



# Influence of feed efficiency and physiological state on rumen VFA and microbial profiles in cattle

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Y Montanholi<sup>2</sup>



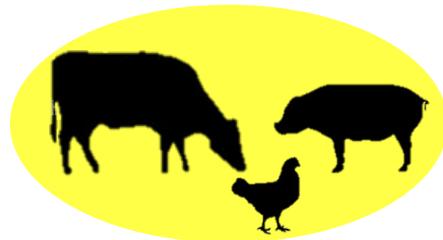
# Outline

- Introduction
- Hypothesis
- Objectives
- Materials and methods
  - Grain-fed study
  - Grass-fed study
- Results and discussion
- Conclusion
- Acknowledgements



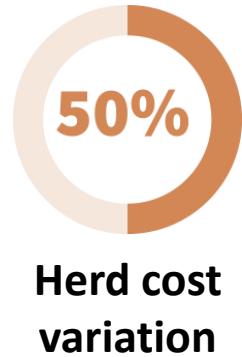
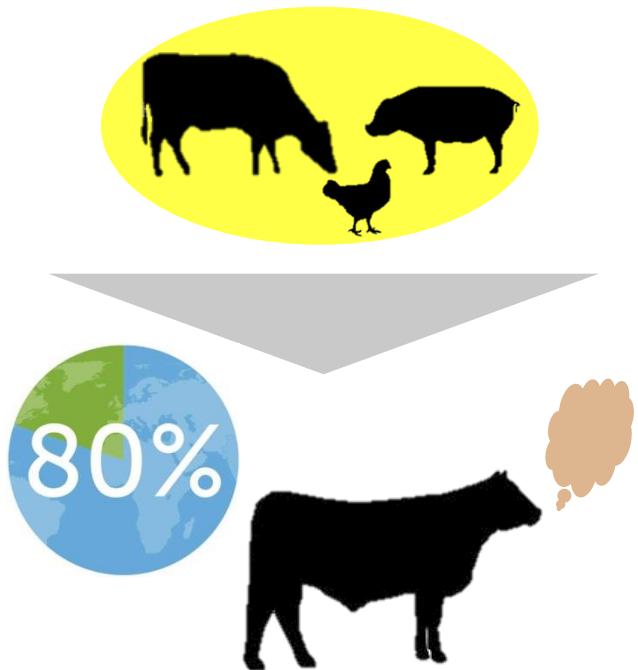
# Introduction: Industry challenges

**30-50% agriculture GHG**



# Introduction: Industry challenges

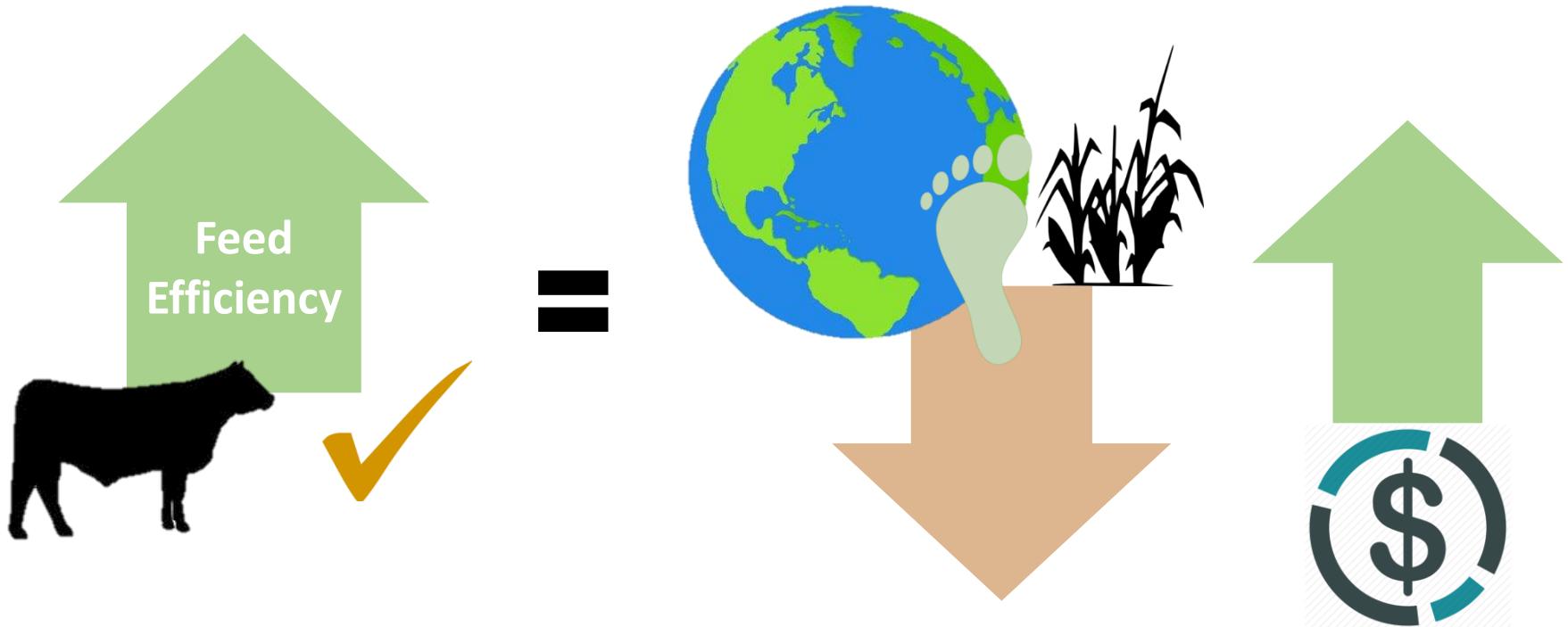
30-50% agriculture GHG



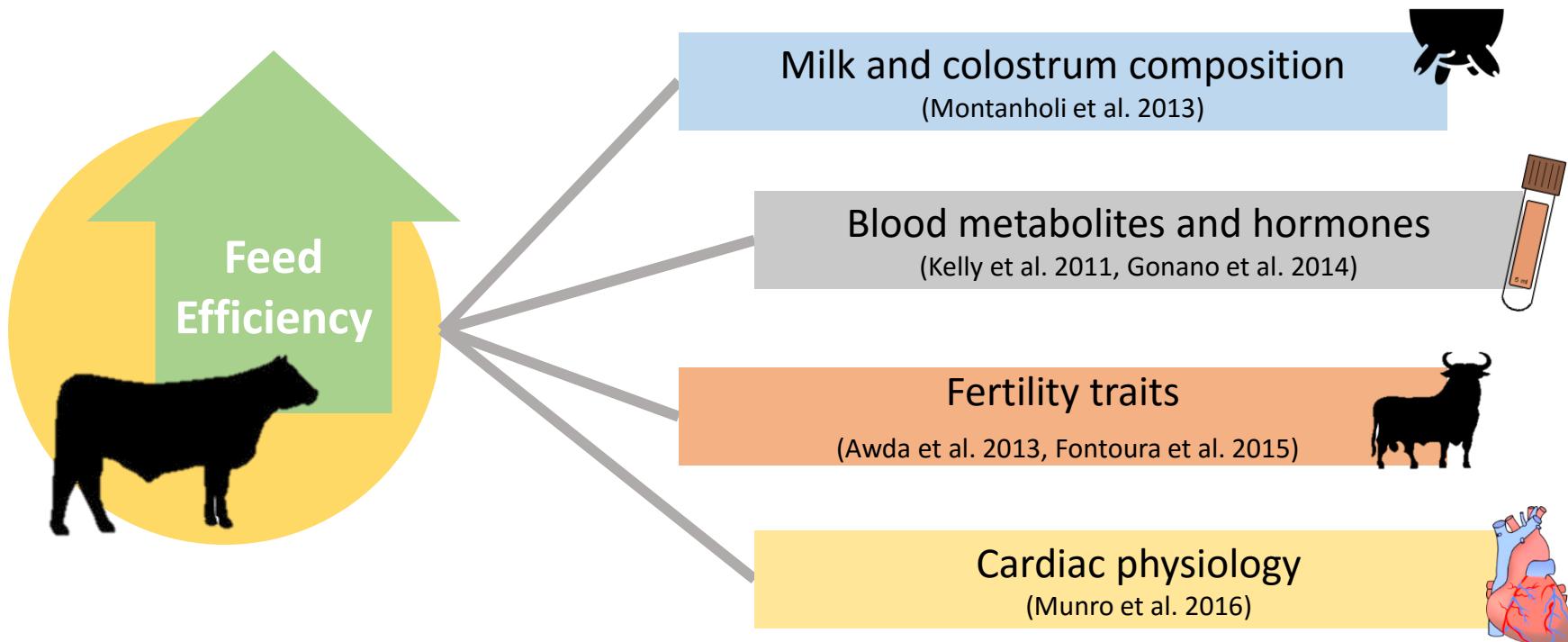
# Introduction: Feed efficiency



# Introduction: Feed efficiency



# Introduction: Feed efficiency



# Introduction: Rumen metabolism

%

10% of the biological variation of RFI due to digestibility

(Richardson and Herd 2004)



Rumen and reticulum = 75% total digestive tract

(Baldwin 1980)



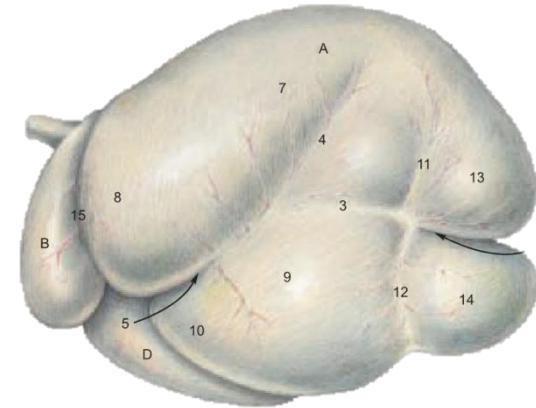
Large energetic sink with high energy demand

(Hungate 1960)



Area of energy absorption - 75% total VFA

(Bergman 1990)



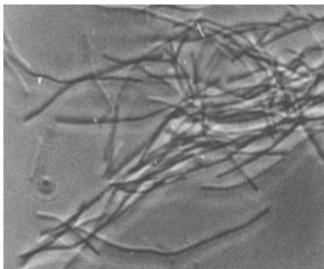
# Introduction: Microbiology



**Bacteria ( $10^{11}$  cells/ml)**  
75% feed particle digestion



**Fungi ( $10^3$ - $10^4$  cells/ml)**  
Fibrolytic particle digestion



**Methanogens ( $10^4$ - $10^6$  cells/ml)**  
Methanogen ecology associated with methane emissions

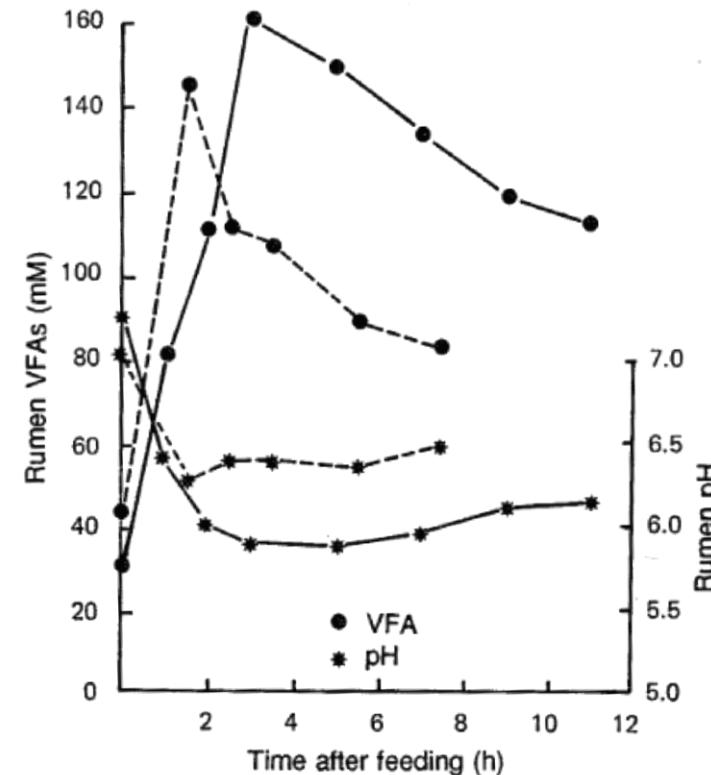
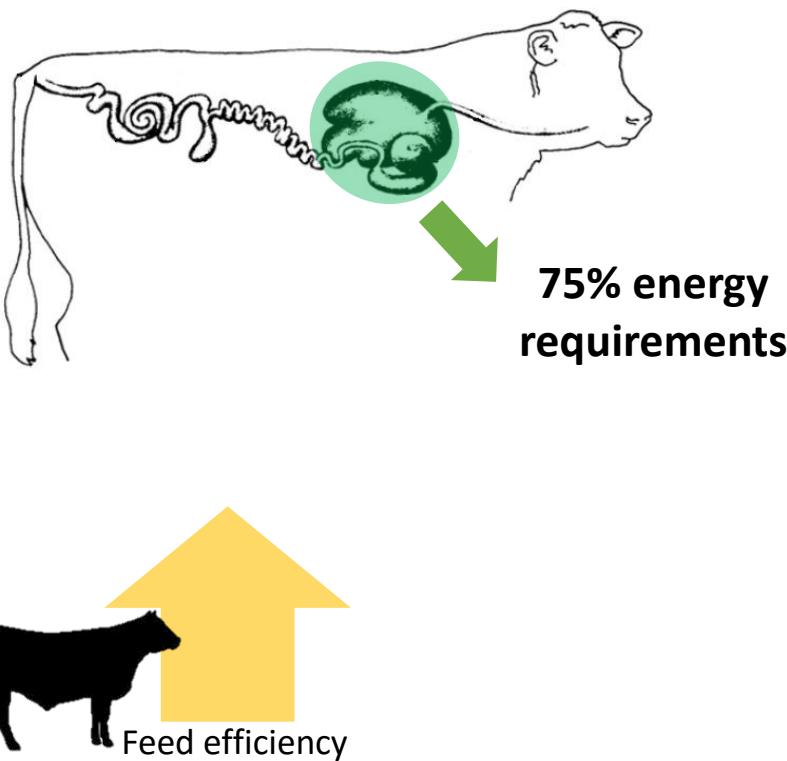


**Protozoa ( $10^4$ - $10^6$  cells/ml)**  
Ciliate species digesting suspended and colonized feed particles

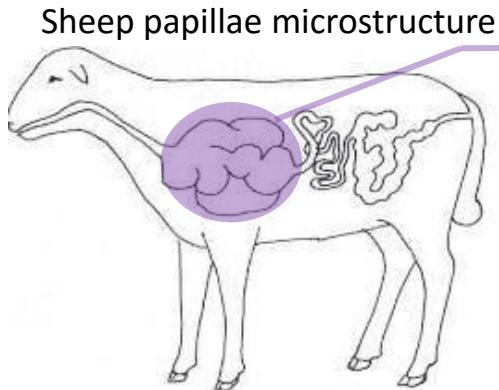


Feed efficiency (Guan et al. 2008, Zhou et al. 2009, Hernandez et al. 2012, Carberry et al. 2014)

# Introduction: Volatile fatty acids (VFA)

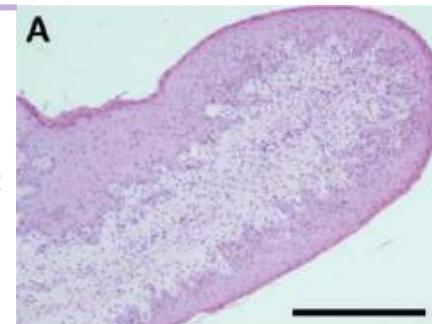


# Introduction: Rumen microstructure

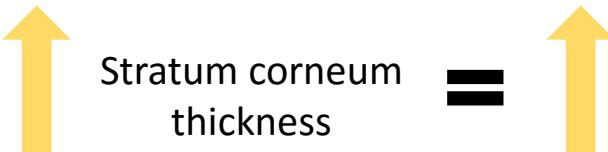
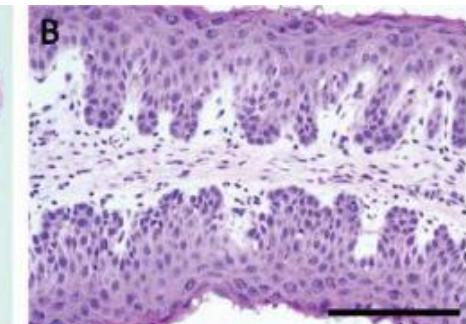
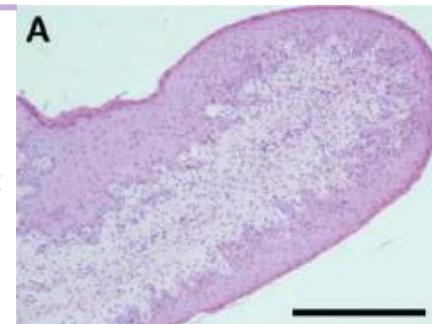
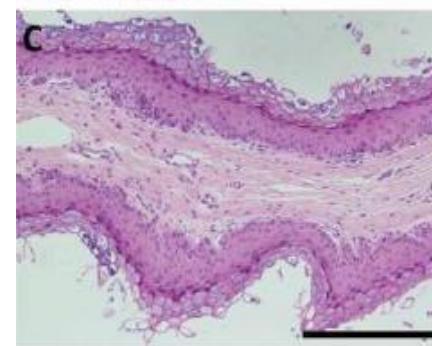


Sheep papillae microstructure

Low energy diet:



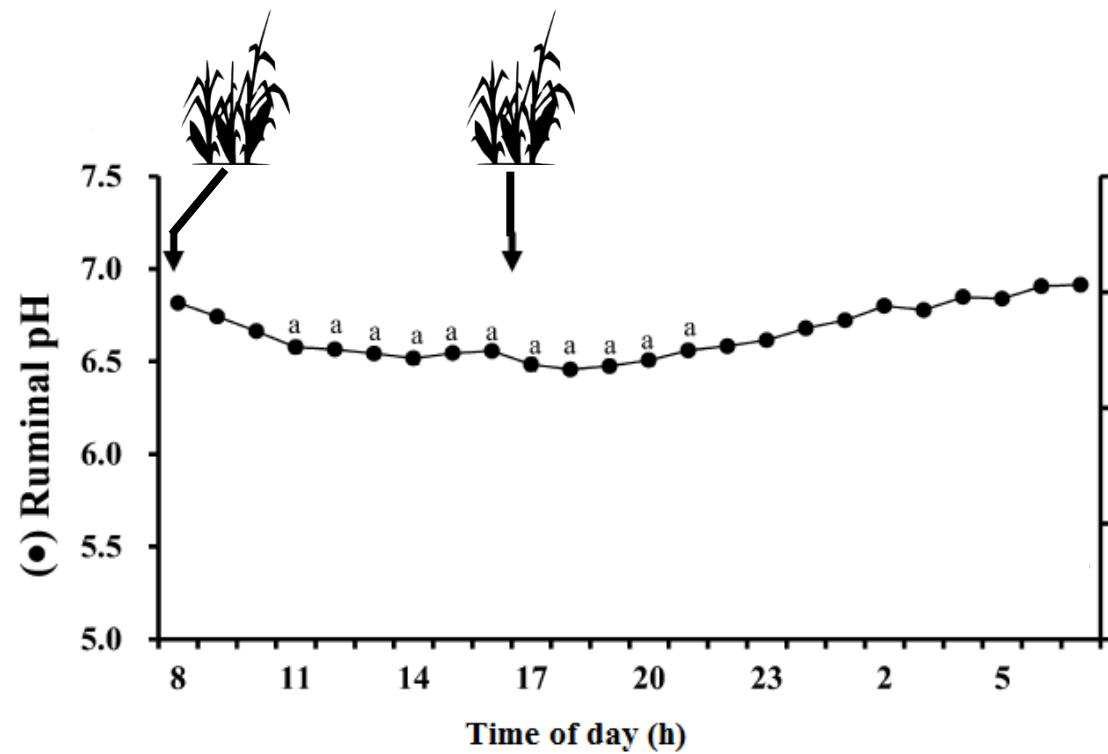
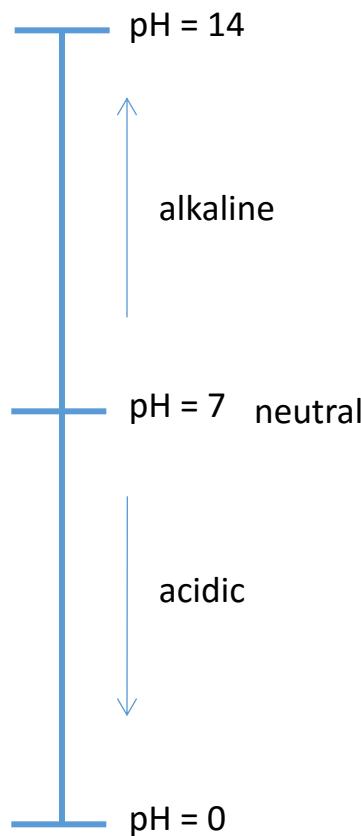
High energy diet:



Energy in diet

(Steele et al. 2012)

# Introduction: Rumen pH



(Kimura et al. 2016)

# Hypothesis

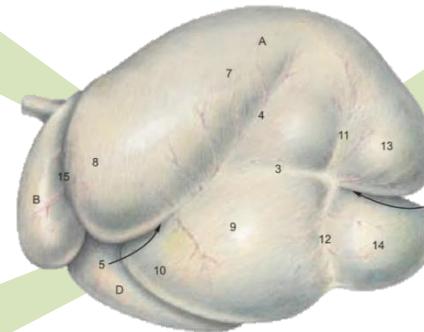
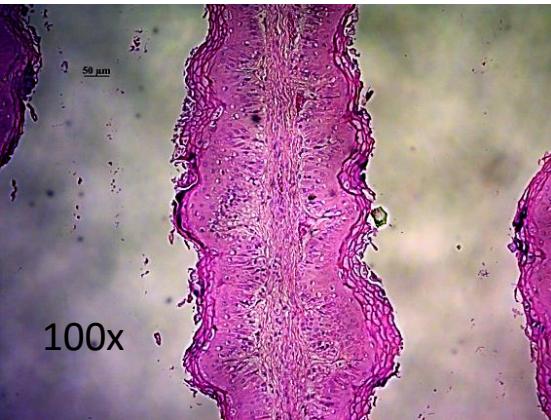
Feed efficiency is associated with energetic processes and the rumen is a highly metabolically active organ. Therefore, the variability in rumen metabolism across feed efficiency phenotypes and dietary treatments may be featured through rumen functional and structural assessments.

# Objectives

Microbiology



Papillae epithelium



Volatile fatty acids (VFA)



Rumen pH

# Objectives

Microbiology



Pa

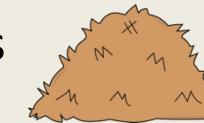
Efficient vs Inefficient



Grain-fed animals



Grass-fed animals



100x



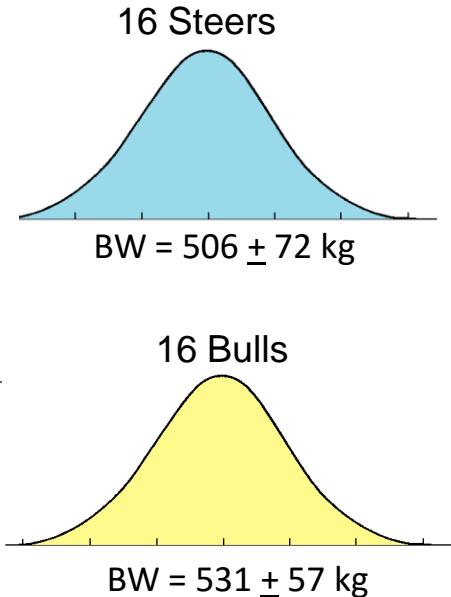
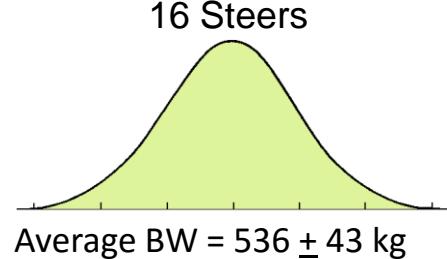
Rumen pH

# Materials: Experimental conditions

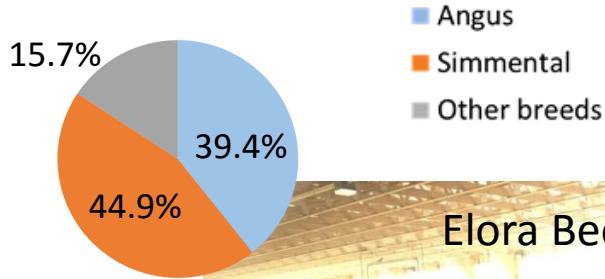


## Feedlot study

- 48 crossbred cattle
- Trial length: 112 d



Overall breed composition



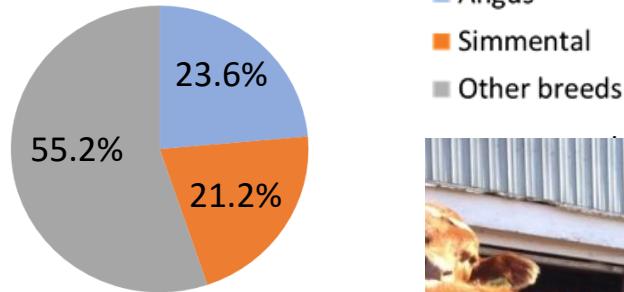
# Materials: Experimental conditions



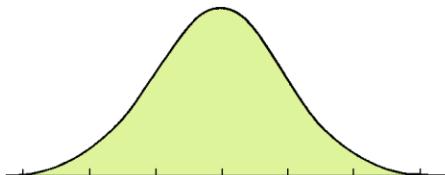
## Grass-fed study

- 141 crossbred cattle
- Trial length: 124 d

Overall breed composition



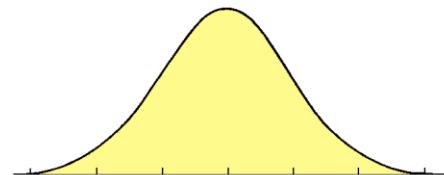
107 heifer calves



$BW = 253 \pm 38 \text{ kg}$

$\text{Age} = 403 \pm 27 \text{ d}$

36 pregnant heifers



$BW = 406 \pm 42 \text{ kg}$

$\text{Age} = 594 \pm 95 \text{ d}$

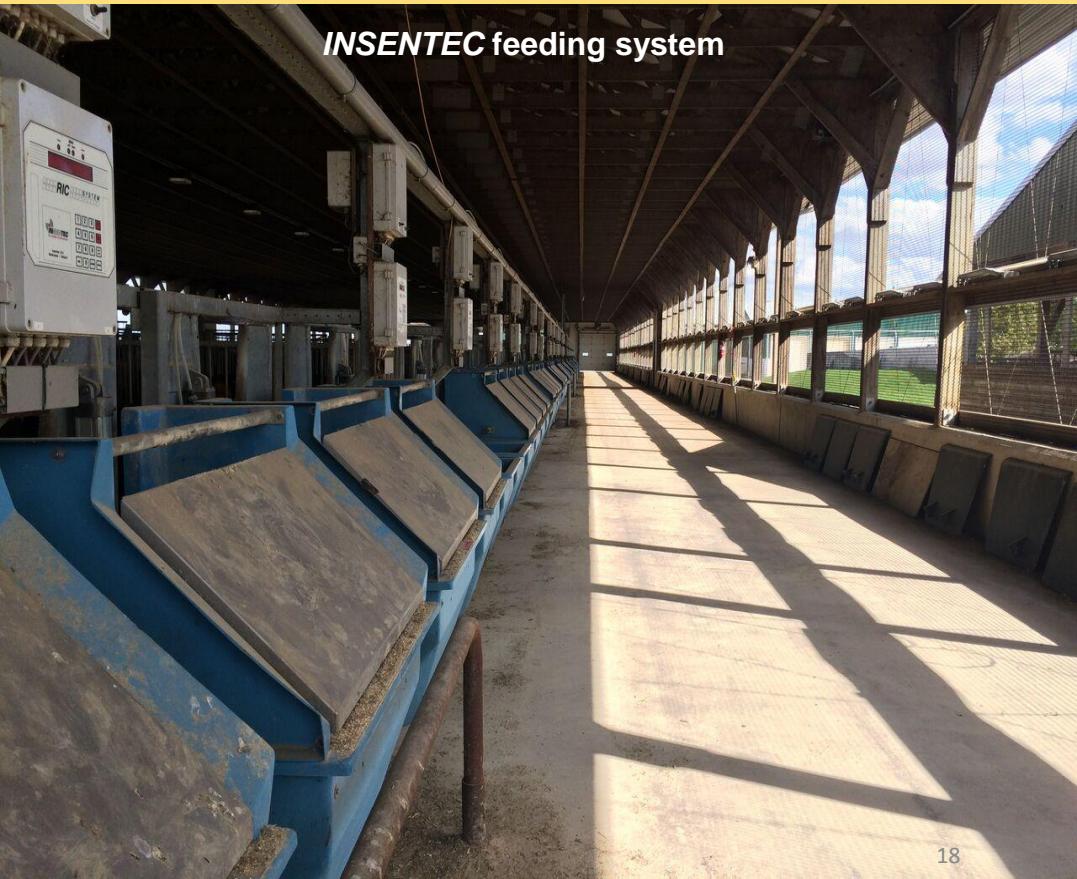


Maritime Beef Test Station

# Methods: Diet



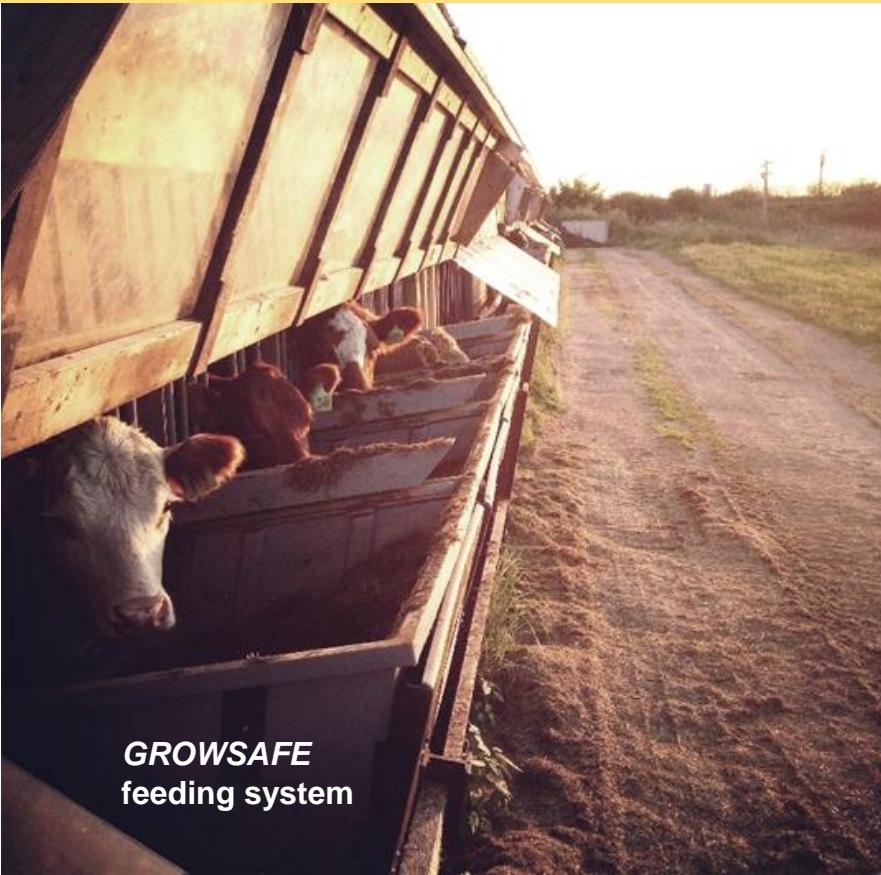
**INSENTEC feeding system**



<b>Chemical Composition</b>	<b>Dry Basis (%)</b>
Dry Matter	53.8
Crude Protein	13.9
Acid Detergent Fibre	10.9
Neutral Detergent Fibre	22.2
Starch	45.0
Total Digestible Nutrients	86.0
<b>Ingredient Composition</b>	<b>Dry Basis (%)</b>
High-moisture corn	52.5
Haylage	42.3
Soybean meal	3.5
Premix*	1.7

\*Contains 40% of calcium phosphate, 60% trace mineralized salt

# Methods: Diet

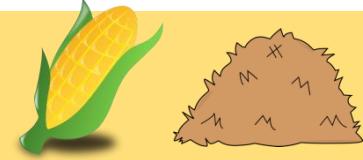


**GROWSAFE**  
feeding system

<b><i>Chemical Composition</i></b>	<b><i>Dry Basis (%)</i></b>
Dry matter	36.1
Crude Protein	15.5
Acid Detergent Fibre	29.6
Neutral Detergent Fibre	53.7
Starch	6.5
Total Digestible Nutrients	70.3
<b><i>Ingredient Composition</i></b>	<b><i>Dry Basis (%)</i></b>
Haylage	99.5
Premix*	0.5

\*Contains 37.4% of calcium phosphate, 62.7% trace mineralized salt

# Methods: RFI models



Grain-fed ( $R^2 = 0.74$ ):



$$\text{Feed intake} = [\mu + (\beta_1 \times \text{body weight})] + (\beta_2 * \text{ADG}) - (\beta_3 * \text{ribeye area}) +$$

*Body Size*      *Rate of Gain*      *Leanness*

$$(\beta_4 * \text{back fat}) - (\beta_5 * \text{marbling}) + \text{subpopulation} + \text{RFI}$$

*Fatness*

Grass-fed ( $R^2 = 0.61$ ):



$$\text{Feed intake} = [\mu + (\beta_1 * \text{body weight})] + (\beta_2 * \text{ADG}) - (\beta_3 * \text{ribeye area}) +$$

*Body Size*      *Rate of Gain*      *Leanness*

$$(\beta_4 * \text{rump fat}) - (\beta_5 * \text{age}) + \text{subpopulation} + \text{RFI}$$

*Fatness*

# Methods: Sample collection



**5.5±1 d prior slaughter**



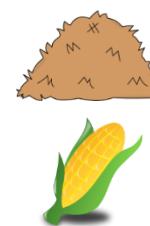
pH logger insertion



**End of performance test**

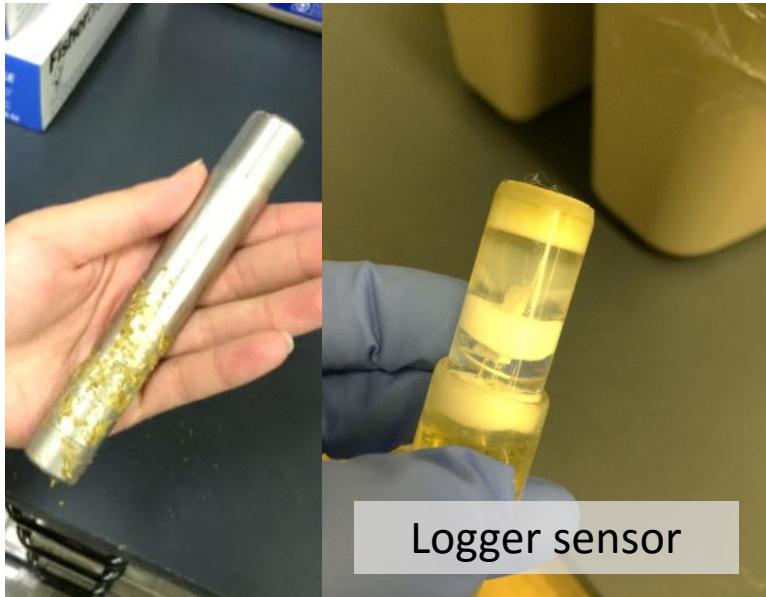


Rumen fluid



Rumen tissue

# Methods: Logger insertion



**Rumen pH loggers**

T9 LRCpH Data Logger Dascor

**Method:** esophageal tubing

**Recording:** 5 minute intervals  
(~2,600 data points/animal)

# Methods: Rumen fluid collection



## Method:

Oro-ruminal probe with suction

## Evaluating:

Microbiology

Volatile fatty acid profiles



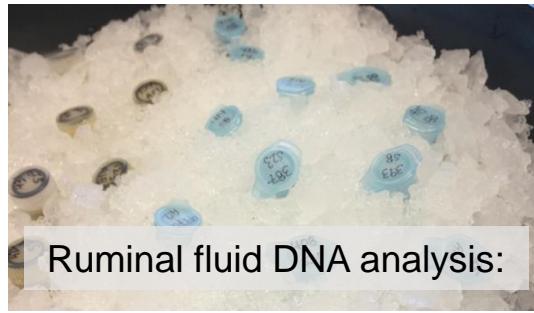
# Methods: Microbiology



## Method:

Rumen fluid DNA isolation

RT-qPCR



Ruminal fluid DNA analysis:

## Evaluating:

Bacteria



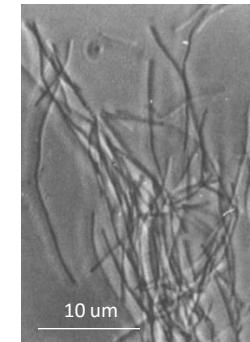
Protozoa



Fungi



Methanogen



# Methods: VFA



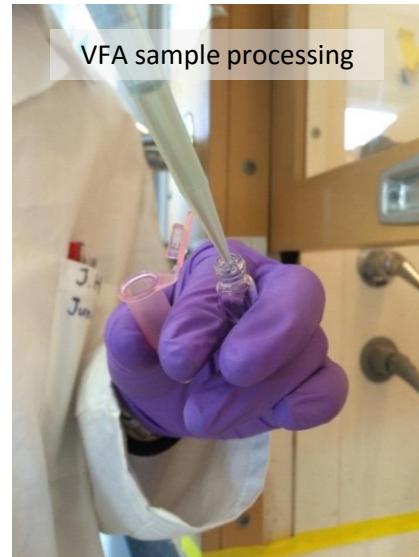
## Evaluated:

VFA molar concentrations

- **Acetate**
- **Propionate**
- **Butyrate**
- Valerate
- Isovalerate
- Isobutyrate
- Caproate

Total VFA concentration

## Method:



# Methods: Rumen tissue collection

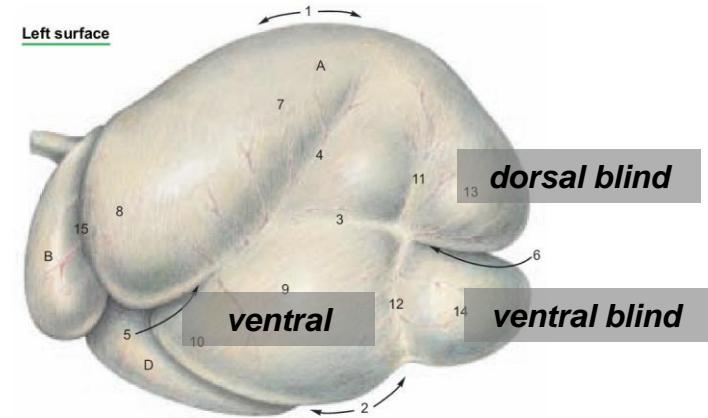


## Method:

Tissue collection

Processed for histomorphology

## Evaluating: Papillae epithelial thickness

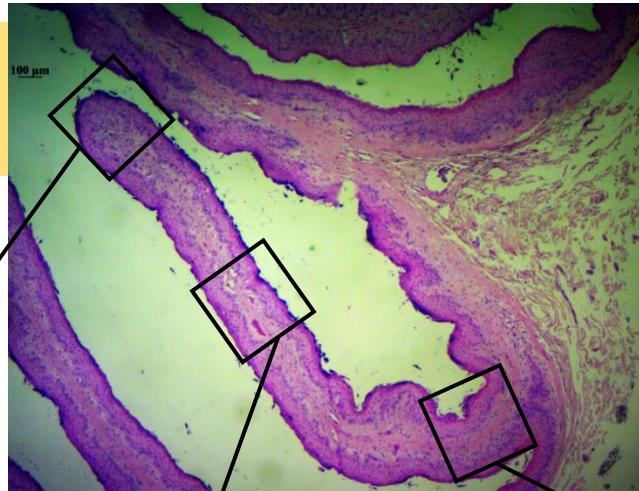
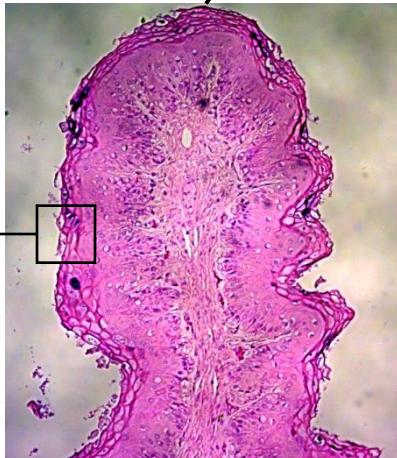
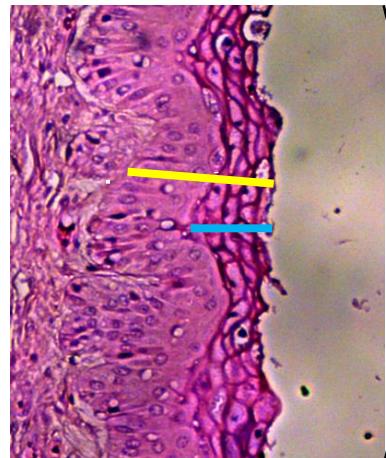


# Methods: Histomorphometry



Histology traits:

- Stratum corneum ●
- Papillae width ●

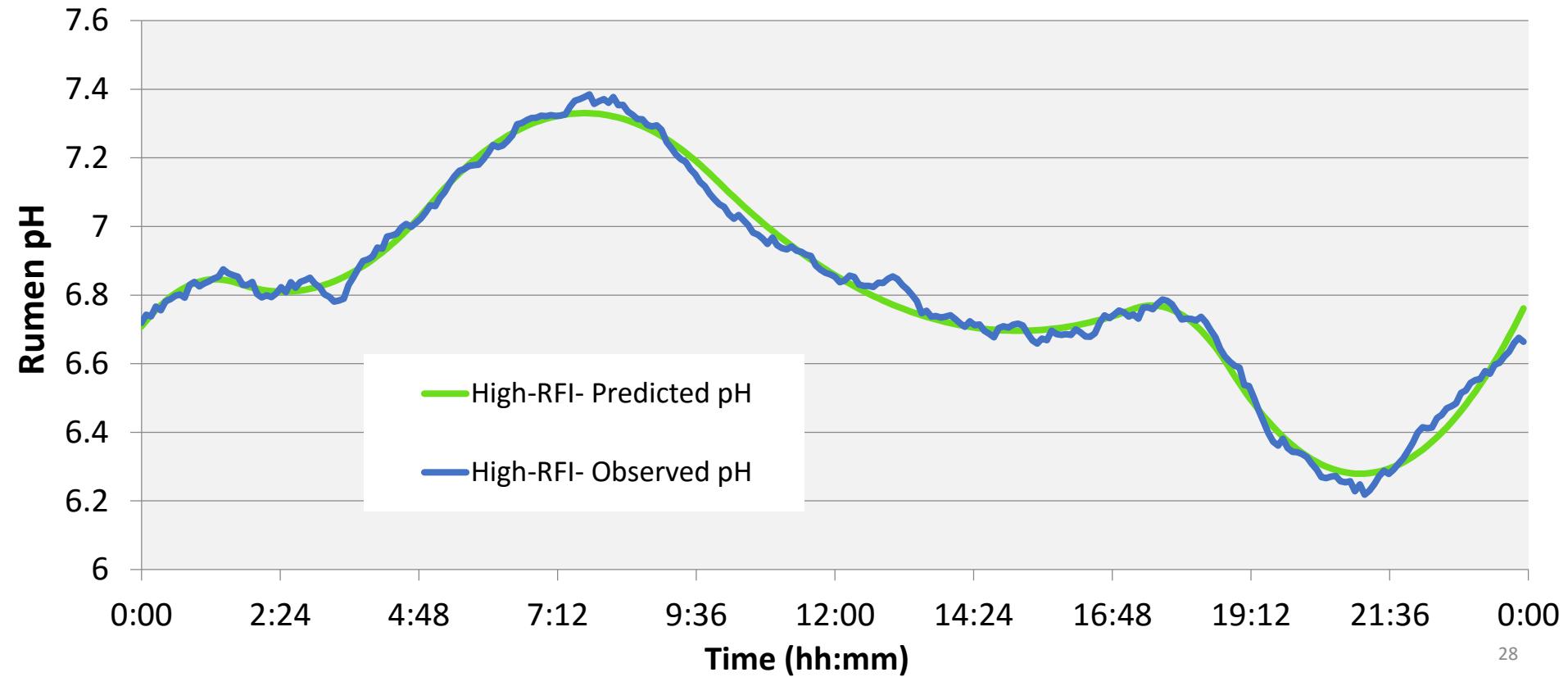


Tip

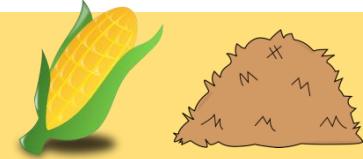
Middle piece

Base

# Methods: pH measurements



# Methods: Statistical analysis



## Univariate Normality Procedure

- Skewness, Kurtosis, Anderson-Darling Test
- Transformations
  - logarithm
  - squared

## GLM Select procedure

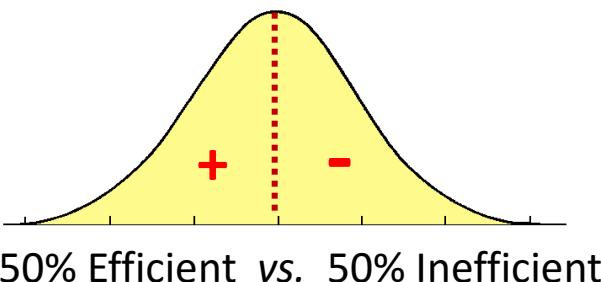
- Determine model effects

## General Linear Model (GLM) Procedure

- Rumen traits

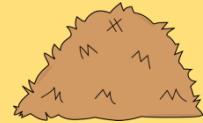
## Partial Least Square procedure

- Determine % contribution to RFI



$$Y_{ijkl} = \mu + \text{efficiency group}_i + \text{subpopulation}_j + \text{breeds}_k + \epsilon_{ijkl}$$

# Results: Physiological status



Trait (%/total VFA)	Heifer calves	Pregnant heifers	<i>P-value</i>
<b>Acetate</b>	71.68	74.62	<b>&lt;0.01</b>
<b>Propionate</b>	18.30	16.53	<b>&lt;0.01</b>
Isobutyrate	0.97	0.89	0.051
<b>Butyrate</b>	7.29	6.47	<b>&lt;0.01</b>
Isovalerate	0.90	0.90	0.952
<b>Valerate</b>	0.58	0.41	<b>&lt;0.01</b>
<b>Caproate</b>	0.28	0.18	<b>&lt;0.01</b>
<b>Total VFA (<math>\mu\text{mol}/\text{ml}</math>)</b>	43.52	37.39	<b>&lt;0.01</b>

# Results: Physiological status



## Pregnant heifers



Bacteria population

Metabolic activity throughout gestation

VFA metabolism and energy demand

(Church, 1988; Drackley et al. 2001)



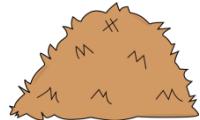
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Total VFA ( $\mu\text{mol}/\text{ml}$ )	43.52	37.39	<0.01

# Results: RFI – Microbial profiles

Trait (16s rRNA copy/ml)	Inefficient	Efficient	P-value
<b>Total bacteria</b>	$4.3 \times 10^{11}$	$7.6 \times 10^{11}$	<0.05
<b>Methanogen</b>	$4.9 \times 10^9$	$2.3 \times 10^9$	<0.05
Protozoa	$4.3 \times 10^7$	$1.5 \times 10^7$	0.18
Fungi	$6.3 \times 10^4$	$3.8 \times 10^4$	0.37

Trait (16s rRNA copy/ml)	Inefficient	Efficient	P-value
Total bacteria	$6.0 \times 10^{10}$	$5.3 \times 10^{10}$	0.16
<b>Methanogen</b>	$2.6 \times 10^7$	$3.1 \times 10^7$	<0.05
Protozoa	$1.2 \times 10^5$	$1.6 \times 10^5$	0.30
Fungi	$1.3 \times 10^5$	$1.9 \times 10^5$	<0.05

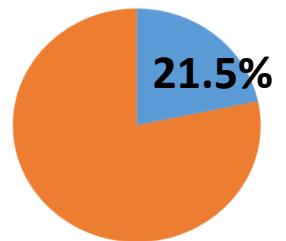
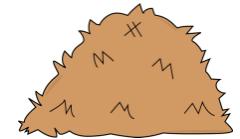
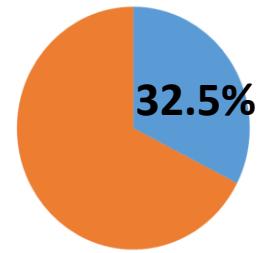


# Results: RFI – VFA profiles

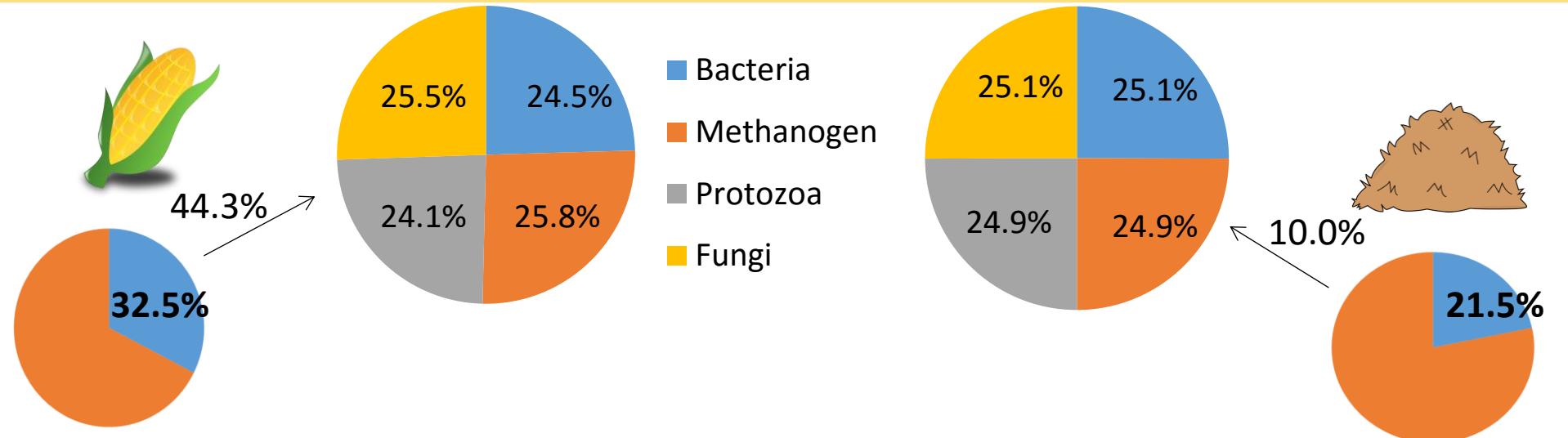


Trait (%/total VFA)	Inefficient	Efficient	P-value	Inefficient	Efficient	P-value
Acetate	54.4	54.5	0.97	73.48	72.95	0.55
Propionate	29.0	29.3	0.89	17.25	17.45	0.38
Isobutyrate	1.2	1.0	0.11	0.90	0.95	<b>&lt;0.10</b>
Butyrate	8.9	8.6	0.75	6.81	6.91	0.44
Isovalerate	3.0	2.7	0.41	0.87	0.94	<b>&lt;0.05</b>
Valerate	2.6	2.9	0.29	0.47	0.49	0.43
Caproate	0.4	0.5	0.40	0.22	0.22	0.87
Total VFA ( $\mu\text{mol}/\text{ml}$ )	78.0	80.3	0.66	38.94	41.83	<b>&lt;0.10</b>

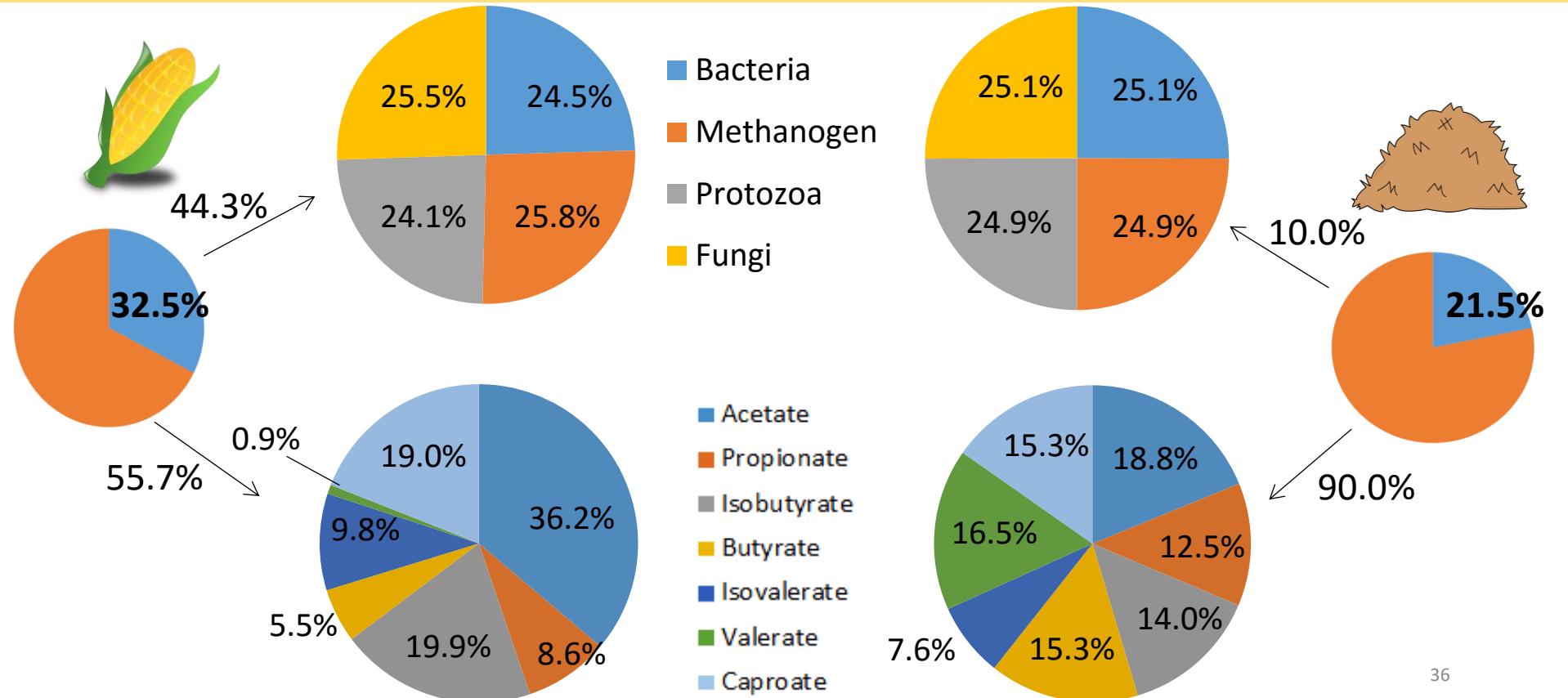
# Results: Variance analysis



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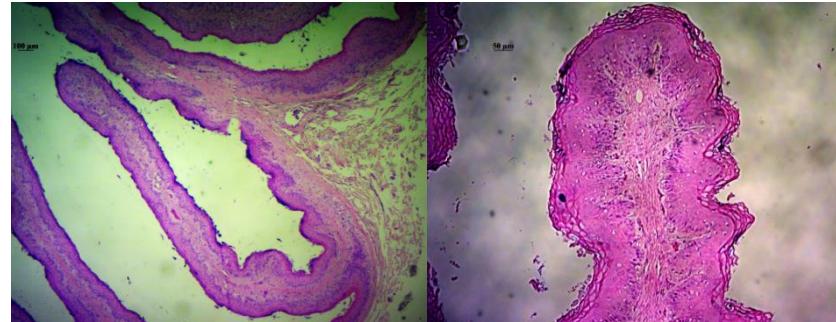
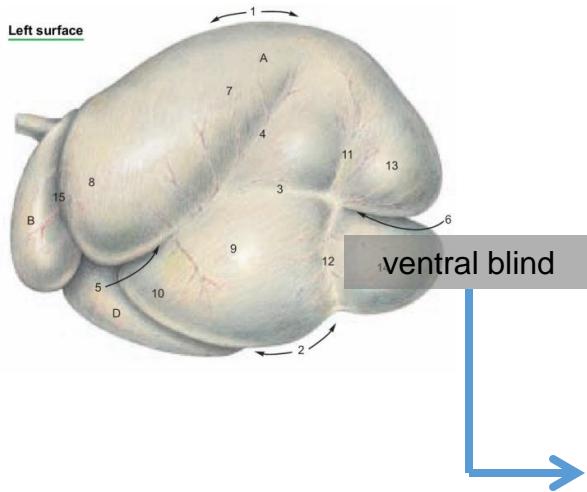


# Results: Variance analysis



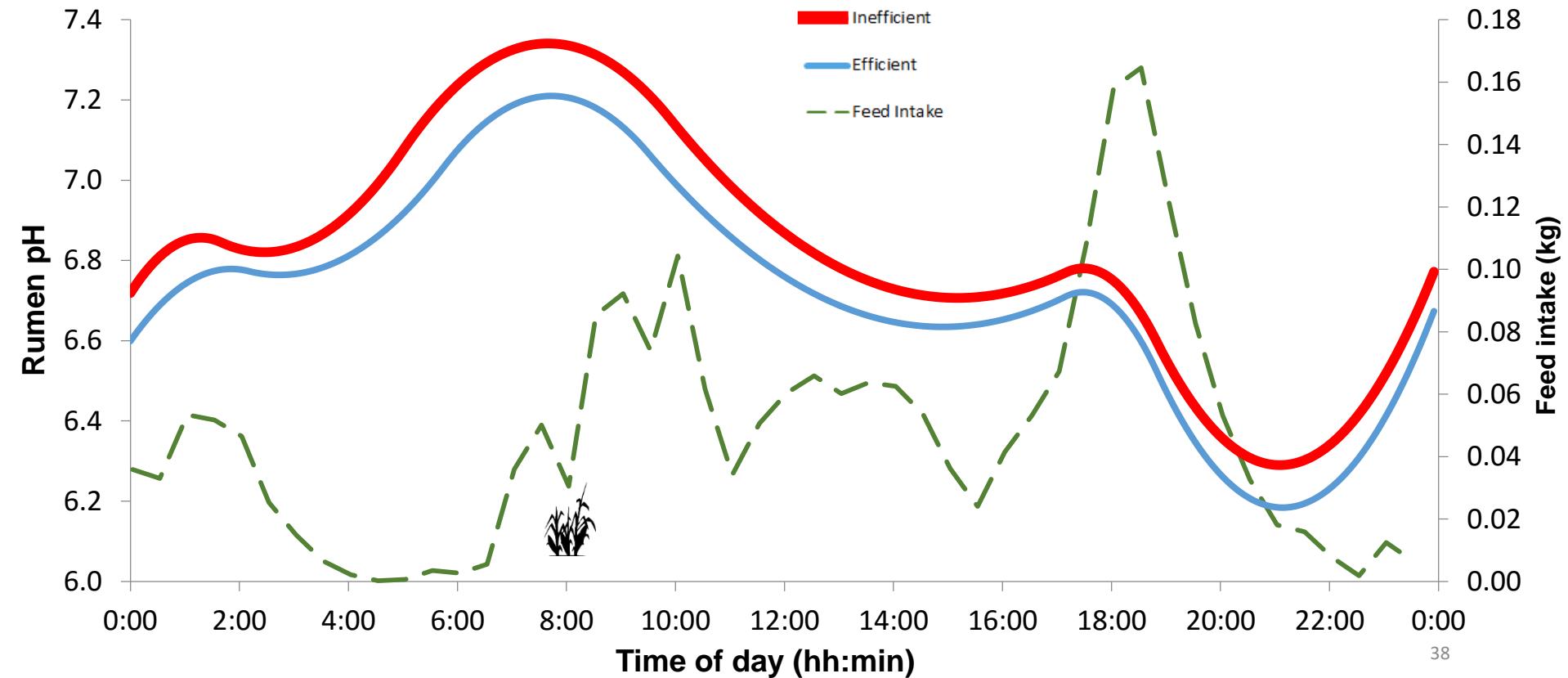


# Results: Papillae histomorphometry

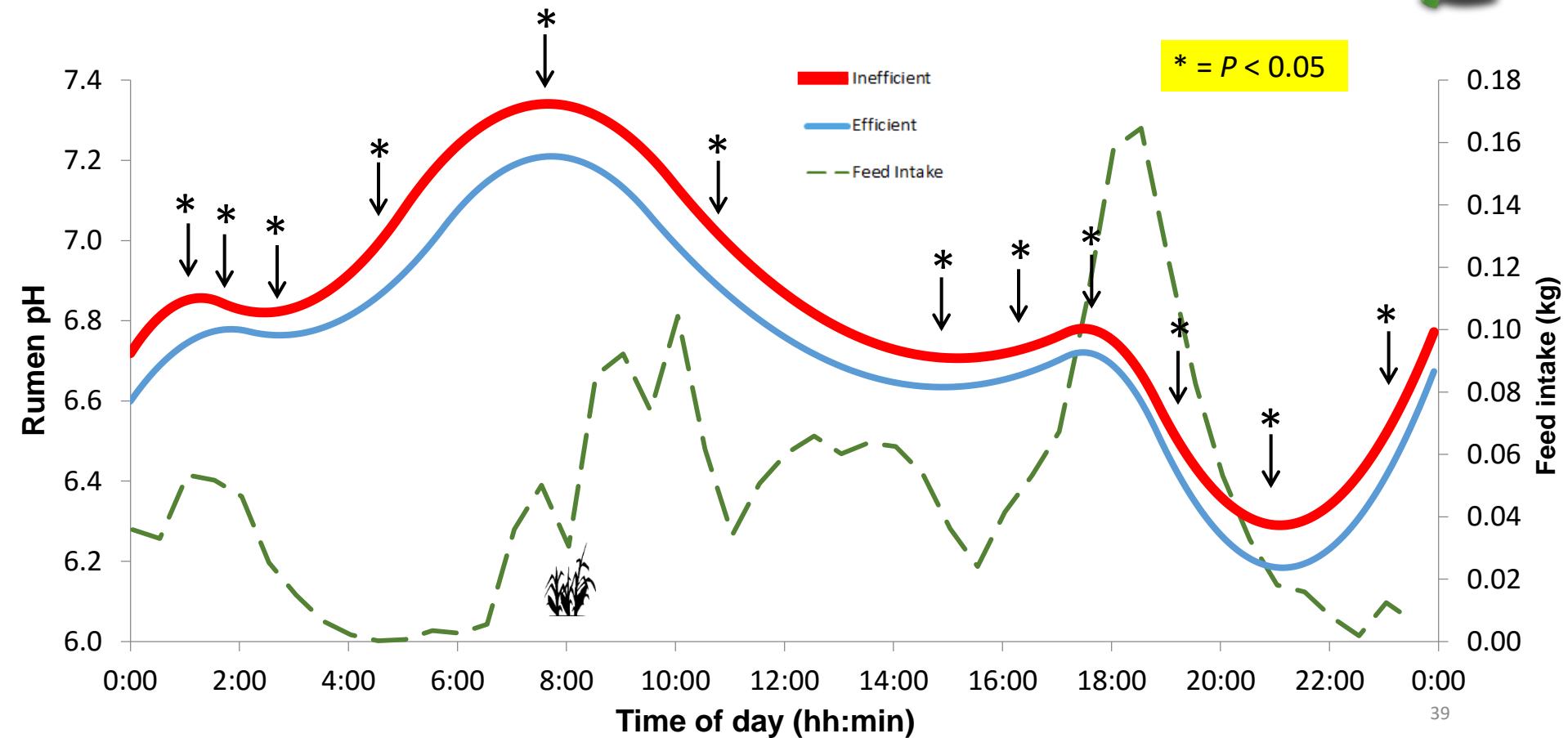


Papillae width (μm)	Papillae area	Inefficient	Efficient	P
	Base	110.1	113.4	0.39
	Middle	119.5	139.4	<0.05
	Tip	125.0	148.4	<0.01

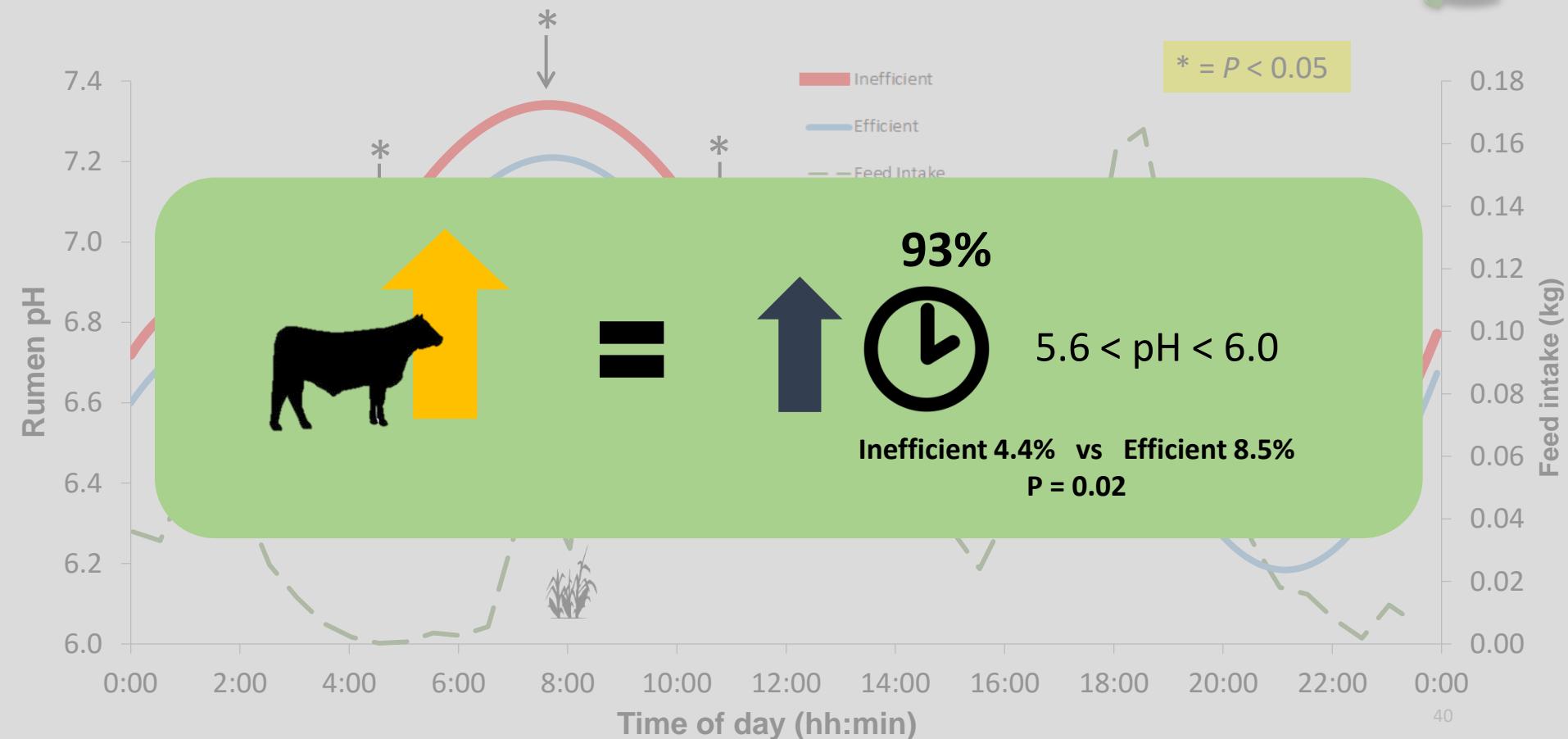
# Results: Predicted rumen pH curves



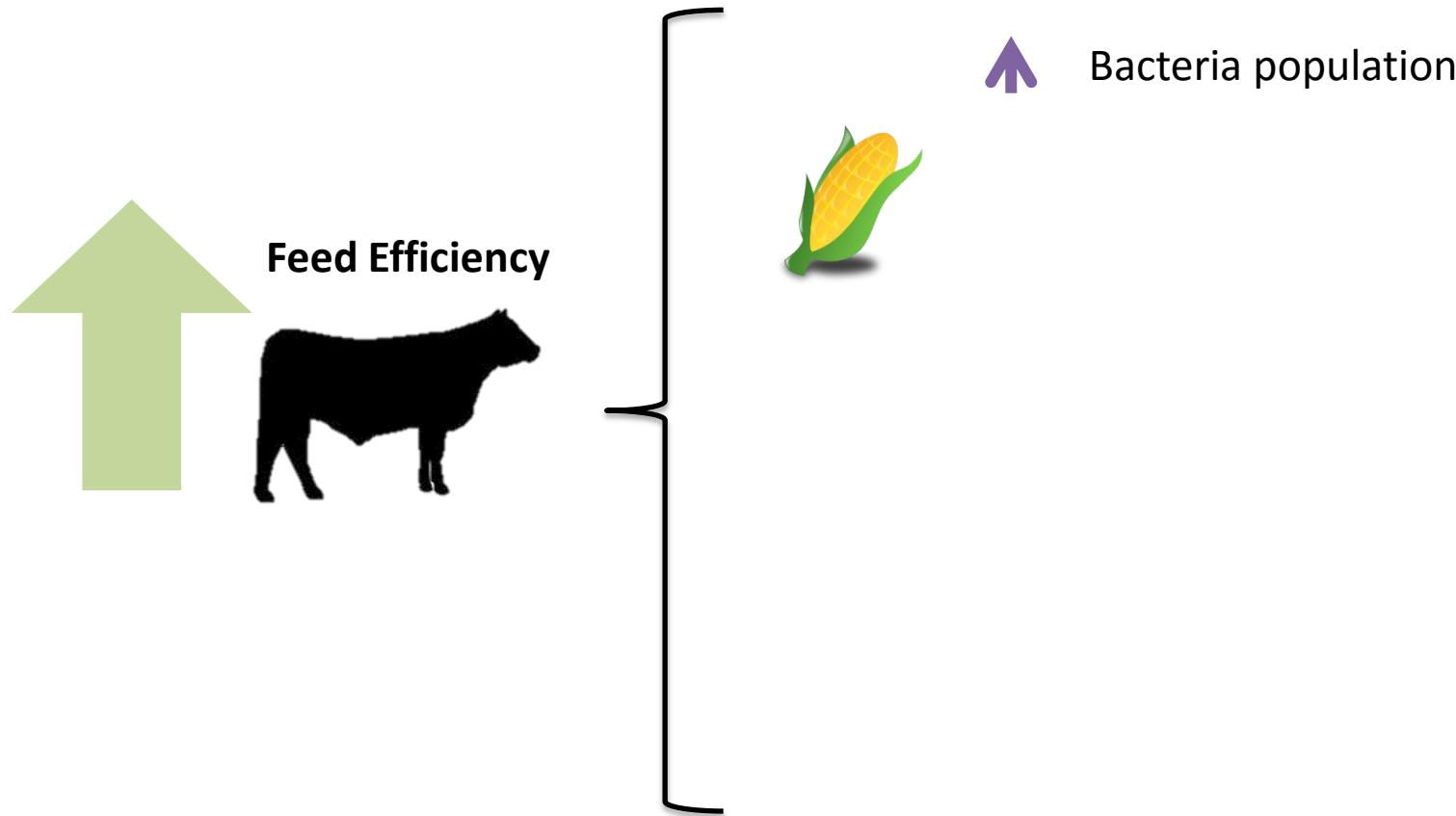
# Results: Predicted rumen pH curves



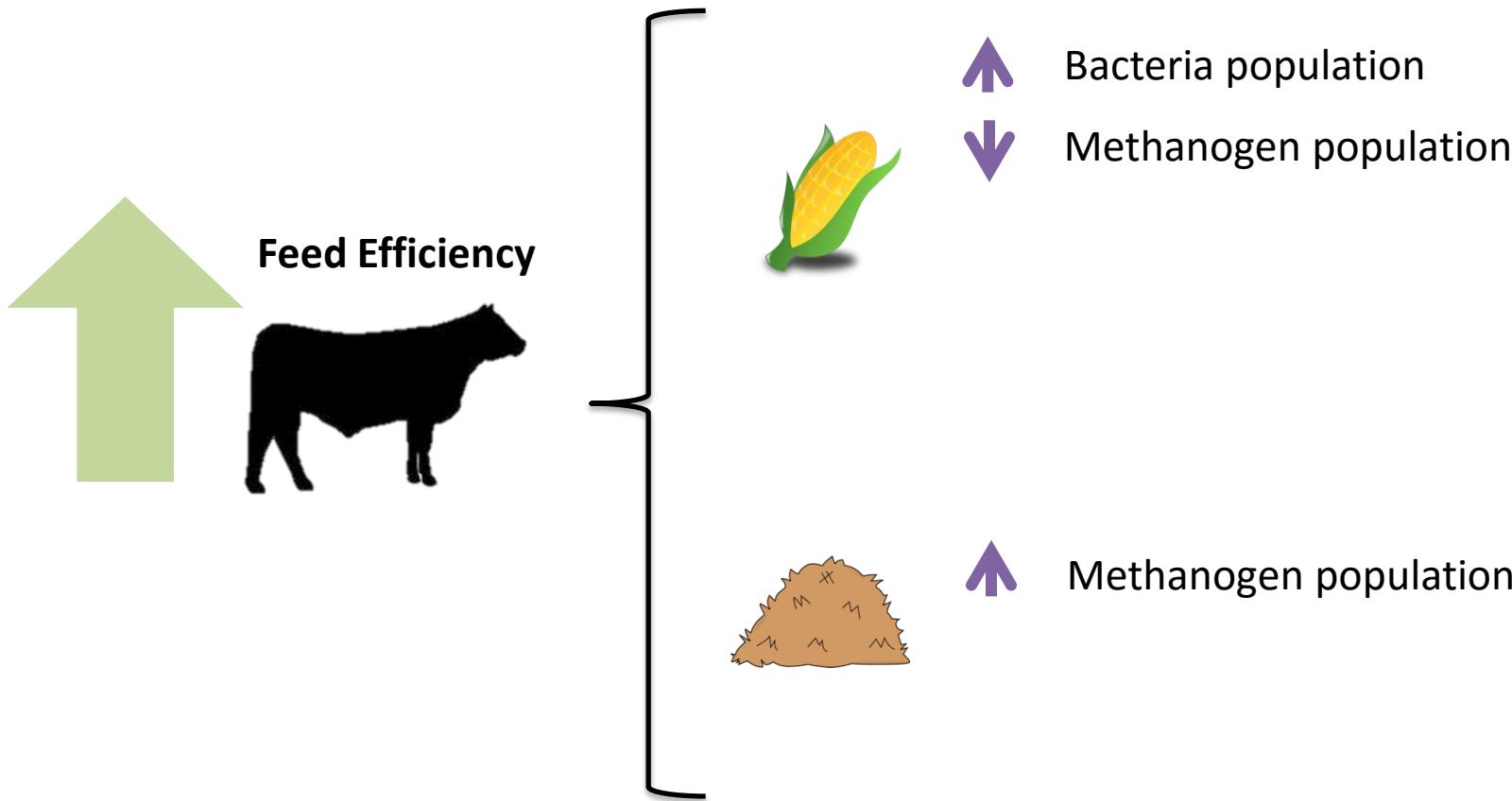
# Results: Predicted rumen pH curves



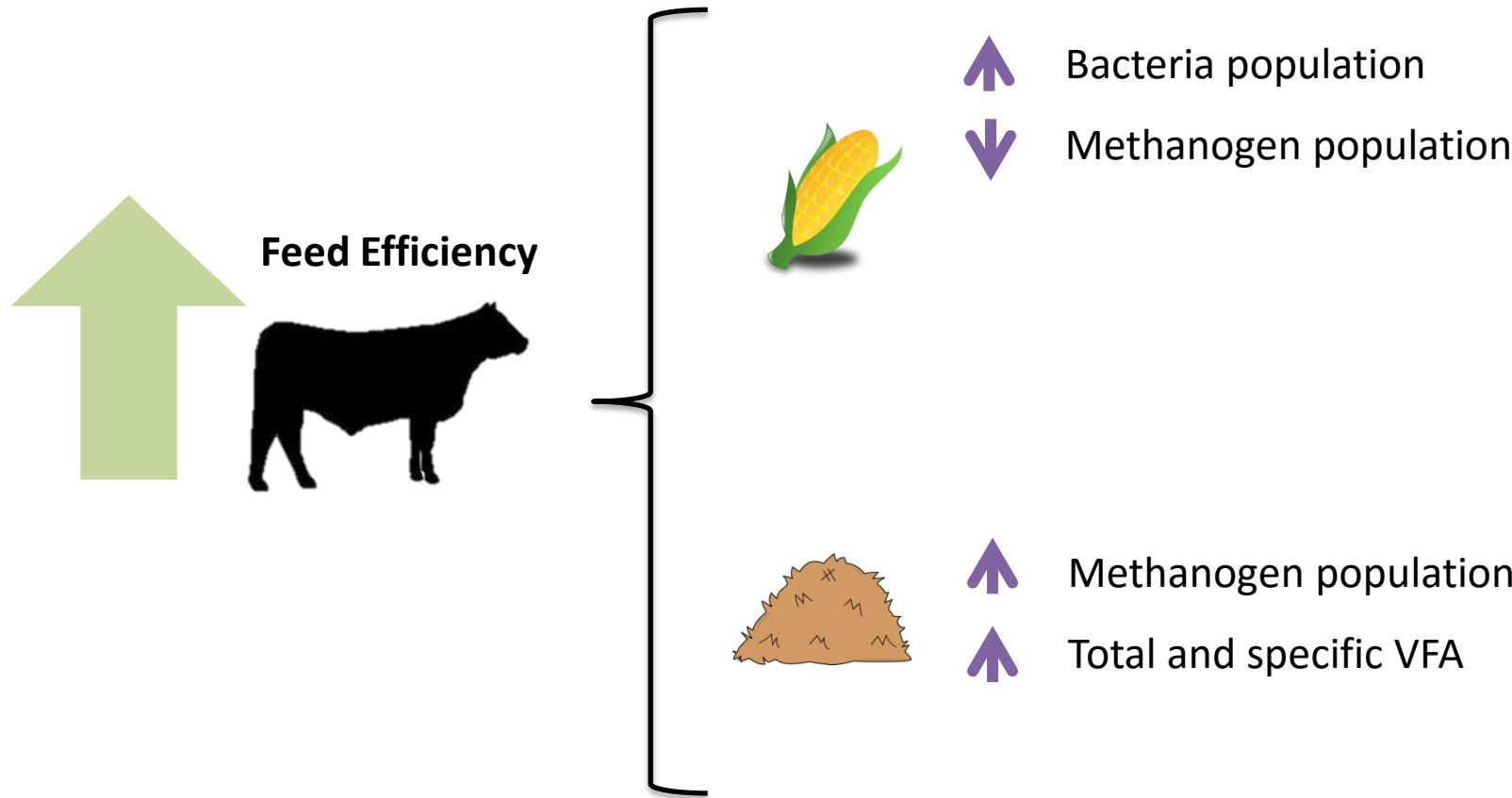
# Summary



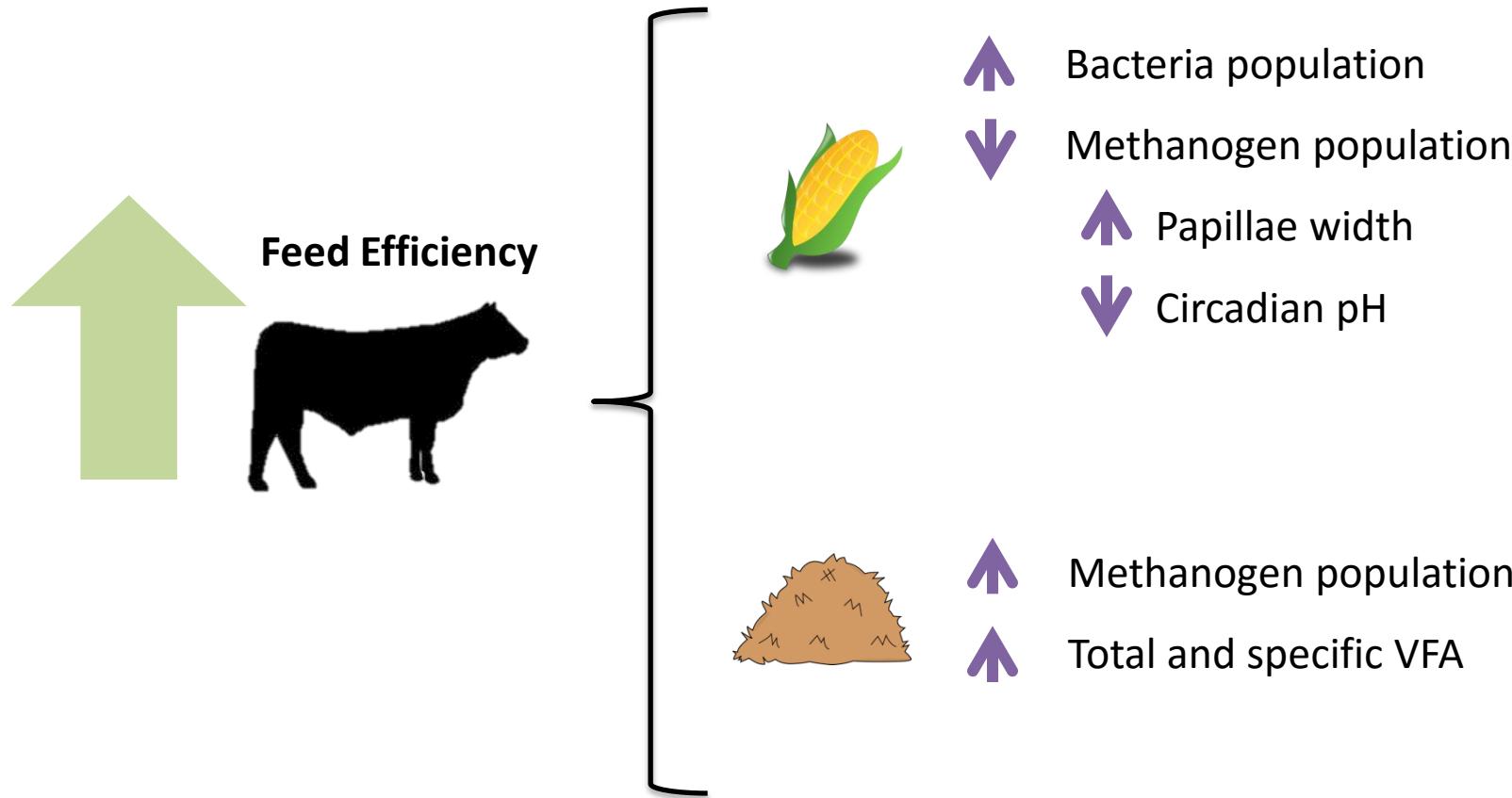
# Summary



# Summary



# Summary



# Conclusions

- Rumen microbiology, functional and structural parameters are important in assessing the underlying digestive biology of feed efficiency
- Dietary treatment has an impact on the relevance of rumen parameters used for indicating feed efficiency



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