Impact of moderate CP restriction and additive combination on N balance and hindgut microbiota in pigs

L. Sarri [1], E. Fuertes [1], E. Pérez-Calvo [2], A.R. Seradj [1], J. Balcells [1] and G. de la Fuente [1]

[1] Universitat de Lleida – Agrotecnio - CERCA, Spain

[2] dsm-firmenich, Animal Nutrition and Health, Switzeland

Nutritional strategies

- **Reducing Crude Protein content with balanced crystalline AA supply** **Precision feeding**
  - Reduce feeding costs and environmental impact
  - Improve nutrient efficiency

(Halas et al., 2010; Torrallardona et al., 2007; Xu et al., 2018)

Nutritional strategies

- **Reducing Crude Protein content with balanced crystalline AA supply** **Precision feeding**
  - Reduce feeding costs and environmental impact
  - Improve nutrient efficiency

- **Addition of Feed Additives** (e.g. exogenous enzymes, acidifying agents and plant extracts)
  - Increase nutrient use efficiency
  - Improve immune system
  - Modulate microbial population and intestinal morphology

(Halas et al., 2010; Torrallardona et al., 2007; Xu et al., 2018)

Objectives

Evaluate the effect of dietary CP level and the presence or absence of a blend of feed additives on:

- Apparent ileal digestibility
- Nitrogen balance
- Hindgut microbiota and fecal excretion of microbial biomass

In two feeding phases: **GROWING** and **FINISHING** pigs

Animals

A total of **32** entire male pigs

Pietrain × (Landrace x Large White)

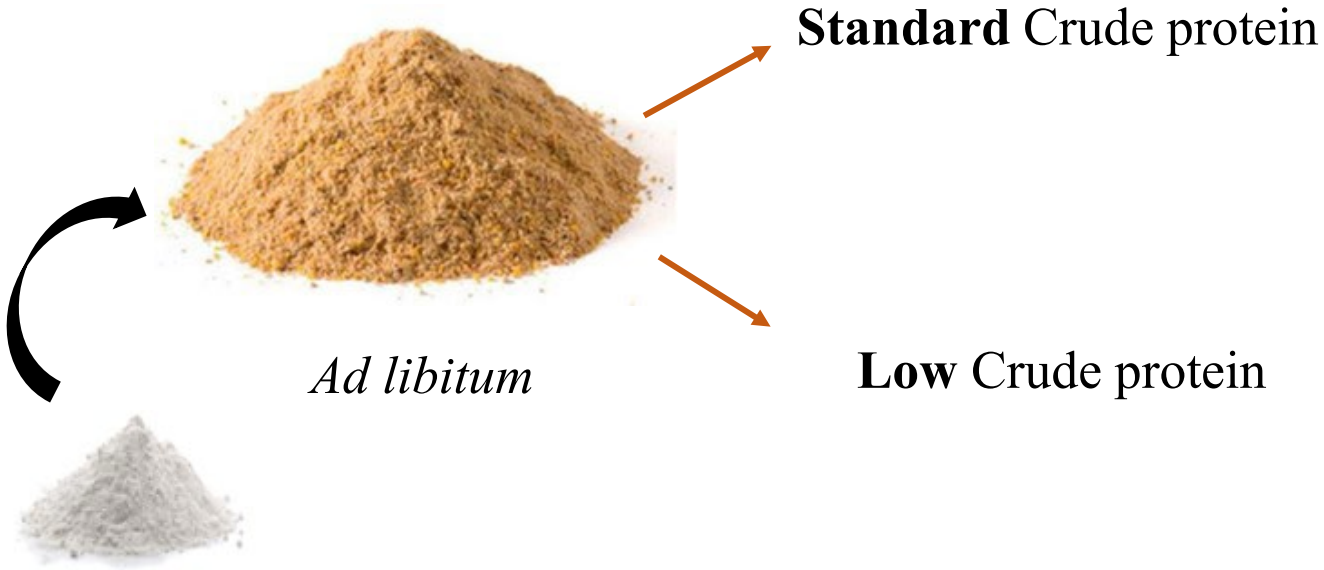
16 GROWING pigs (**68.4** ± 2.18 kg BW)

16 FINISHING pigs (**108.8** ± 1.30 kg BW)

* Pigs of each feeding phase were assigned in 4 dietary treatments (4 pigs/treatment):

Diets

2 × 2 factorial design **Crude Protein level**



(1.3 g TiO_2 /kg DM)

Diets

2 × 2 factorial design

Crude Protein level

Feed Additives

Ad libitum

(1.3 g TiO_2 /kg DM)

Standard Crude protein

Low Crude protein

- **Control** → **SP**
- **Additive** → **SPA**
- **Control** → **LP**
- **Additive** → **LPA**

- ❑ Carbohydrases (i.e., xylanases)
- ❑ Organic acids (e.g., benzoic acid)
- ❑ Essential oils

Table 1. Chemical composition of the experimental diets g/100 g DM (%)

	GROWING PIGS				FINISHING PIGS			
	SP	**SPA**	**LP**	**LPA**	**SP**	**SPA**	**LP**	**LPA**
DM (g/100 g FM)	90.18	89.89	90.30	89.62	89.83	89.47	89.64	88.99
OM	95.69	95.24	96.09	95.90	95.95	95.81	95.91	95.70
Crude Protein	**16.0**	**16.0**	**14.5**	**14.5**	**15.0**	**15.0**	**13.5**	**13.5**
Crude Fiber	3.22	3.85	3.32	3.96	3.34	3.95	3.43	4.07
NDF	11.8	13.2	12.4	13.9	12.5	13.8	13.1	14.6
EE	4.93	4.05	4.41	3.42	4.61	3.58	4.09	3.0

1.5 % reduction in CP following recommendations of:

FEDNA
Fundación Española para el Desarrollo de la Nutrición Animal

Pigs were allotted individually in **metabolic cages** during 5 days to obtain:

- Fresh **feces** and **urine** (10 % H_2SO_4)
  - N balance
  - Diaminopimelic acid (DAPA) → Bacterial cell wall amino acid

On the last day, animals were euthanized for obtaining samples:

- **Ileum**
  - Apparent ileal digestibility (AID)
- **Colon**
  - Volatile fatty acids (VFA)
  - Microbiota characterization

Table 2. Apparent ileal digestibility (%)

	SP	SPA	LP	LPA	SEM	*P*-value		
						CP	Additives	CP × Additives
GROWING PHASE								
DM	70.38	74.48	71.96	68.20	4.133	NS	NS	NS
OM	72.60	76.98	74.33	70.90	3.910	NS	NS	NS
CP	71.47	79.21	69.43	70.22	4.967	NS	NS	NS
Lys	88.70	92.31	87.79	90.20	2.404	NS	NS	NS
FINISHING PHASE								
DM	81.90 ^{a}	71.14 ^{b}	78.38 ^{ab}	80.76 ^{a}	2.766	NS	NS	**0.037**
OM	83.65 ^{a}	73.49 ^{b}	80.40 ^{ab}	82.25 ^{a}	2.666	NS	NS	**0.046**
CP	83.33 ^{ab}	73.36 ^{b}	79.64 ^{ab}	84.17 ^{a}	2.350	NS	NS	**0.011**
Lys	92.71 ^{ab}	90.43 ^{b}	92.17 ^{ab}	94.98 ^{a}	0.945	NS	NS	**0.021**

* NS, non-significant

Table 2. Apparent ileal digestibility (%)

	SP	SPA	LP	LPA	SEM	*P*-value		
						CP	Additives	CP × Additives
GROWING PHASE								
DM	70.38	74.48	71.96	68.20	4.133	NS	NS	NS
OM	72.60	76.98	74.33	70.90	3.910	NS	NS	NS
CP	71.47	79.21	69.43	70.22	4.967	NS	NS	NS
Lys	88.70	92.31	87.79	90.20	2.404	NS	NS	NS
FINISHING PHASE								
DM	81.90 ^{a}	71.14 ^{b}	78.38 ^{ab}	80.76 ^{a}	2.766	NS	NS	**0.037**
OM	83.65 ^{a}	73.49 ^{b}	80.40 ^{ab}	82.25 ^{a}	2.666	NS	NS	**0.046**
CP	83.33 ^{ab}	73.36 ^{b}	79.64 ^{ab}	84.17 ^{a}	2.350	NS	NS	**0.011**
Lys	92.71 ^{ab}	90.43 ^{b}	92.17 ^{ab}	94.98 ^{a}	0.945	NS	NS	**0.021**

* NS, non-significant

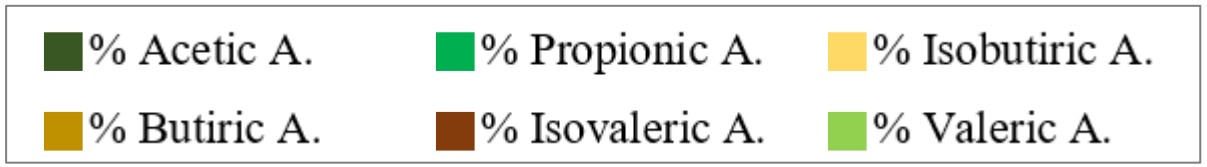
LP diets did not alter AID, although it was reduced in SPA diet

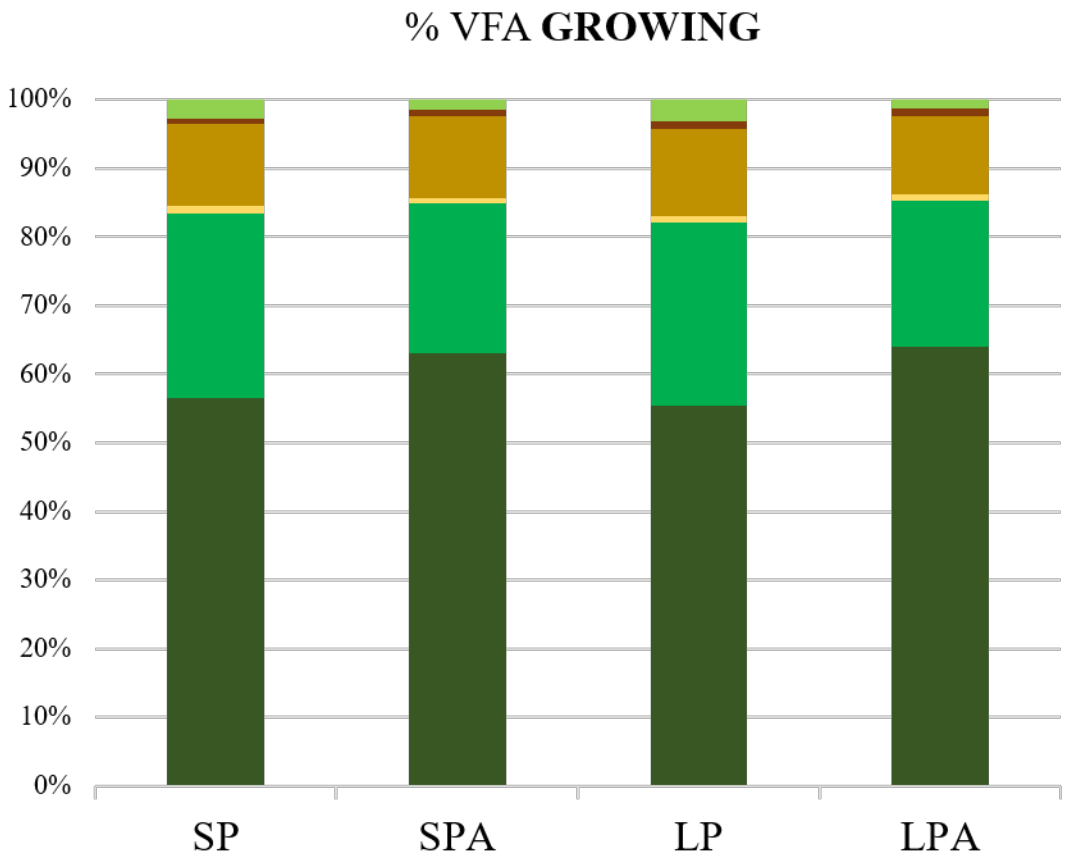
Table 3. Nitrogen balance expressed as g N/day

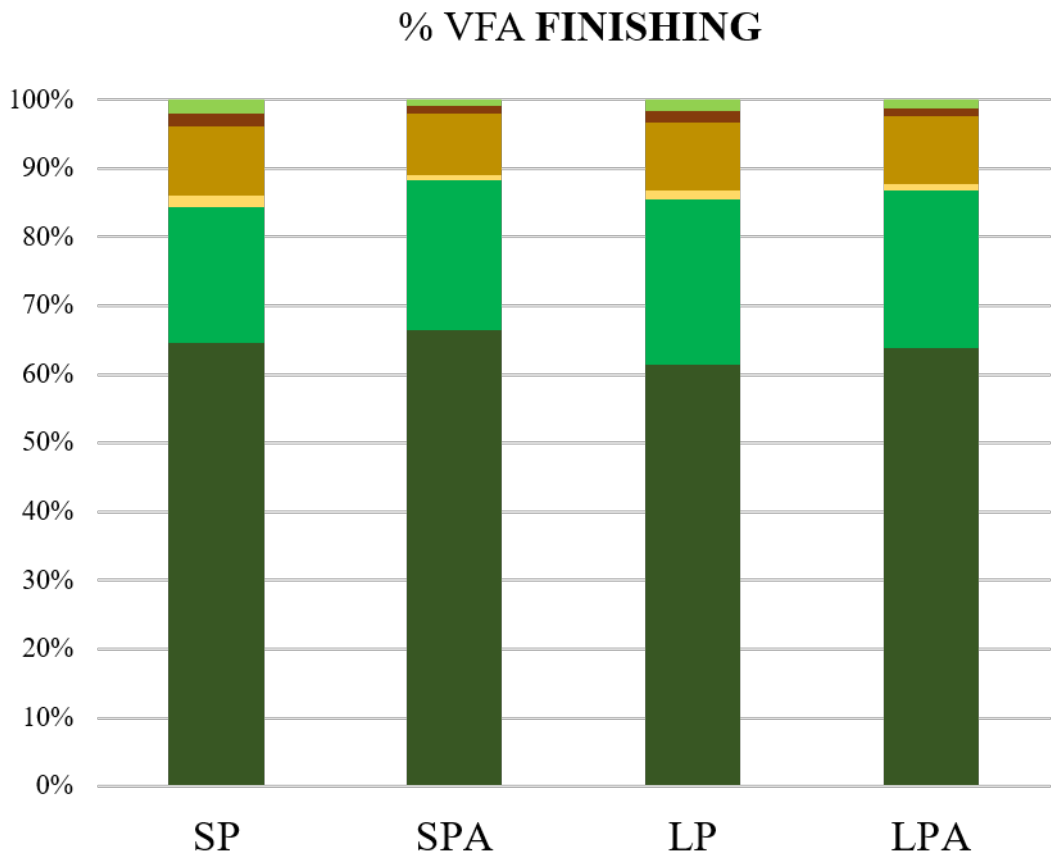
	SP	SPA	LP	LPA	SEM	*P*-value		
						CP	Additives	CP × Additives
GROWING PHASE								
Intake	51.2	51.5	51.7	49.3	1.72	NS	NS	NS
Fecal excretion	5.5	5.7	6.4	6.2	0.79	NS	NS	NS
Urinary excretion	6.4	7.1	5.6	5.6	0.87	NS	NS	NS
Retention	39.3	38.8	39.7	37.5	1.35	NS	NS	NS
N retained/N intake (%)	76.6	75.3	77.1	76.1	1.74	NS	NS	NS
FINISHING PHASE								
Intake	52.8	45.1	51.2	49.3	2.64	NS	NS	NS
Fecal excretion	4.8	4.5	5.6	5.7	0.70	NS	NS	NS
Urinary excretion	6.4	5.9	5.4	4.4	1.25	NS	NS	NS
Retention	41.6	34.7	40.3	39.2	2.33	NS	NS	NS
N retained/N intake (%)	79.01	76.86	78.76	79.50	3.196	NS	NS	NS

No significant differences were found between groups

Volatil Fatty acids (VFA)





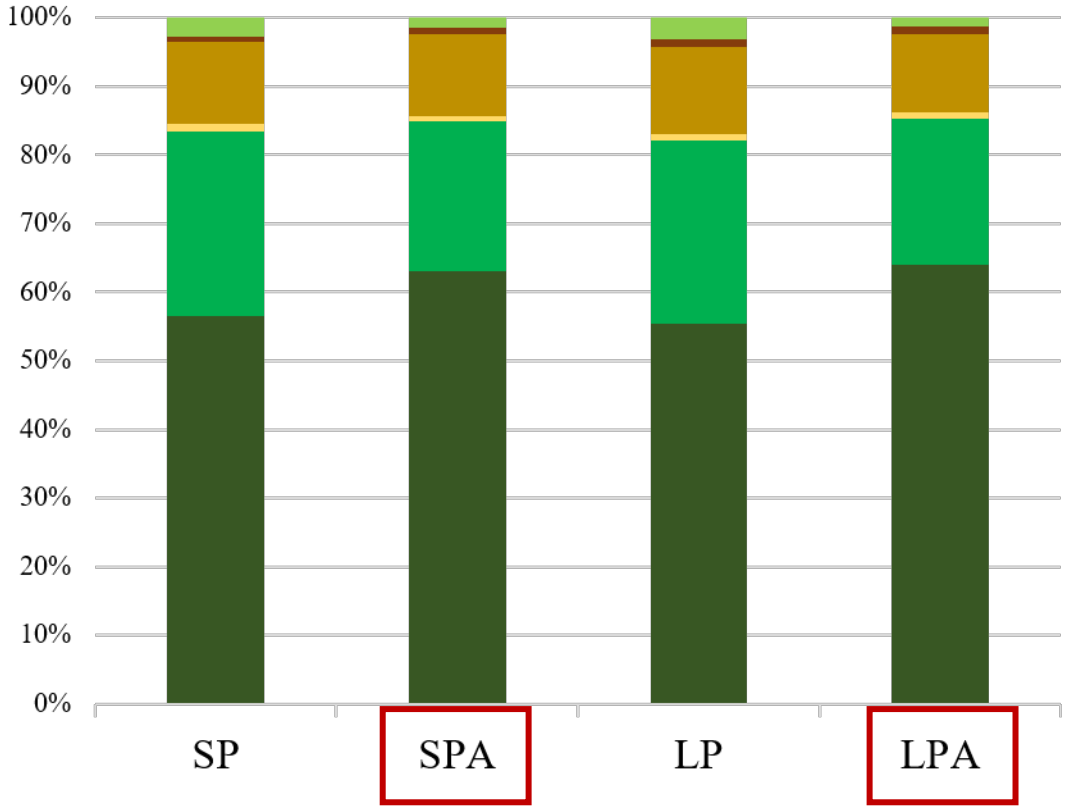


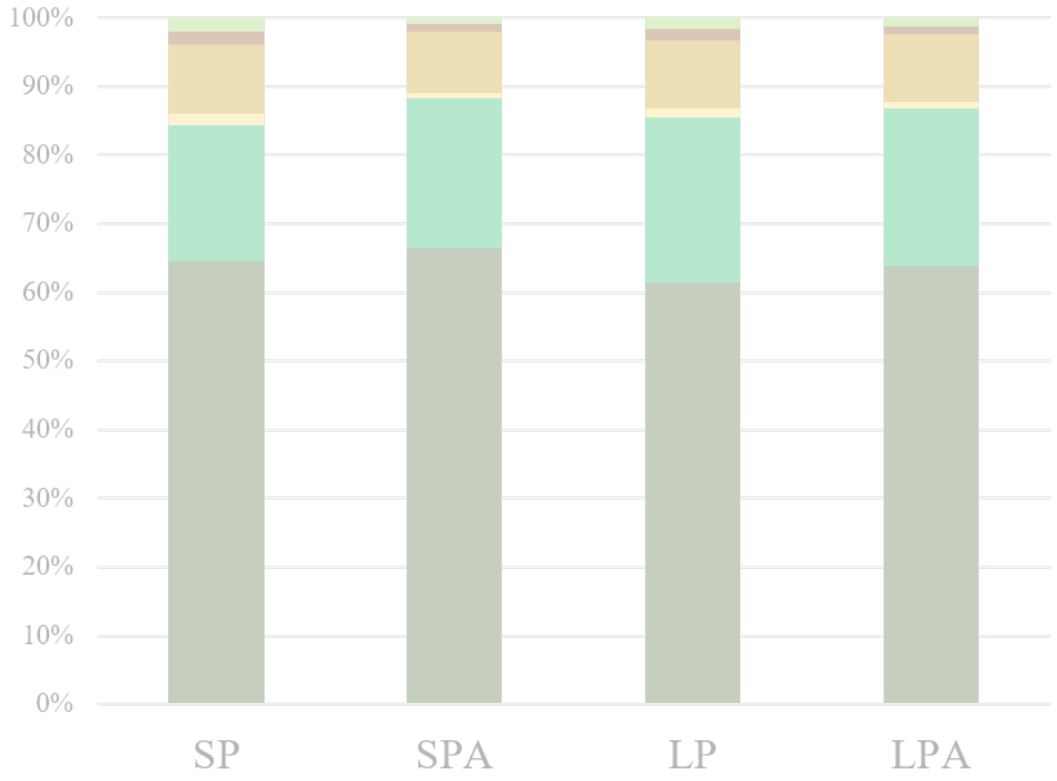
Volatil Fatty acids (VFA)

% Acetic A. ↑ | % Propionic A. ↓ | % Isobutiric A.
% Butiric A. | % Isovaleric A. | % Valeric A. ↓

$P < 0.05$

Additives





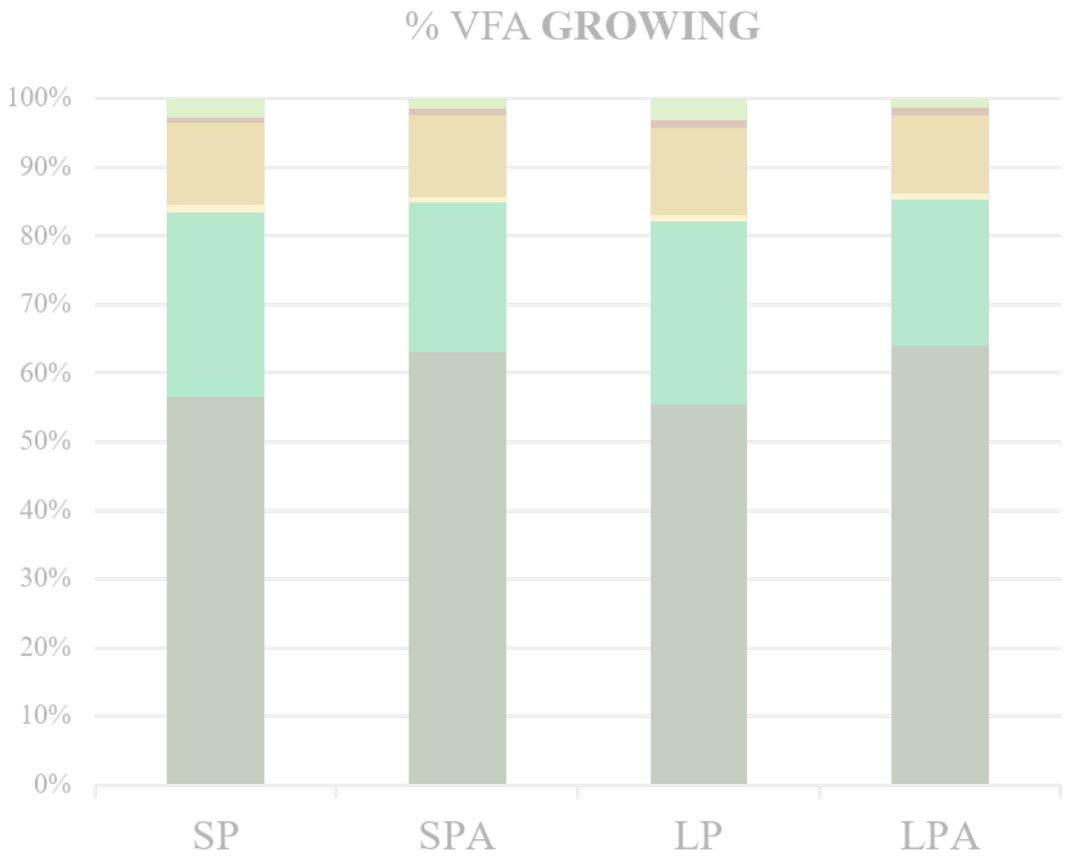
Volatil Fatty acids (VFA)

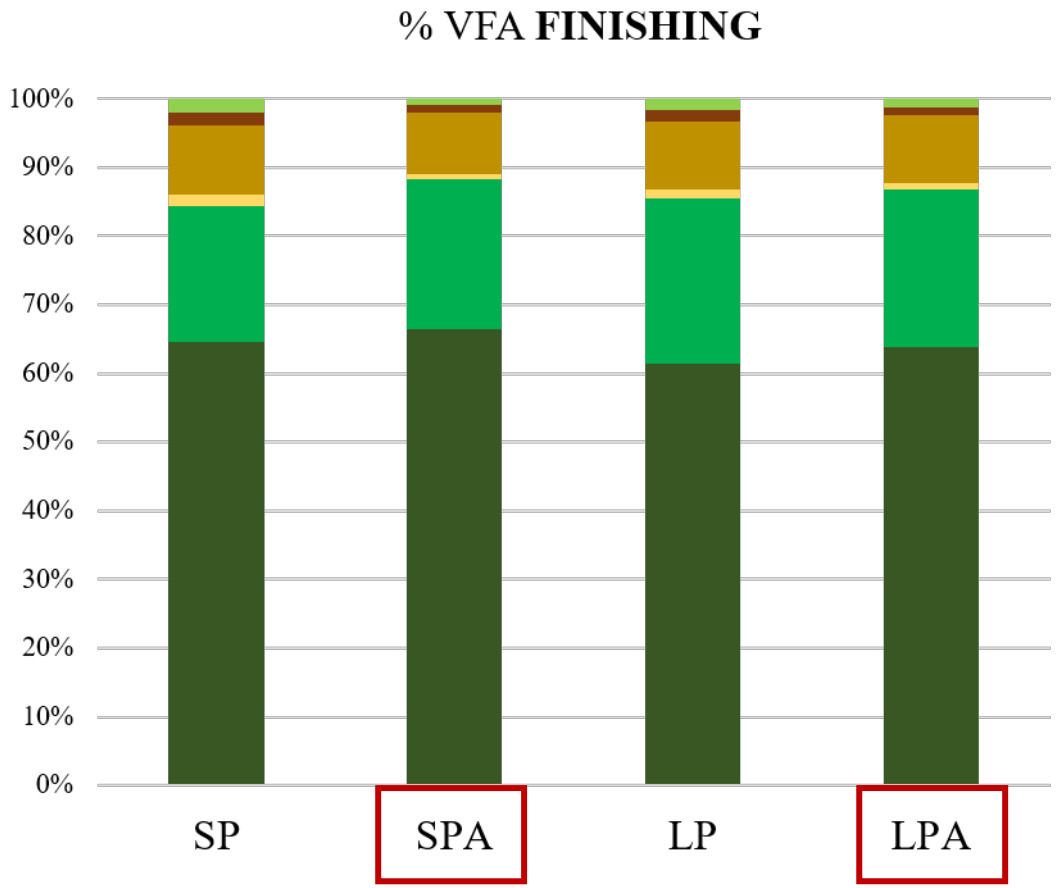
↑ % Acetic A. | % Propionic A. | ↓ % Isobutiric A.

% Butiric A. | ↓ % Isovaleric A. | ↓ % Valeric A.

$P < 0.05$

Additives



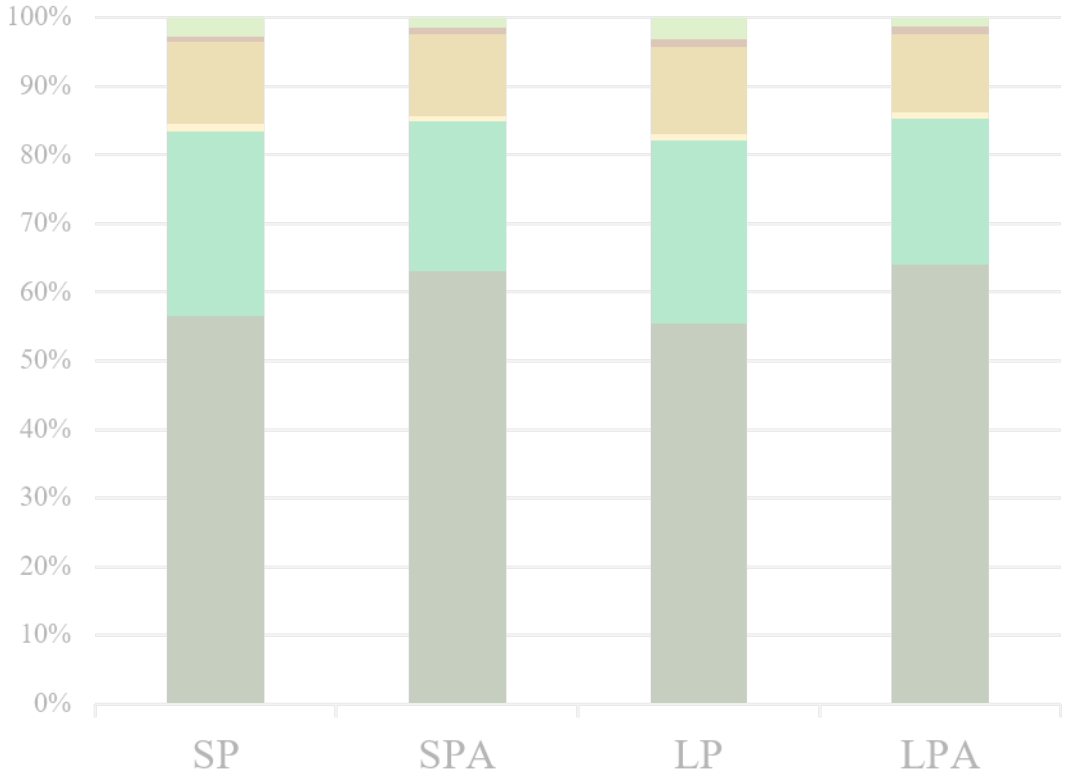


Volatil Fatty acids (VFA)

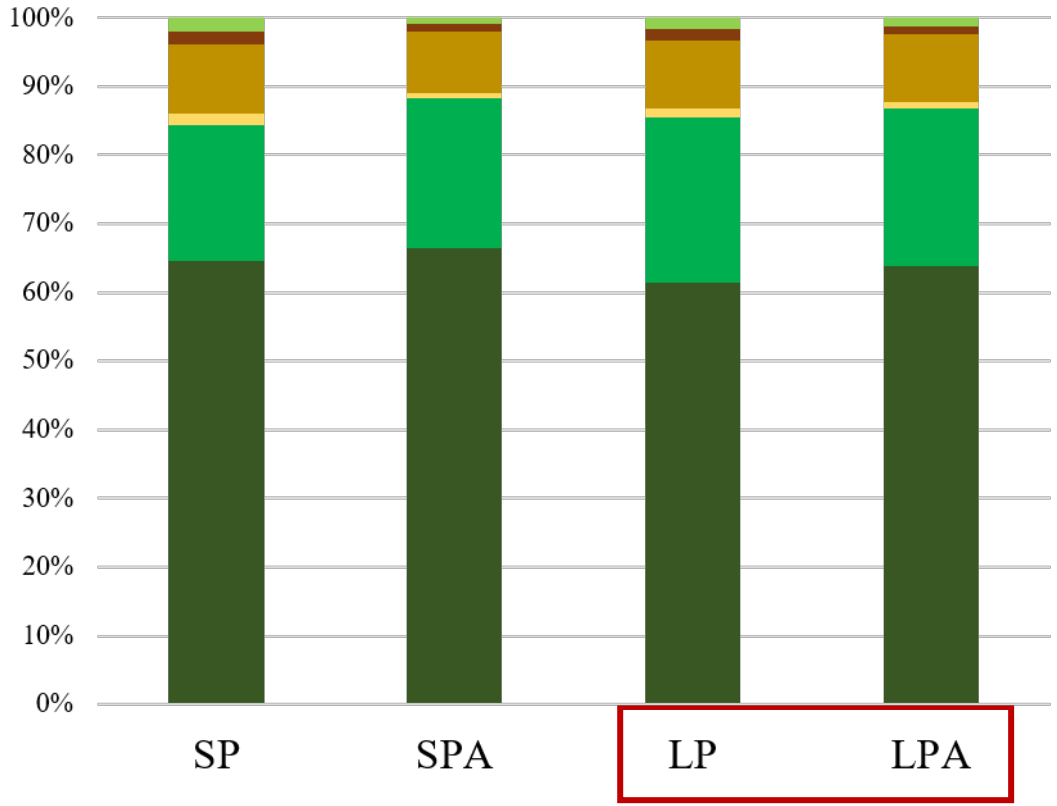
Crude protein

% Acetic A. ↓ | % Propionic A. ↑ | % Isobutiric A. | % Butiric A. | % Isovaleric A. | % Valeric A.

↓ Acetic / Propionic ratio $P = 0.001$

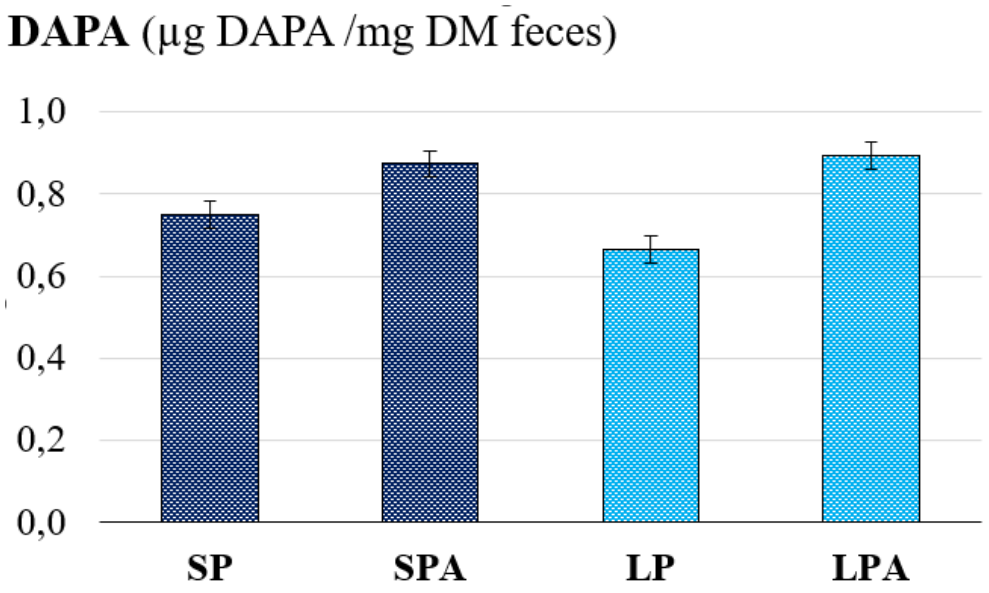


% VFA **FINISHING**

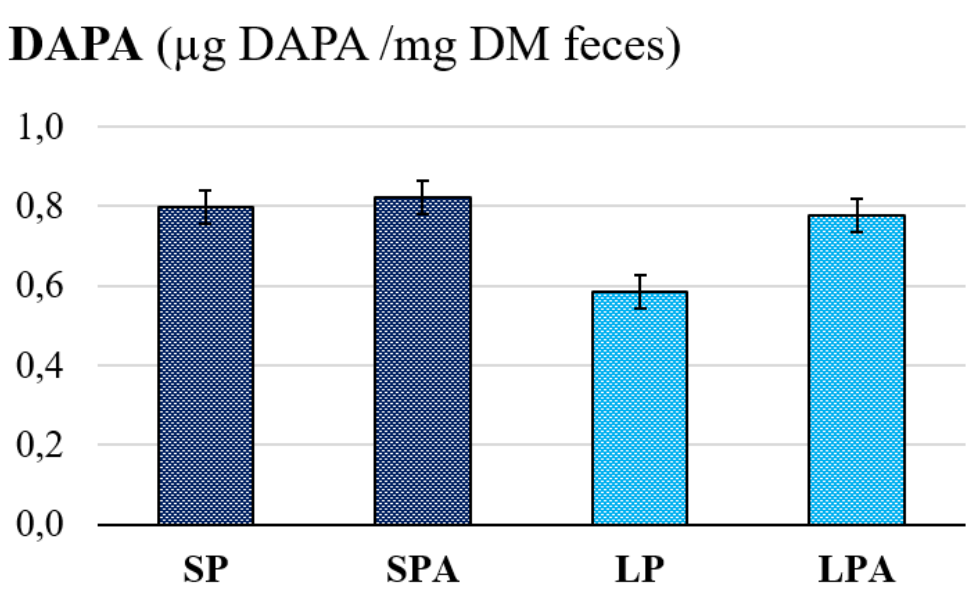


Diaminopimelic acid (DAPA)

GROWING phase



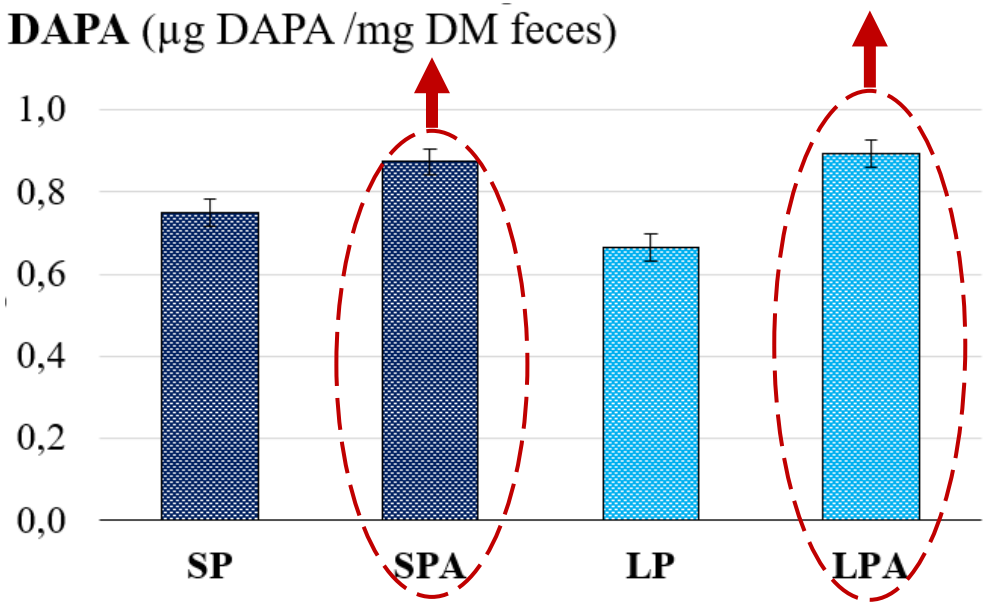
FINISHING phase



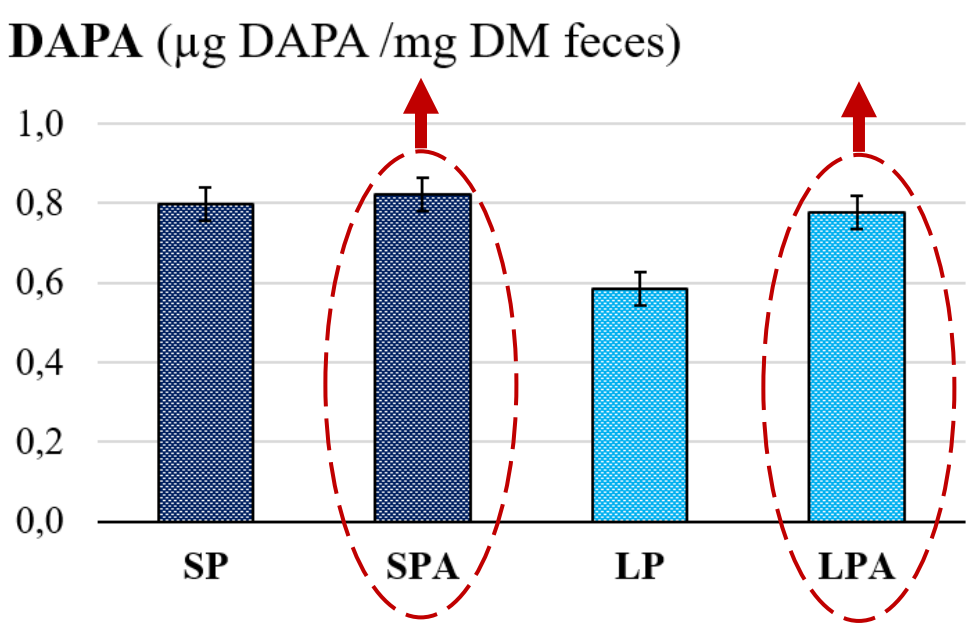
Diaminopimelic acid (DAPA)

Additives

GROWING phase



FINISHING phase

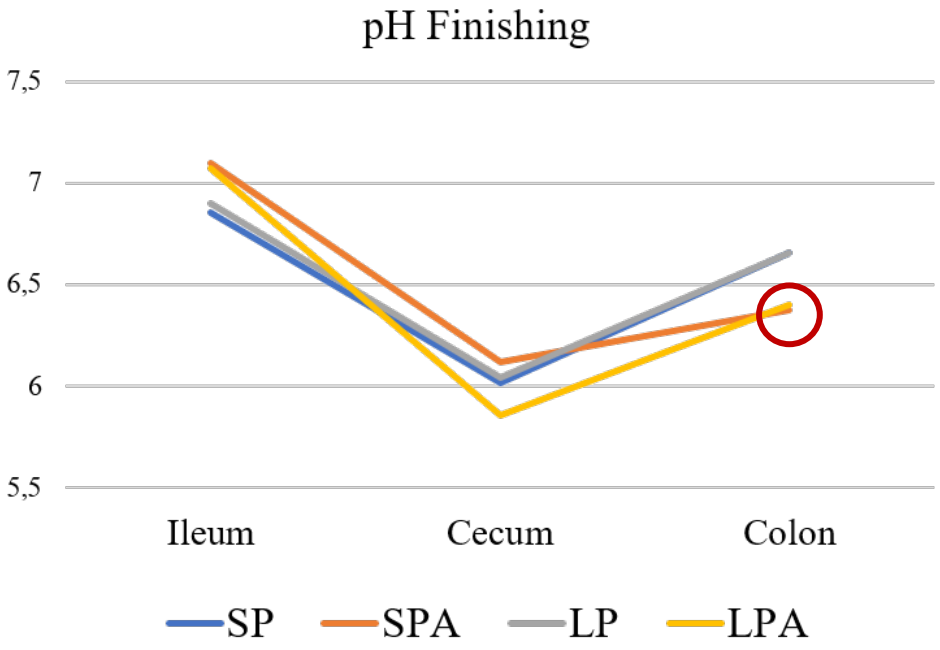


Growing phase ***P*** = **0.0002**

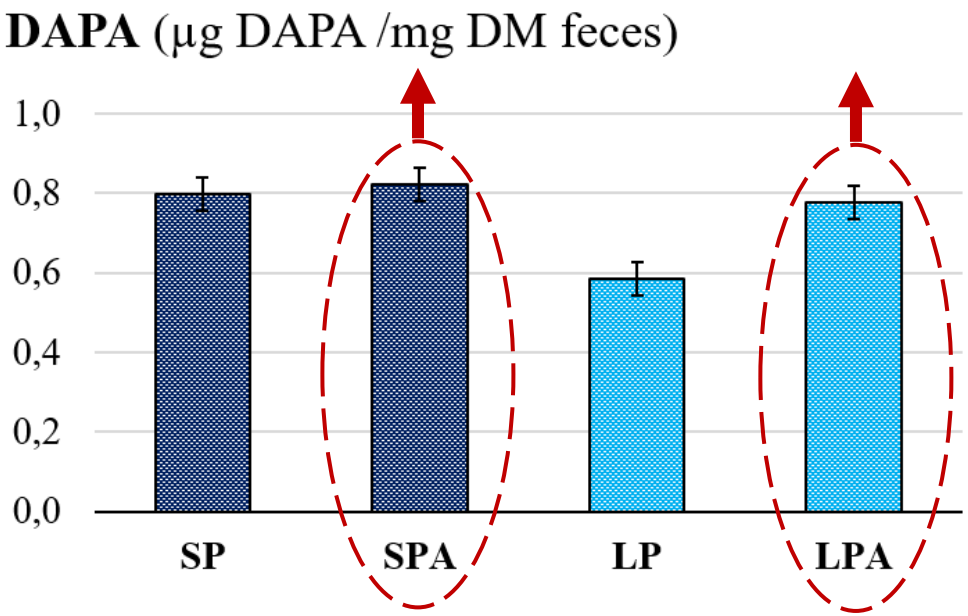
Finishing phase ***P*** = **0.024**

Feed additives significantly increased DAPA concentration in feces

Diaminopimelic acid (DAPA)



FINISHING phase

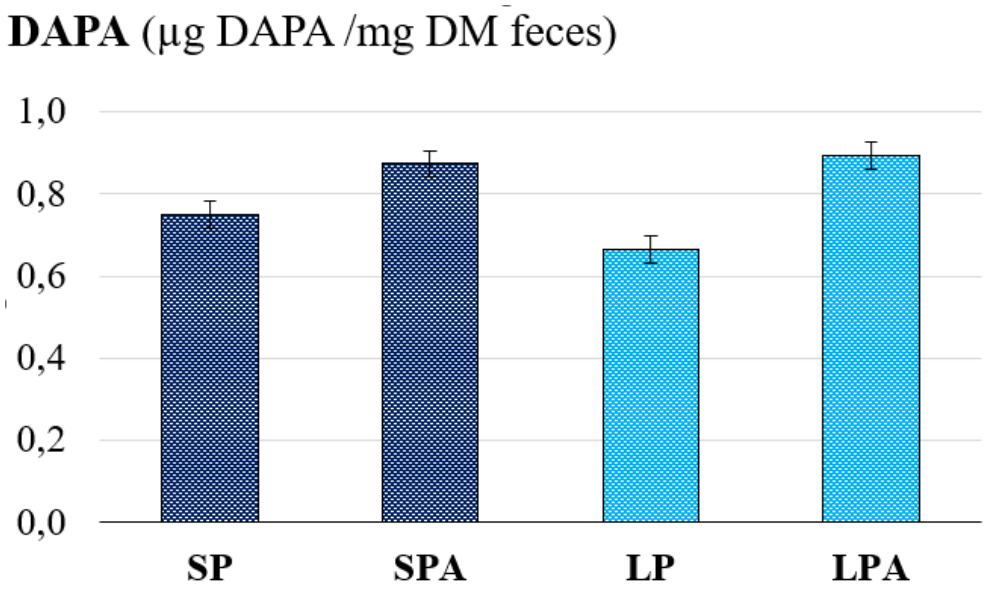


$P < 0.05$

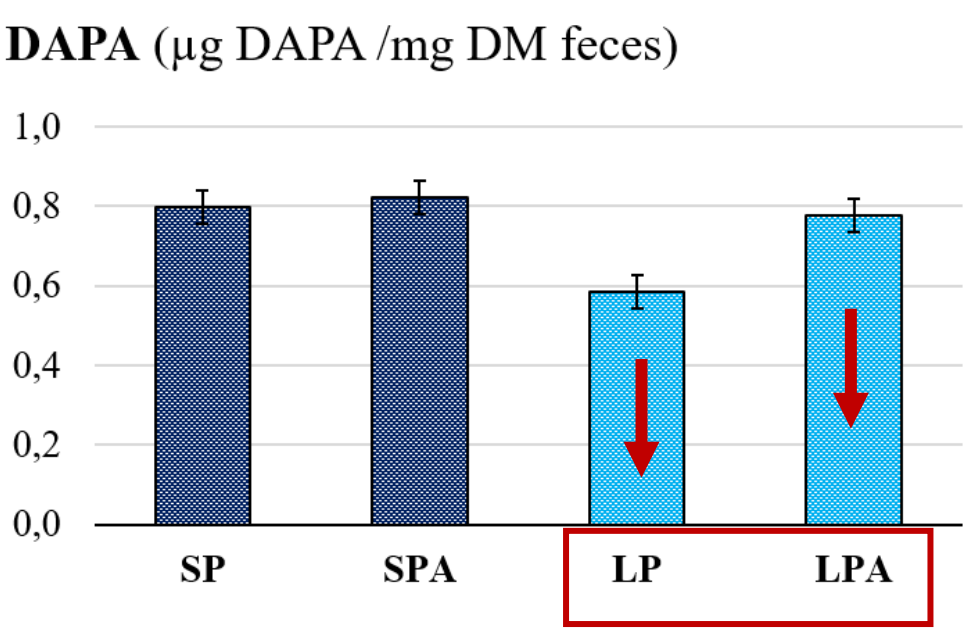
Diaminopimelic acid (DAPA)

Crude protein

GROWING phase



FINISHING phase



Growing phase ***P*** **NS**

Finishing phase ***P*** = **0.010**

LP diet dereased DAPA concentration in feces of finishing pigs

Alpha microbial diversity

Table 4. Microbial **alpha diversity** indices (based on OTUs) in colon

	SP	SPA	LP	LPA	SEM	*P*-value		
						CP	Additives	CP × Additives
GROWING PHASE								
Shannon index	4.02	3.97	3.89	4.13	0.094	NS	NS	NS
Simpson index	0.96	0.96	0.95	0.97	0.007	NS	NS	NS
Richness	346 ^{ab}	309 ^{b}	318 ^{b}	364 ^{a} ↑	11.0	NS	NS	**0.001**
FINISHING PHASE								
Shannon index	3.95	3.79	3.79	3.97	0.208	NS	NS	NS
Simpson index	0.96	0.94	0.93	0.96	0.017	NS	NS	NS
Richness	333	339	319	329	22.8	NS	NS	NS

Microbial richness index increased significantly when LP diet was supplemented with feed additives

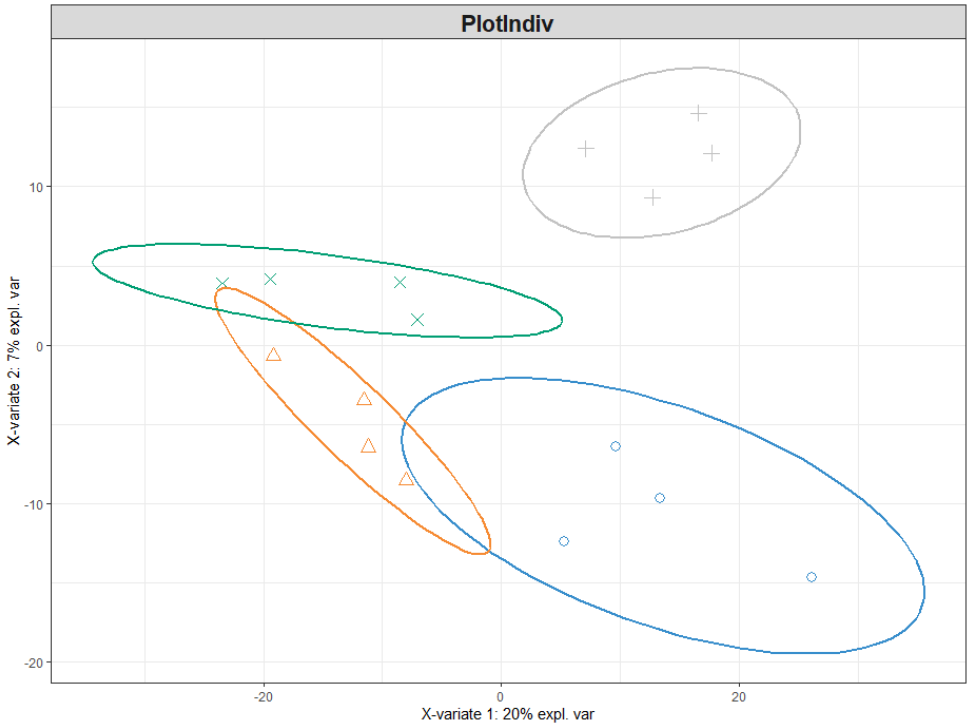
Beta microbial diversity analisis

Fig. 6 Graphical representation of partial least squares-discriminant analysis (PLS-DA) in the colon

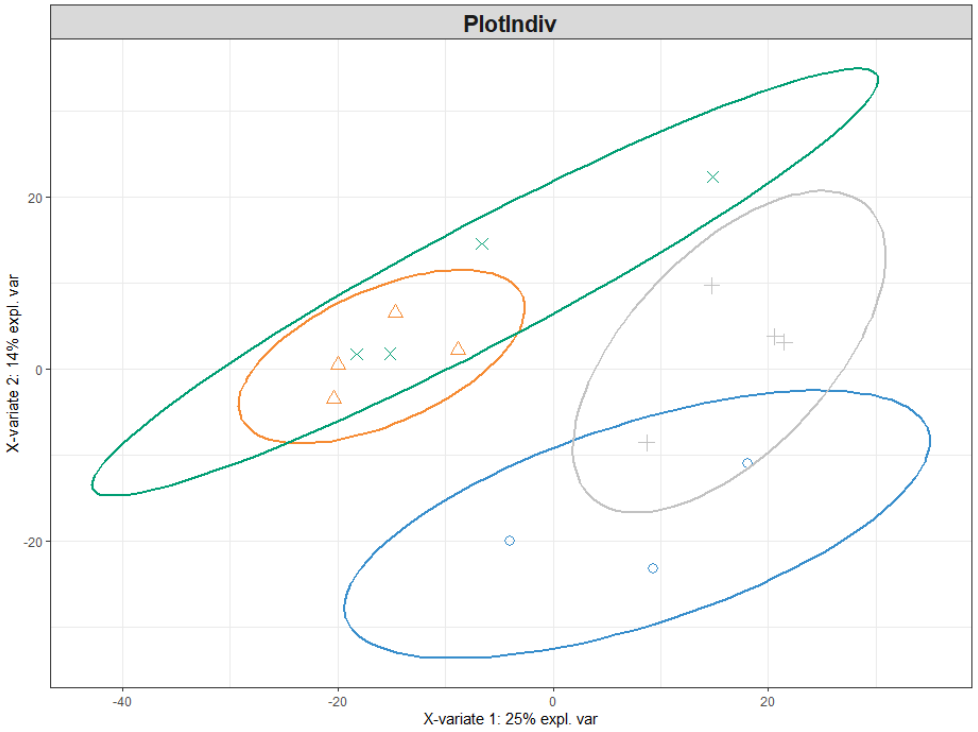
Additives

SP
SPA
LP
LPA

GROWING PHASE



FINISHING PHASE



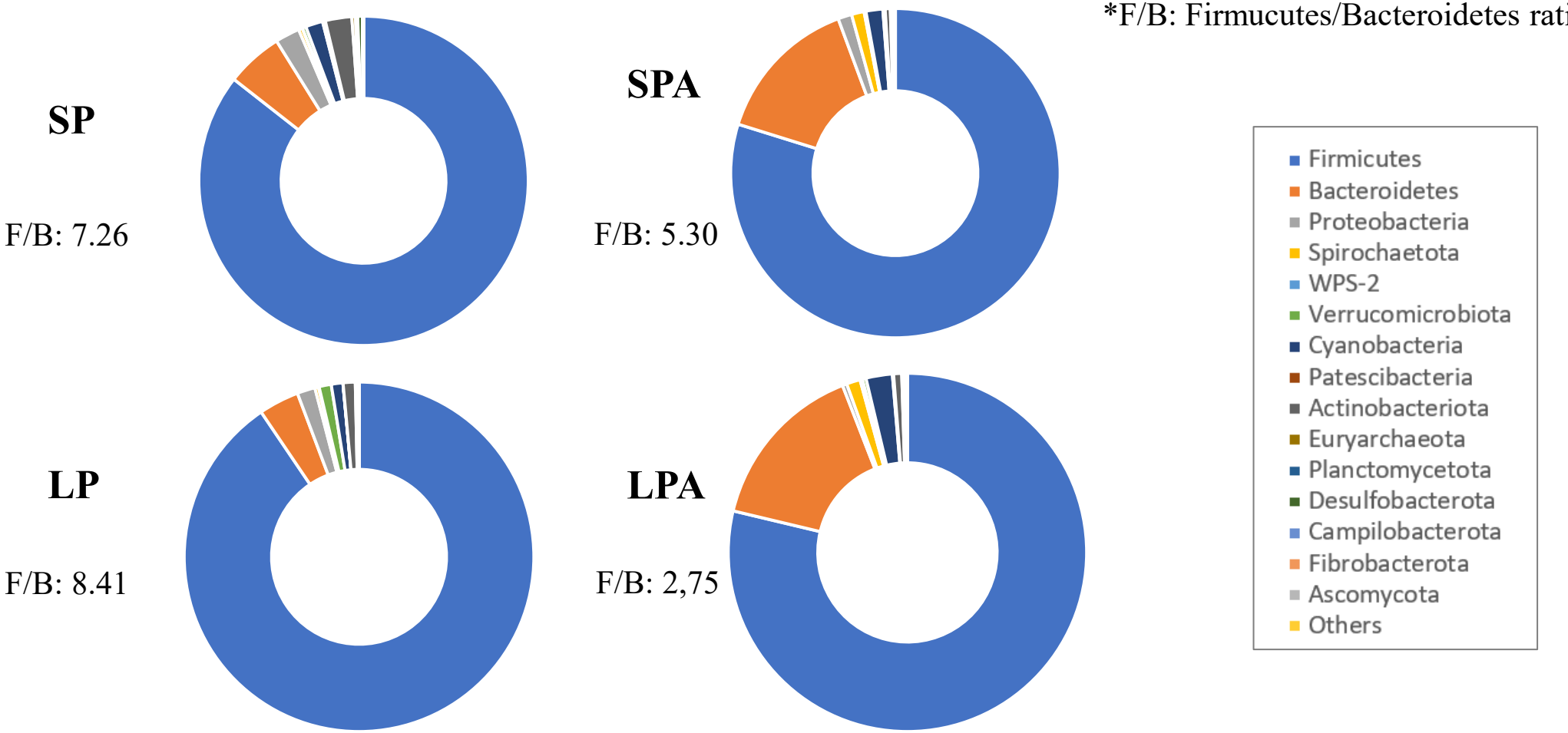
PERMANOVA analysis: ***P*** = **0.0014** ***P*** = **0.0294**

Microbial composition clustered separately between diets supplemented and non-supplemented with additives

Abundance of microbial phyla

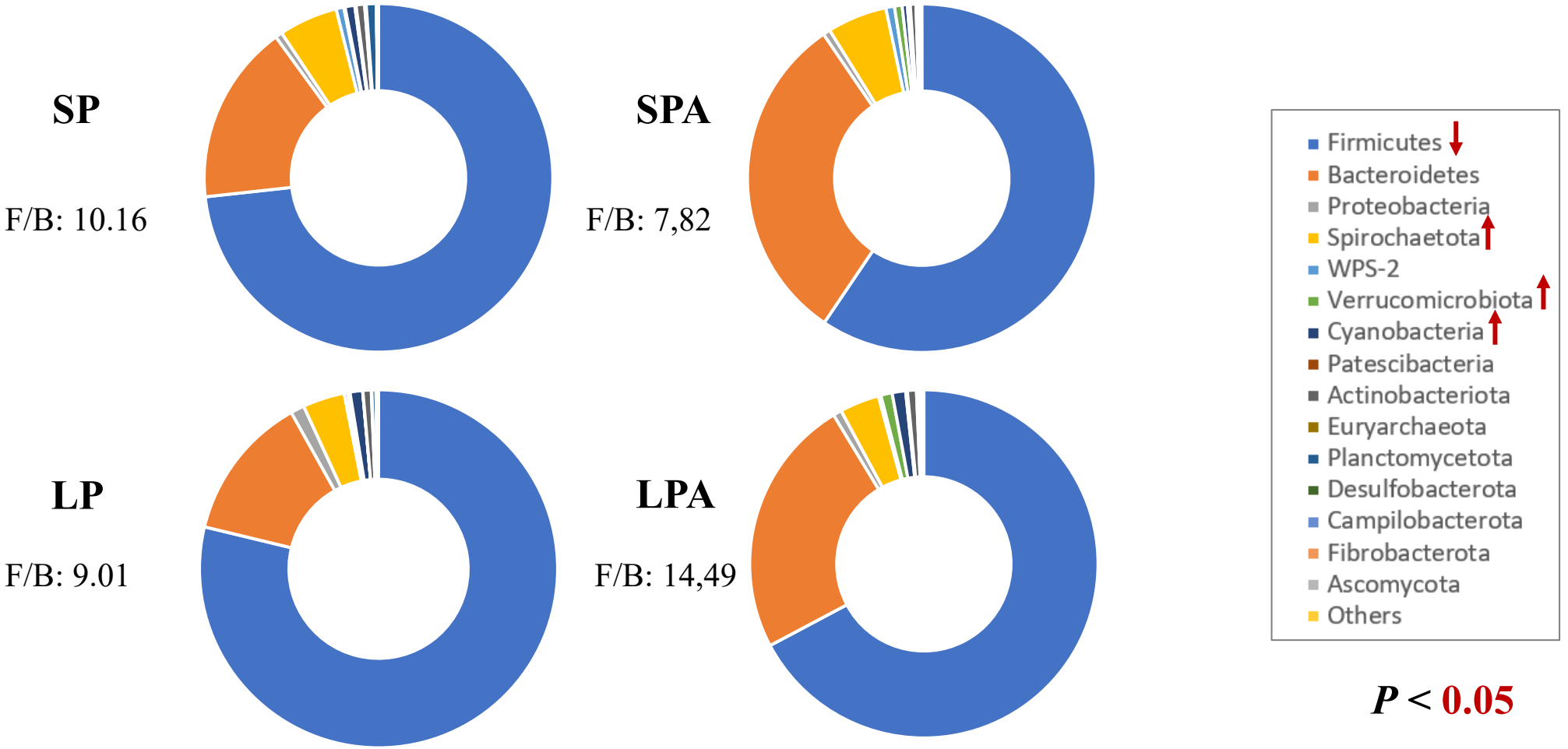
Fig. 7 Phyla abundance in the colon of **GROWING** pigs

*F/B: Firmucutes/Bacteroidetes ratio **NS**



Additives

Fig. 8 Phyla abundance in the colon of **FINISHING** pigs



$P < 0.05$

Conclusions

APPARENT ILEAL DIGESTIBILITY

- AID was slightly improved in LP diets when were supplemented with feed additives

VOLATIL FATTY ACIDS

- Additives increased Acetic acid and decreased valeric acid in both feeding phases
- LP diets decreased Acetic/Propionic ratio in finishing pigs

DAPA

- Additives increased microbial load
- LP diets reduced microbial load in finishing

MICROBIOTA

- Microbial richness increased in the LPA diet in growing pigs

Take home message:

The addition of feed additives in the diet seemed to increase microbial activity and microbial richness especially in LP diets, thus altering the volatile fatty acids profile. However, they had no relevant effects on N balance.

Universitat de Lleida

Thank you for your attention!

agrotecnio

EXCELLENCE IN FOOD AND AGRICULTURE RESEARCH

dsm-firmenich