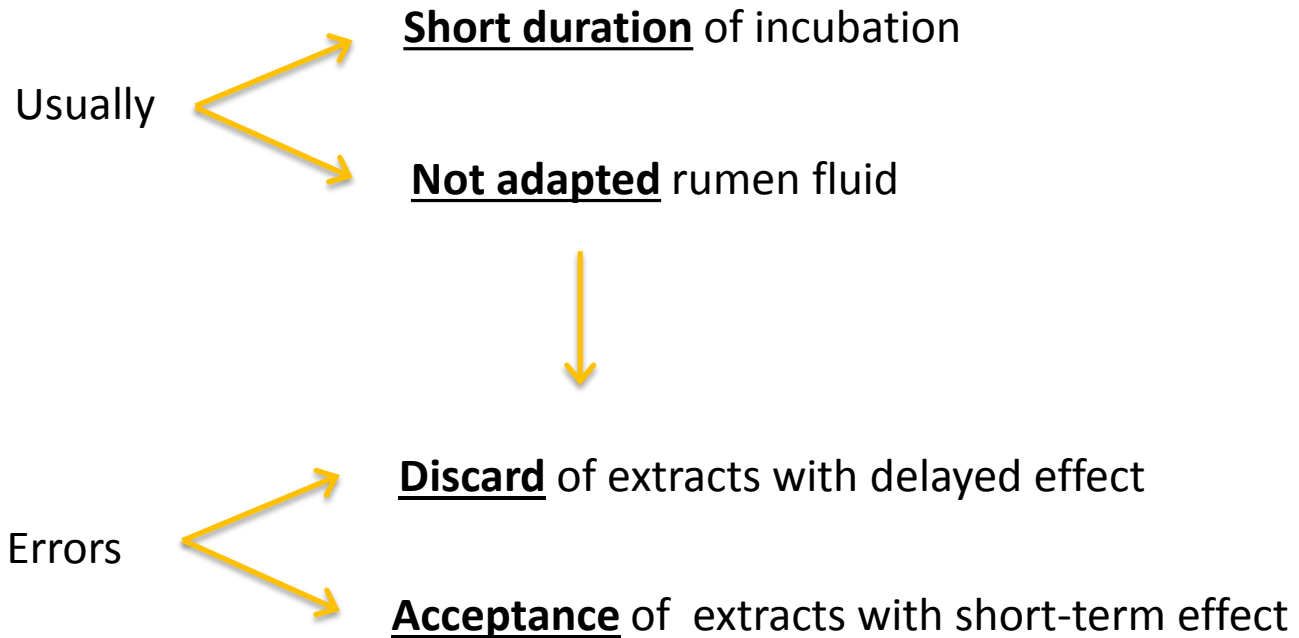
# Effect of pure extracts on *in vitro* CH<sub>4</sub> production using rumen fluid of cows fed or not the same extracts

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August 31<sup>st</sup>, Belfast, UK

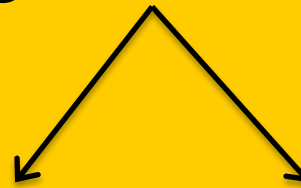
# BACKGROUND

Many *in vitro* studies have been performed to evaluate the effects of plant extracts on CH<sub>4</sub> emissions and rumen fermentation



# AIM

To compare effects on *in vitro* rumen fermentation of different plant extracts using rumen fluid:



not adapted

adapted

# **MATERIALS AND METHODS**

# THE PURE PLANTS EXTRACTS

- Antimicrobial activity
- Effective on rumen fermentation

***Cinnamaldehyde***



***Limonene***

***Allyl-sulfide***



# 1° STEP: RUMEN FLUID DONORS

*LATIN SQUARE 4 X 4*

Period	Cow 1	Cow 2	Cow 3	Cow 4
1 <sup>st</sup>	NOT ADAPTED	CINNAMALDEHYDE	LIMONENE	ALLYL-SULFIDE
2 <sup>nd</sup>	ALLYL-SULFIDE	NOT ADAPTED	CINNAMALDEHYDE	LIMONENE
3 <sup>rd</sup>	LIMONENE	ALLYL-SULFIDE	NOT ADAPTED	CINNAMALDEHYDE
4 <sup>th</sup>	CINNAMALDEHYDE	LIMONENE	ALLYL-SULFIDE	NOT ADAPTED

**Period:** 21 d = 7 transition days + 14 treatment days

**Pure extract:** 1 g/d per cow

## 2° STEP: IN VITRO EXPERIMENT

*Donors rumen fluid*

**NAF = NOT Adapted  
Fluid**

**AF<sub>CIN</sub> = Adapted Fluid to  
Cinnamaldehyde**

**AF<sub>LIM</sub> = Adapted Fluid  
to Limonene**

**AF<sub>ALL</sub> = Adapted Fluid  
to Allyl-Sulfide**

## 2° STEP: IN VITRO EXPERIMENT

<i>Rumen fluid</i>	<i>In vitro</i> *
NAF	NAF
AF <sub>CIN</sub>	
AF <sub>LIM</sub>	
AF <sub>ALL</sub>	

\* Extract dosage: 30 mg added to 1 g of incubated diet



## 2° STEP: IN VITRO EXPERIMENT

<i>Rumen fluid</i>	<i>In vitro</i> *	Tested effect
NAF	NAF	Not adapted
AF <sub>CIN</sub>		
AF <sub>LIM</sub>		
AF <sub>ALL</sub>		

\* Extract dosage: 30 mg added to 1 g of incubated diet

## 2° STEP: IN VITRO EXPERIMENT

<i>Rumen fluid</i>	<i>In vitro</i> *	Tested effect
NAF	NAF	Not adapted
	NAF + CIN	
	NAF + LIM	
	NAF + ALL	
AF <sub>CIN</sub>		
AF <sub>LIM</sub>		
AF <sub>ALL</sub>		

\* Extract dosage: 30 mg added to 1 g of incubated diet

## 2° STEP: IN VITRO EXPERIMENT

<i>Rumen fluid</i>	<i>In vitro</i> *	Tested effect
NAF	NAF	Not adapted
	NAF + CIN	Not adapted + extract
	NAF + LIM	Not adapted + extract
	NAF + ALL	Not adapted + extract
AF <sub>CIN</sub>		
AF <sub>LIM</sub>		
AF <sub>ALL</sub>		

\* Extract dosage: 30 mg added to 1 g of incubated diet

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## 2° STEP: IN VITRO EXPERIMENT

<i>Rumen fluid</i>	<i>In vitro</i> *	Tested effect
NAF	NAF	Not adapted
	NAF + CIN	Not adapted + extract
	NAF + LIM	Not adapted + extract
	NAF + ALL	Not adapted + extract
AF <sub>CIN</sub>	AF <sub>CIN</sub>	
AF <sub>LIM</sub>	AF <sub>LIM</sub>	
AF <sub>ALL</sub>	AF <sub>ALL</sub>	

\* Extract dosage: 30 mg added to 1 g of incubated diet

# 2° STEP: IN VITRO EXPERIMENT

<i>Rumen fluid</i>	<i>In vitro</i> *	<b>Tested effect</b>
<b>NAF</b>	→ <b>NAF</b>	<b>Not adapted</b>
	→ <b>NAF + CIN</b>	<b>Not adapted + extract</b>
	→ <b>NAF + LIM</b>	<b>Not adapted + extract</b>
	→ <b>NAF + ALL</b>	<b>Not adapted + extract</b>
<b>AF<sub>CIN</sub></b>	→ <b>AF<sub>CIN</sub></b>	<i>Adapted</i>
<b>AF<sub>LIM</sub></b>	→ <b>AF<sub>LIM</sub></b>	<i>Adapted</i>
<b>AF<sub>ALL</sub></b>	→ <b>AF<sub>ALL</sub></b>	<i>Adapted</i>

\* Extract dosage: 30 mg added to 1 g of incubated diet

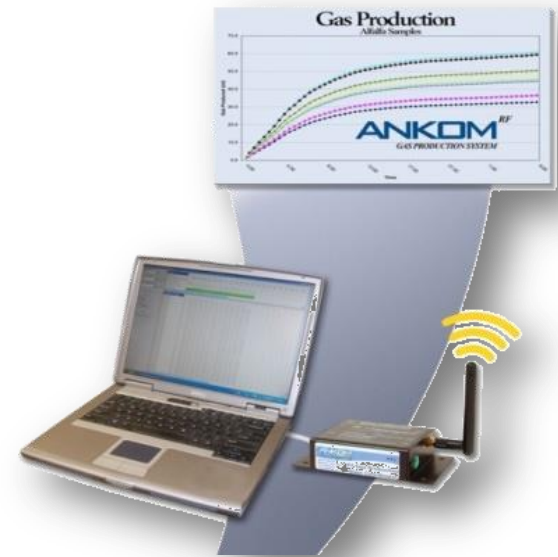
## 2° STEP: IN VITRO EXPERIMENT

<i>Rumen fluid</i>	<i>In vitro</i> *	Tested effect
NAF	NAF	Not adapted
	NAF + CIN	Not adapted + extract
	NAF + LIM	Not adapted + extract
	NAF + ALL	Not adapted + extract
AF <sub>CIN</sub>	AF <sub>CIN</sub>	Adapted
	AF <sub>CIN</sub> + CIN	Adapted + extract
AF <sub>LIM</sub>	AF <sub>LIM</sub>	Adapted
	AF <sub>LIM</sub> + LIM	Adapted + extract
AF <sub>ALL</sub>	AF <sub>ALL</sub>	Adapted
	AF <sub>ALL</sub> + ALL	Adapted + extract

\* Extract dosage: 30 mg added to 1 g of incubated diet

# GAS PRODUCTION SYSTEM: ANKOM<sup>RF</sup>

24 h *in vitro* fermentation



**4 periods x 10 treatments x 4 replicates + 16 blanks  
= 176 incubated bottles**

(Tagliapietra et al, 2010)

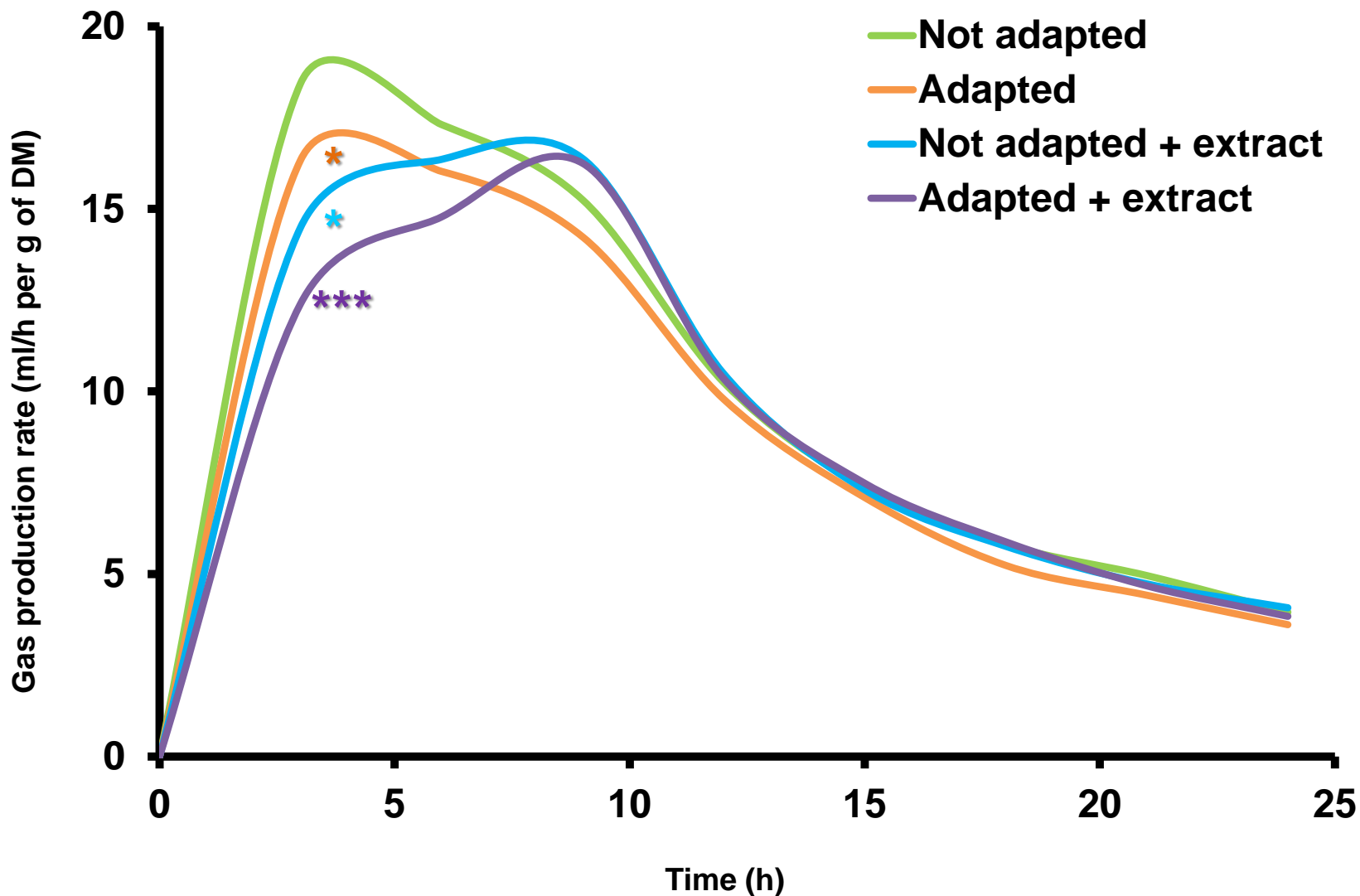
# RESULTS





# CINNAMALDEHYDE: Gas production rate

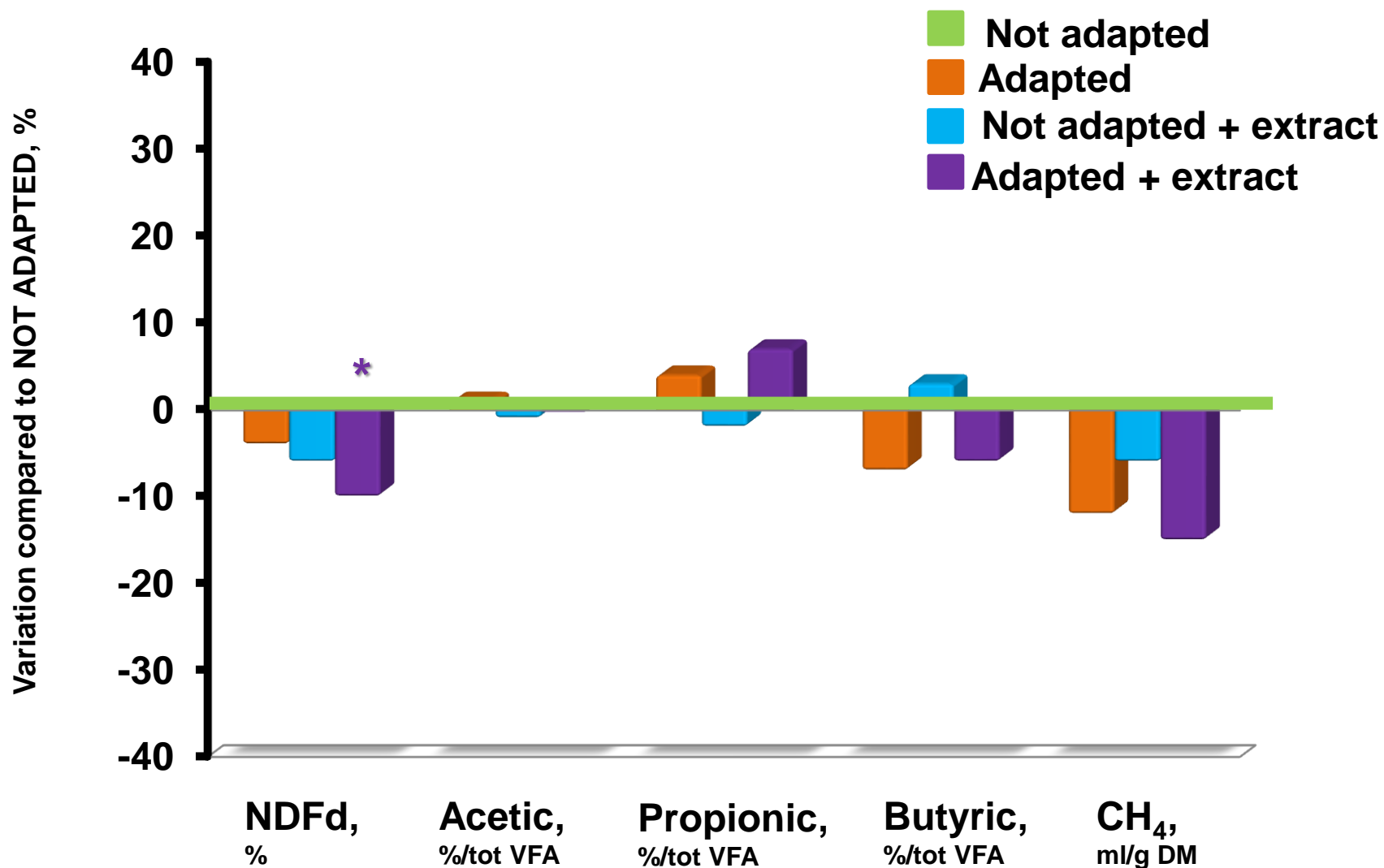
Effects compared to NOT-ADAPTED FLUID: \*  $P \leq 0.05$  \*\*\*  $P < 0.001$





# CINNAMALDEHYDE: Fermentation parameters

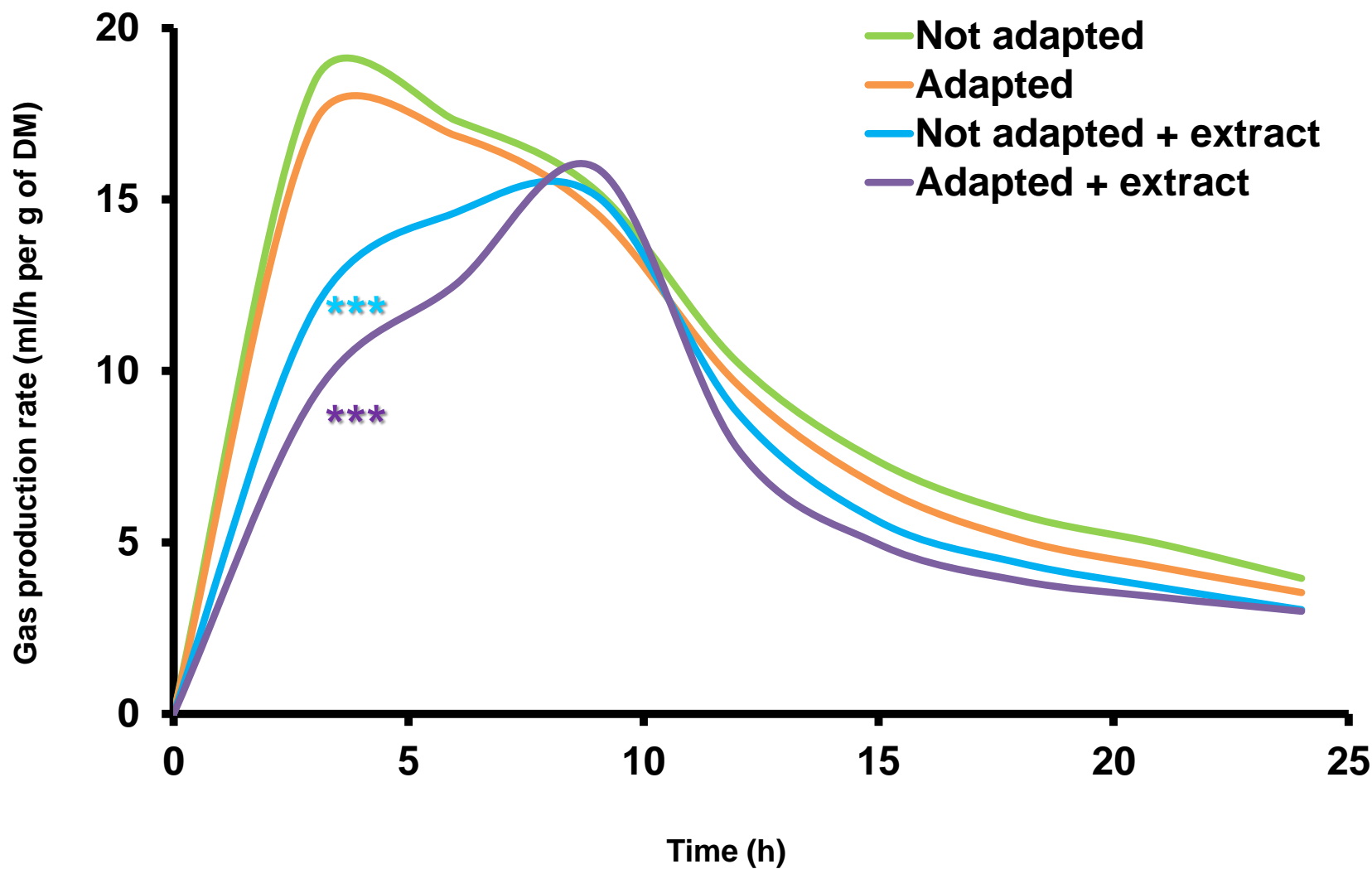
Expressed as differences (%) compared to not-adapted fluid: \*  $P \leq 0.05$





# LIMONENE: Gas production rate

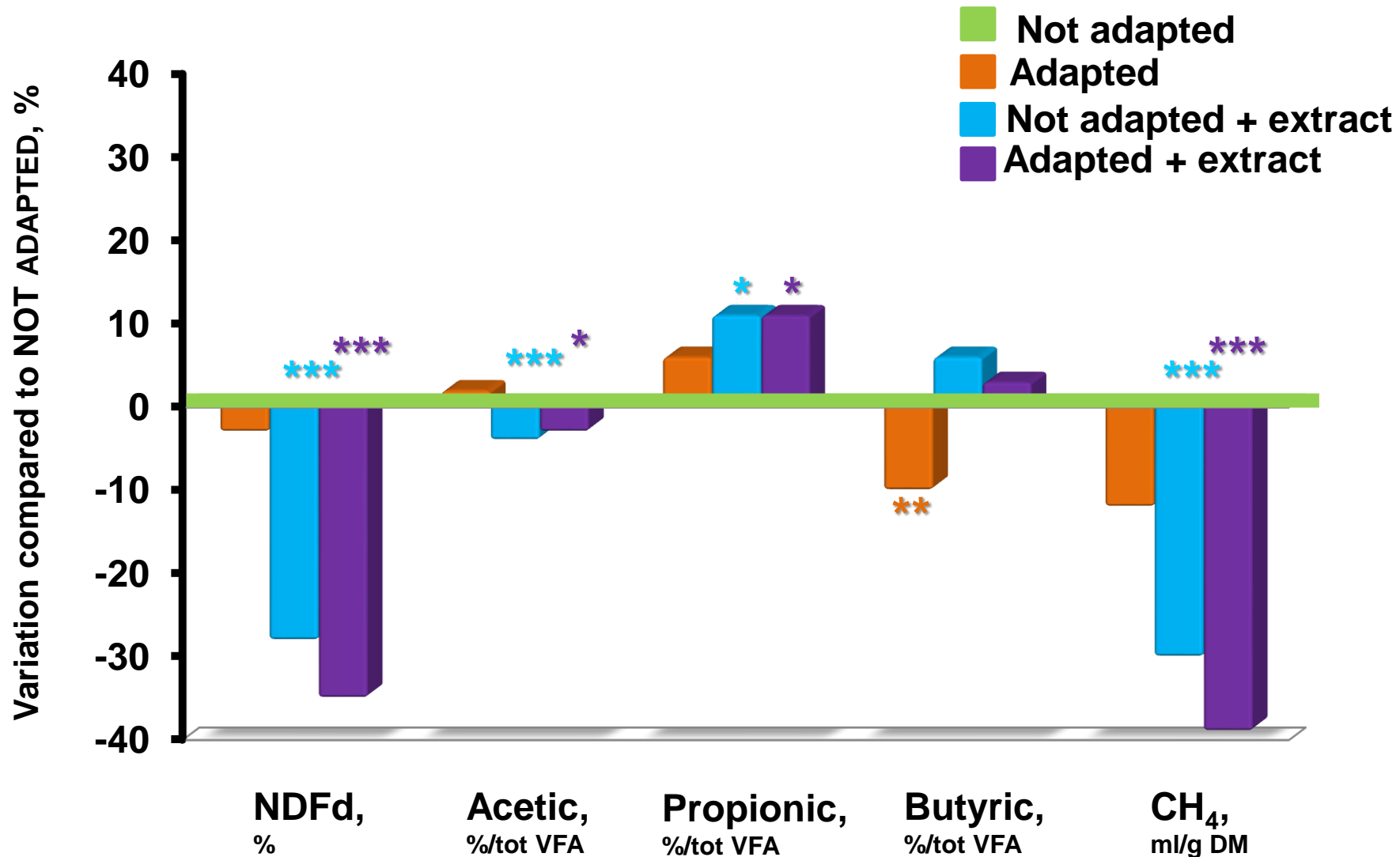
Effects compared to NOT-ADAPTED FLUID: \*\*\*  $P \leq 0.001$





# LIMONENE: Fermentation parameters

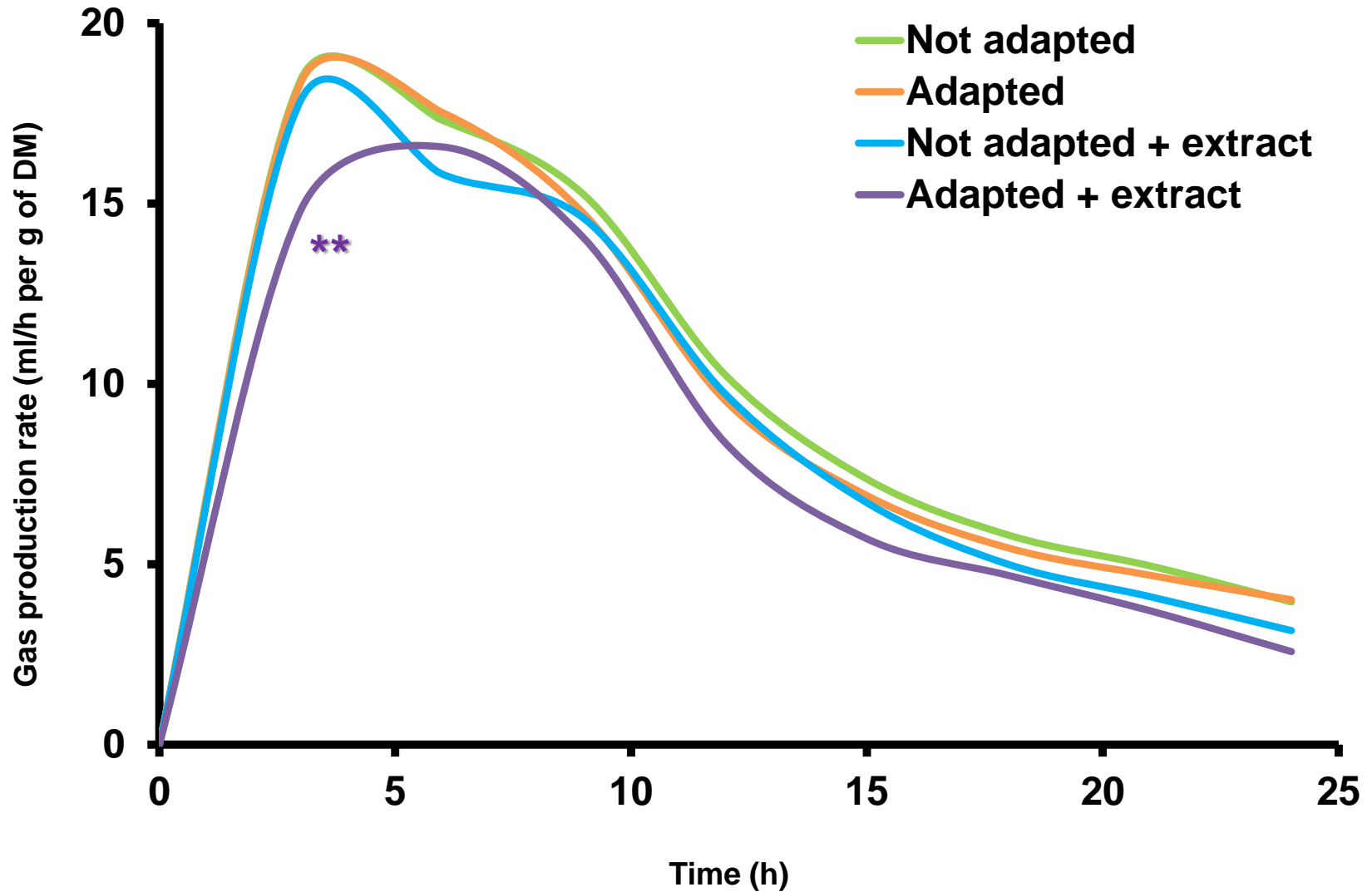
Expressed as differences (%) compared to not-adapted fluid: \*  $P \leq 0.05$  \*\*\*  $P < 0.001$





# ALLYL-SULFIDE: Gas production rate

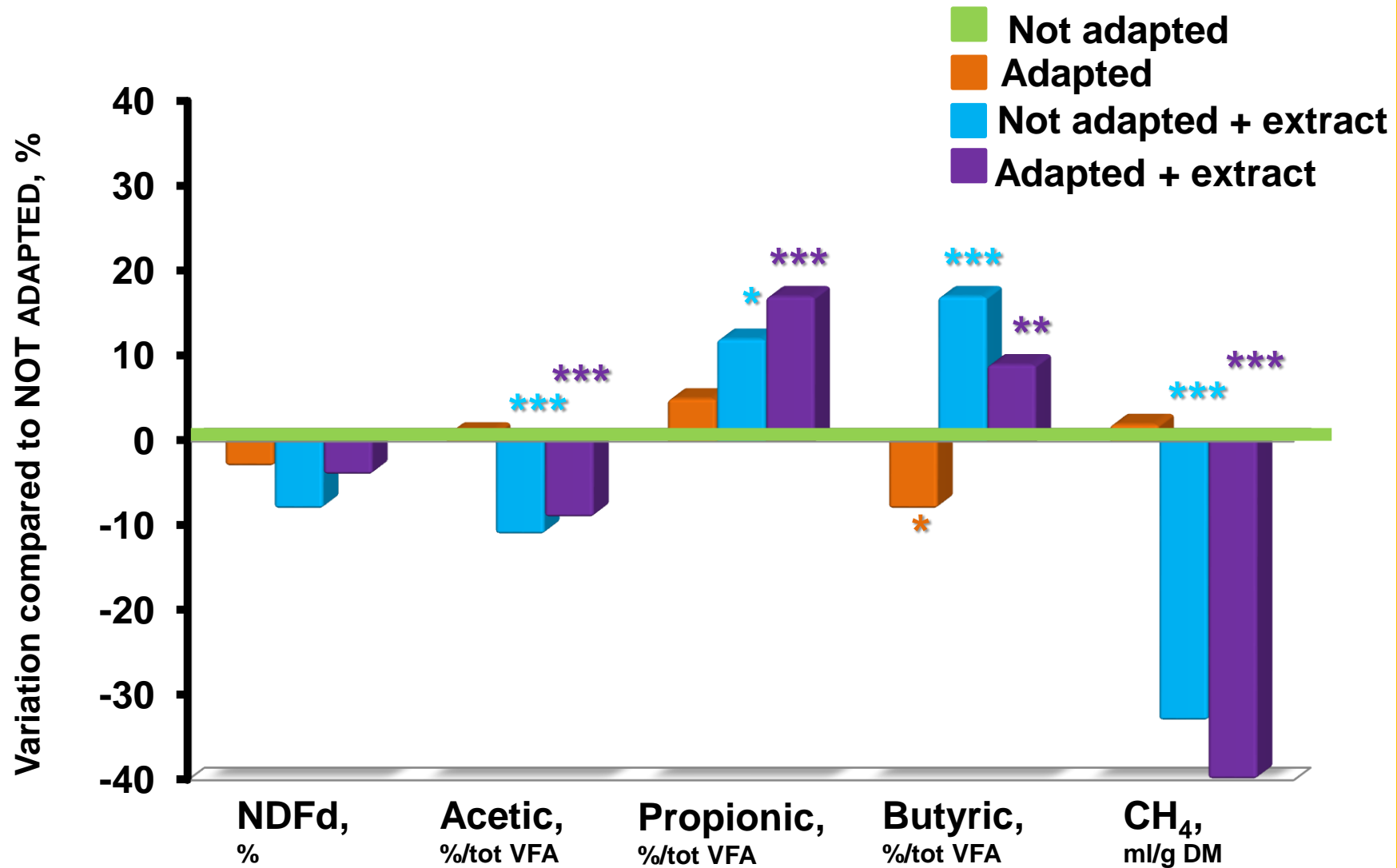
Effects compared to NOT-ADAPTED FLUID: \*\*  $P \leq 0.01$





# ALLYL-SULFIDE: Fermentation parameters

Expressed as differences (%) compared to not-adapted fluid: \*  $P \leq 0.05$  \*\*  $P \leq 0.01$  \*\*\*  $P < 0.001$



The extracts have modified, in different ways, the rumen fermentation:

- CINNAMALDEHYDE ↓ diet degradability = CH<sub>4</sub> production
- LIMONENE ↓ diet degradability ↓ CH<sub>4</sub> production
- ALLYL-SULFIDE = diet degradability ↓ CH<sub>4</sub> production

Under the specific condition of this study, **Allyl-sulfide** turned out to be the best choice within the extracts to mitigate CH<sub>4</sub> emission

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Under the specific condition of this study, **Allyl-sulfide** turned out to be the best choice within the extracts to mitigate CH<sub>4</sub> emission

From a methodological point of view **no statistical differences** have been found between the NOT ADAPTED and the ADAPTED rumen fluid



Possible explanations:

- too low *in vivo* dosage 
- ruminal adaptation of microflora 



**THANK YOU FOR  
YOUR ATTENTION**