

Reduction in crude protein content and amino acid supplementation in piglet diets



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Pré-natal e de Animais Jovens

Content

1. Introduction - development of low protein diet concept

2. Effects of lowering dietary CP level on piglet performance and N excretion

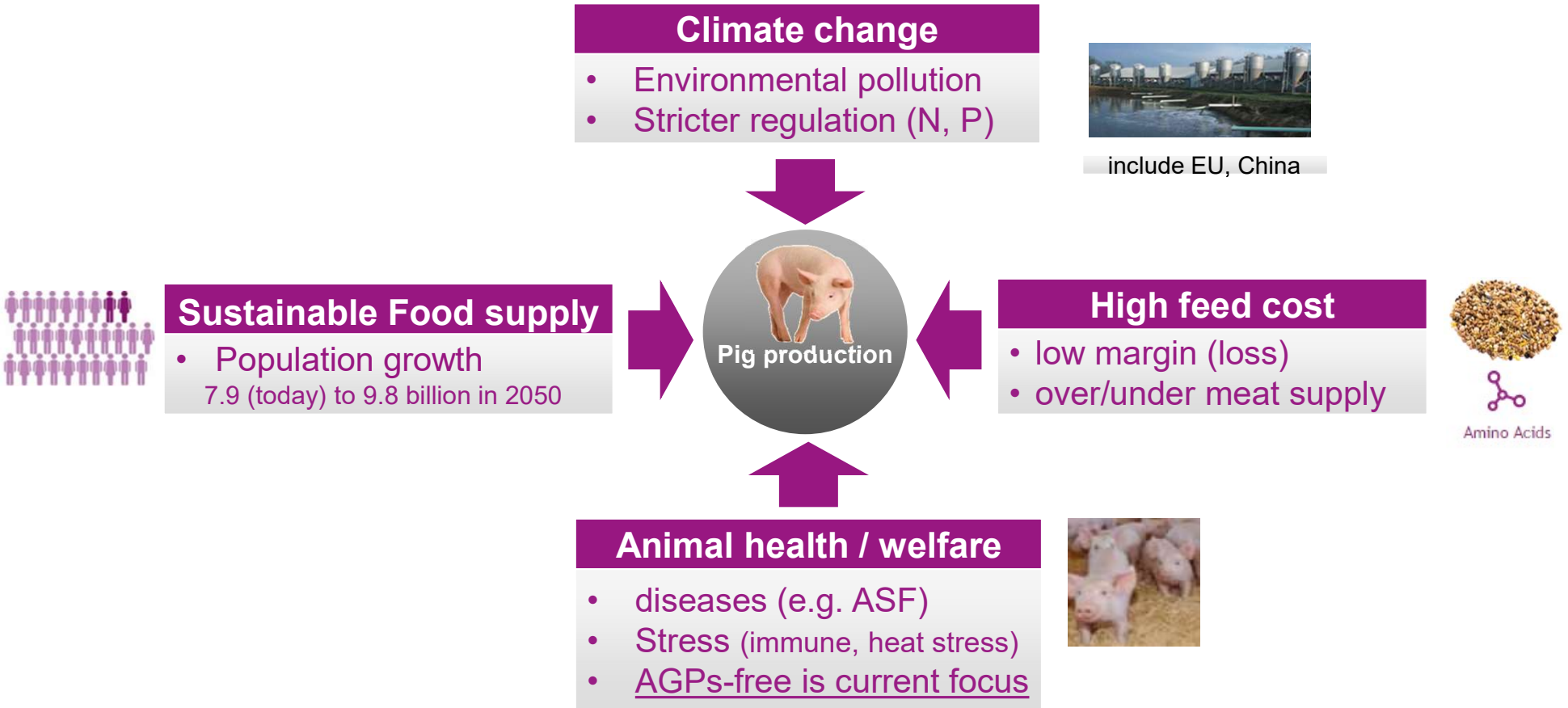
3. Mistakes to be avoided when formulating low protein diets – how low we can go?

4. Advances on low protein diet concept to enhance intestinal health and microbiota

5. Conclusions

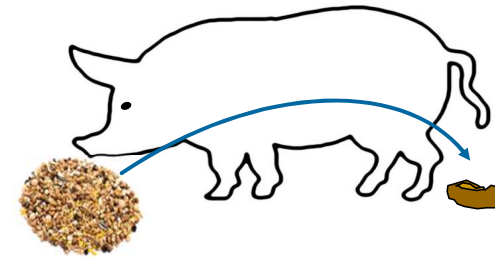


Key challenges which can be tackled by lowering CP levels in pig diets



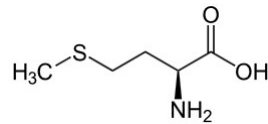
Nutrients in the feed are not completely available to the animals

Not all nutrients are digested and available
 Quality of a protein source is affected by its AA availability
 Needs nutrient availability estimates



SID of AA in a CAA mixture in pigs

Item	AA ^d
Nitrogen	96.0
Indispensable AA	
Lysine	99.3
Tryptophan	97.9
Threonine	98.2 ^e
Methionine	99.2
Histidine	99.3
Leucine	97.2 ^e
Isoleucine	100.1 ^e
Valine	98.5 ^e
Phenylalanine	99.4
Arginine	100.5
Mean	99.0



SID of AA

- good indication of AA availability in ingredients
- used as estimates of available AA in practice by swine nutritionists (AMINODat 6.0; NRC, 2012).

SID of Met (%; AMINODat 6.0)						
DL-Met	Corn	Wheat	Barley	Soybean meal	DDGS	Wheat bran
~100	87	89	83	92	83	82

- Crystalline AA are proven to be completely (100%) digestible
- CAA: higher availability compared with intact protein sources

Chung and Baker (1992; JAS 70:3781-3790)

Low protein diet – an old concept but still relevant today

EFFICACY OF SUPPLEMENTAL LYSINE, METHIONINE AND ROLLED OATS FOR WEANLING PIGS FED A LOW-PROTEIN CORN-SOYBEAN MEAL DIET

R. S. Katz, D. H. Baker, C. E. Sasse¹, A. H. Jensen and B. G. Harmon

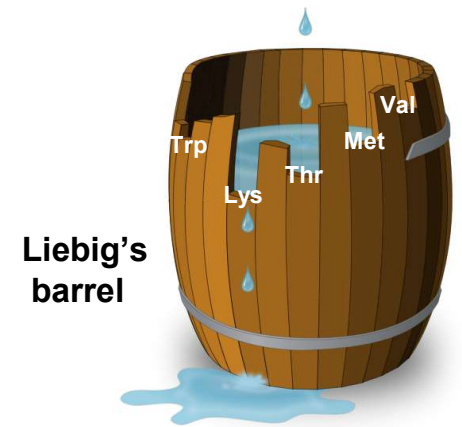
University of Illinois at Urbana-Champaign, Urbana 61801

Katz et al., 1973 -J. Anim. Sci.

- CP level of corn-SBM diets reduced from 19 to 16% without reducing performance of weaned pigs by adding L-Lys.HCl.

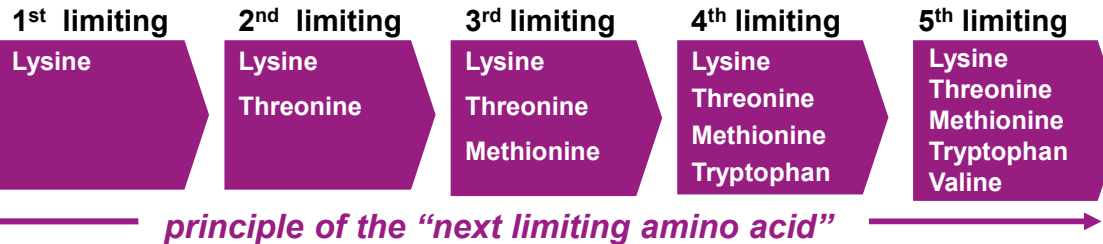


- Reducing dietary CP content by replacing a portion of protein bound AA with crystalline AA
- Balancing adequate level of all AA and energy
- Maintaining the same pig performance



Synergy:

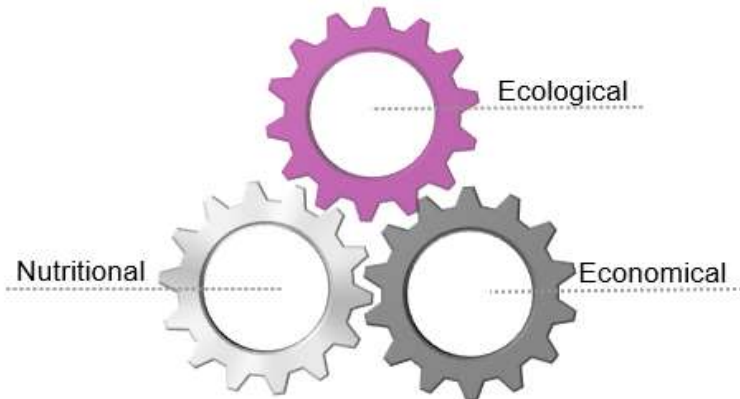
Progress of nutritional concepts:



- Protein synthesis is "all or nothing".
- Lys is the first limiting AA in typical pig diets (before Thr, Met and Trp, Val and Ile)

Advancement in feeding concepts for swine

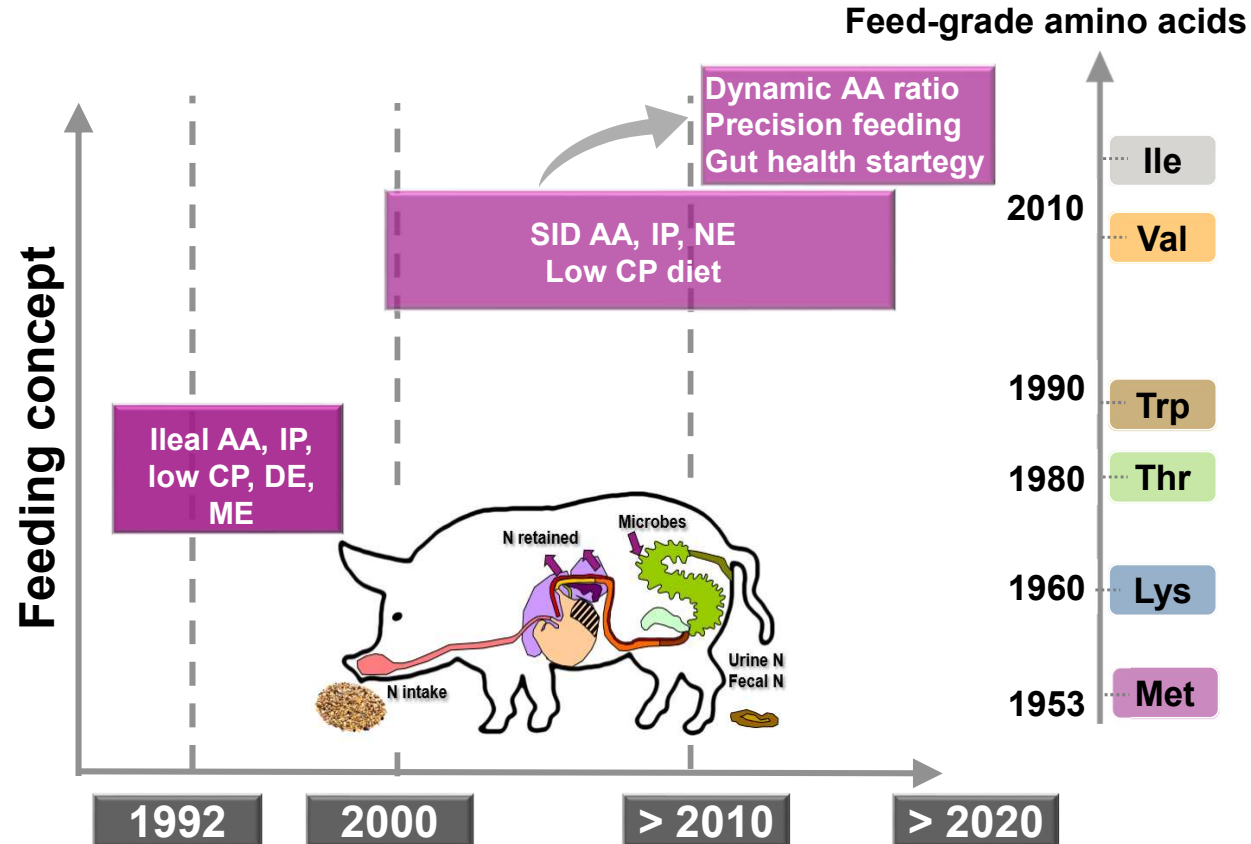
- Advances in ileal AA digestibility and requirements of AA for pigs
- Formulating diets on the basis of SID AA in combination with ideal protein concept and NE allows lowering CP level
- Increased availability of CAA such as Lys, Met, Thr, Trp, Val and Ile (Arg, His)



Brazilian Tables
for Poultry and Swine

NUTRIENT
REQUIREMENTS
OF SWINE

Recommendations for Swine
amino acids and more.



Uncertainty about the formulation of low CP diets?

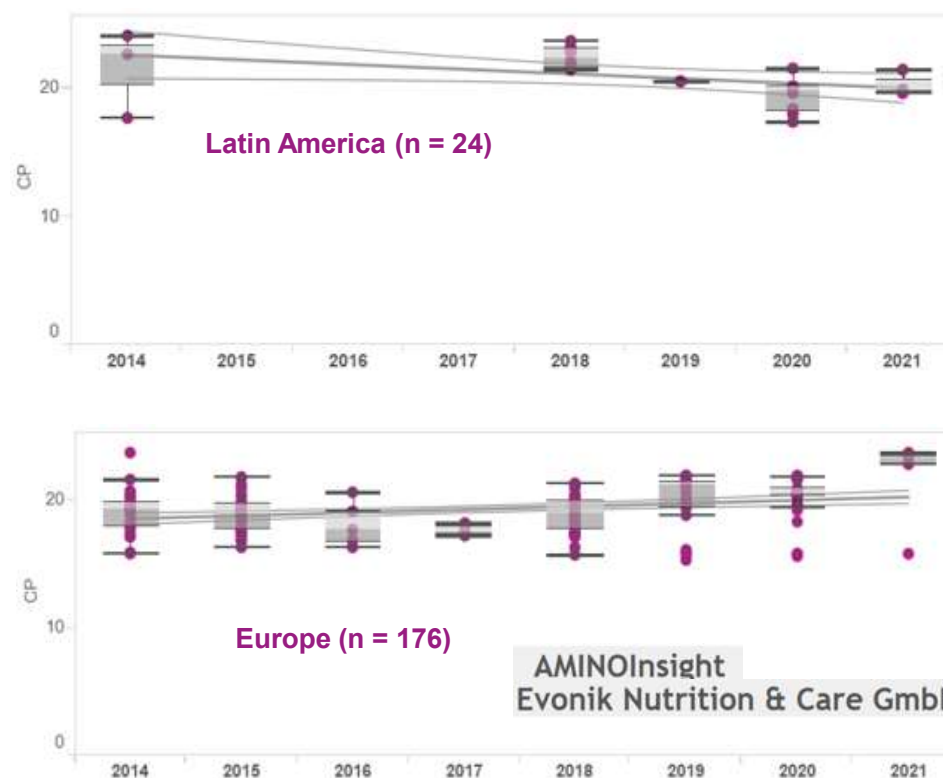
- Reduced pig performance when the dietary CP level was reduced more than 4%-points with increased CAA (Figueroa et al., 2002).
- Uncertainty about the availability of CAA compared with conventional intact protein sources (SBM)
- How much can we lower dietary CP level in pig diets?
- Any potential benefits of Low CP diets beyond N excretion?

How do CP levels in commercial piglet diets look like?

	NRC, 2012	Brazilian Table, 2017	GBT (Chinese)
Pre-starter (5-9 kg)			
SID Lys, %	1.50	1.45	1.42
CP, %	22.7	21.4	21.0
SID Lys:CP, %	6.6	6.8	6.8
Starter 1 (7-11 kg)			
SID Lys, %	1.35	1.35	
CP, %	20.6	19.9	
SID Lys:CP, %	6.6	6.8	
Starter 2 (11-25 kg) (8-25 kg)			
SID Lys, %	1.23	1.12	1.22
CP, %	18.9	18.1	18.5
SID Lys:CP, %	6.5	6.2	6.6

- In Europe often nursery pig diets are formulated to contain only 17–18% CP to avoid PWD (Stein, 2002).
- There is still room to reduce CP level in commercial piglet feed.

Analyzed CP level – Pre-starter commercial piglet feed



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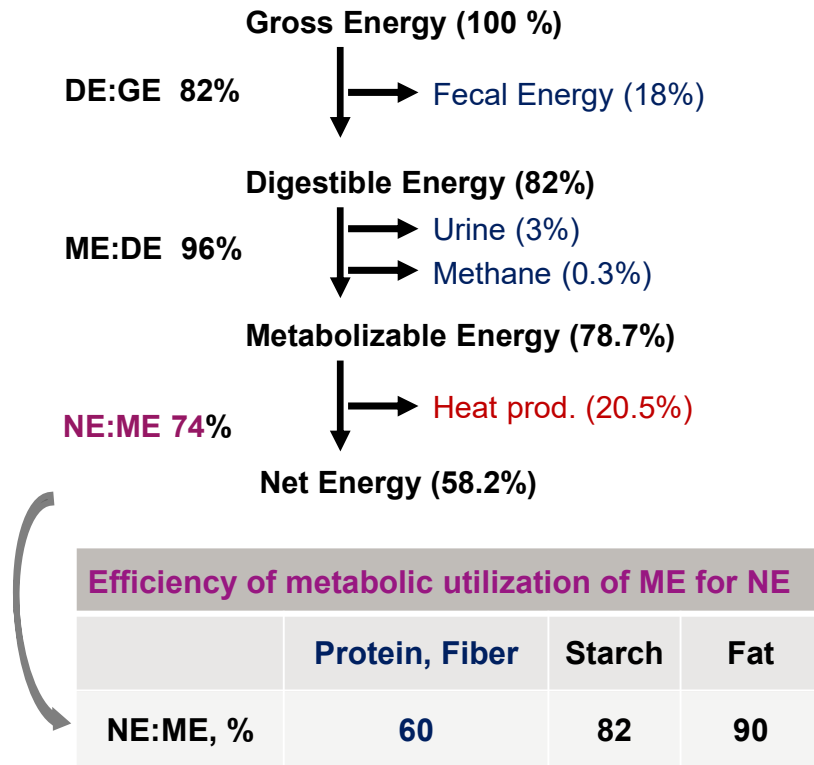
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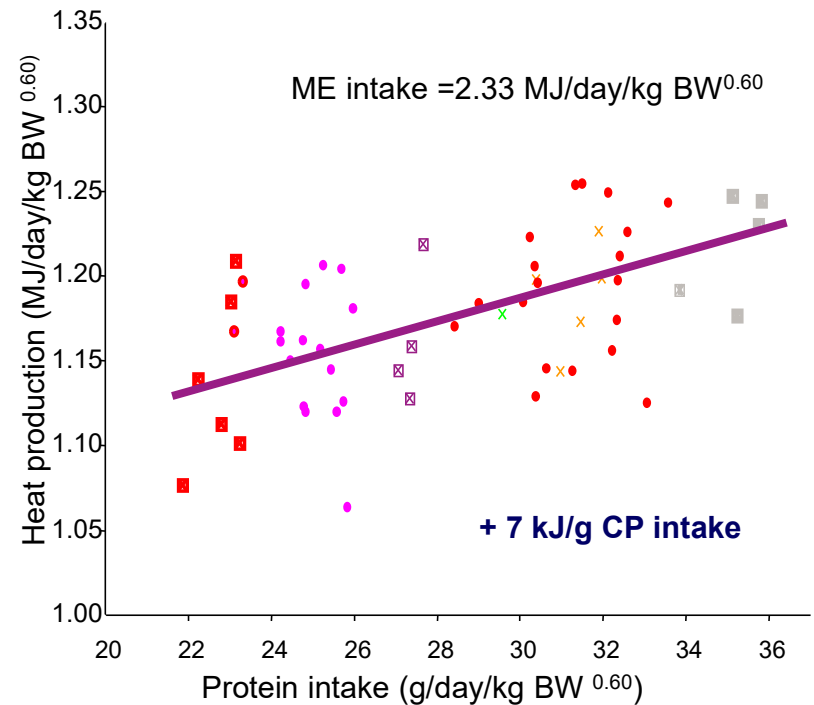


Lowering CP level improves energy utilization efficiency

Noblet et al. (1994)



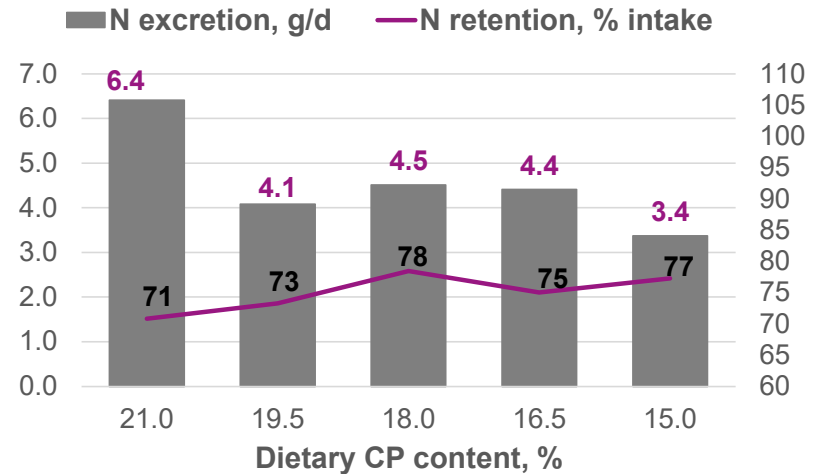
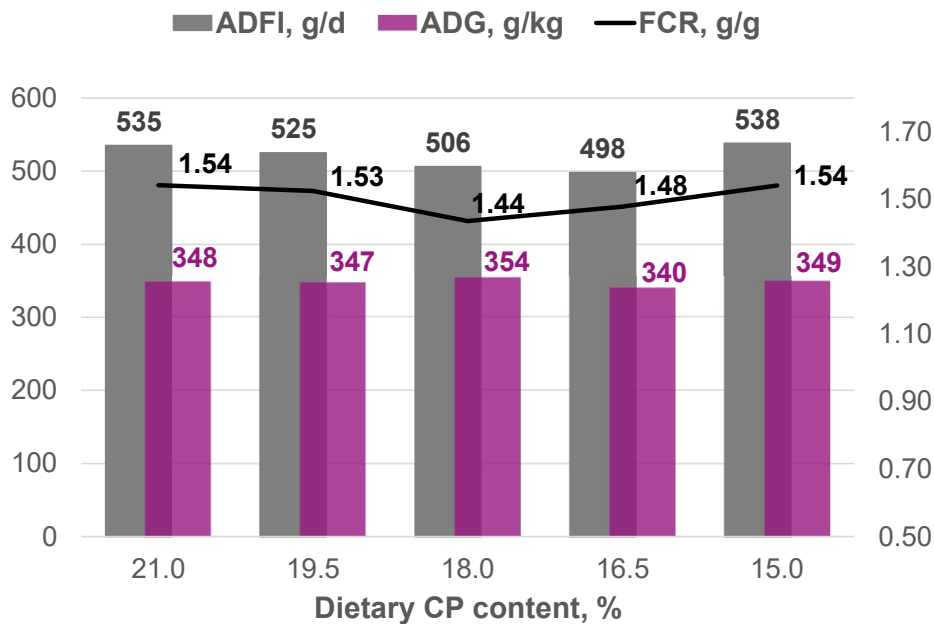
Le Bellego et al. (2001)



Increased fat deposition can be prevented by balancing the same SID Lys:NE.

Reduction of dietary CP (6%-unit) on performance and N excretion in weaned pigs

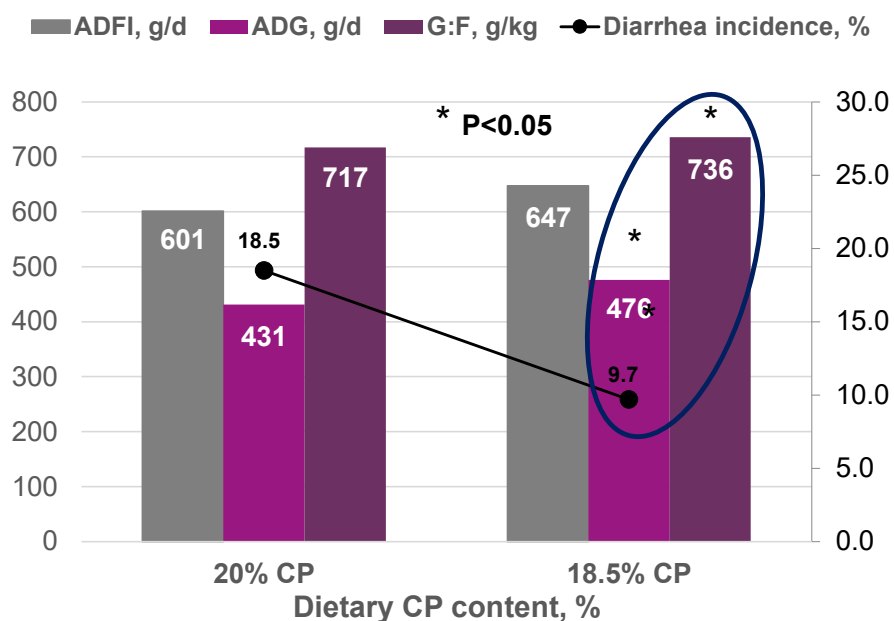
Animals	120 pigs for growth trial (initial BW 6 kg; 28 d) 20 barrows for N-balance (initial BW 10.8 kg)
Treatments	5 CP levels (21, 19.5, 18, 16.5, 15%)
Diets	Corn, SBM, skim milk, Lys, Thr, Met, Trp, Val, Ile
Formulation	Same SID AA, ME and electrolyte balance



Toledo et al. (2014; R. Bras. Zootec.)

Effect of lowering CP and balancing with CAA on performance of weaned pigs

Corn, **SBM**, Whey diets; 20% CP (5 CAA); 18.5% CP (6 CAA); same SID AA (1.23% Lys), piglets (8-20 kg); 4 wk



	20% CP	18.5% CP
Initial BW, kg (d 1)	8.1	8.1
Pig price, EUR/kg*	20	20
Final BW, kg (d 28)	20.2	21.4
Pig price, EUR/kg*	12	12
Revenue per pig, EUR/kg	80.40	94.80
FCR	1.39	1.36
Feed intake per pig, kg	16.88	18.07
Feed cost, EUR/kg*	0.376	0.375
Feed cost per pig, EUR	6.35	6.78
IOFC, EUR/pig	74.05	88.02
Extra value with LCP, EUR/pig		13.97

* Price of ingredients and pigs in China (Sep, 2020; eFeedlink)

Zhou et al. (2019; Anim Sci J. 90(2):237-246)

Effect of reducing CP (replacing fish meal with CAA) on performance of 7-13 kg weaned pigs (d 0-14)

CP, %	Fish meal*	ME, MJ/kg	SID Lys, %	SID EAA	EAA added	NEAA added	SID Lys:CP, %	Lys:CP, %
21.1	4.5	14.10	1.30	Adeq.	6	-	6.2	6.8
20.9	3.6	14.08	1.30	Adeq.	6	Gln, Gly	6.2	6.8
20.8	2.7	14.07	1.30	Adeq.	6	Gln, Gly	6.3	6.9
20.6	1.8	14.05	1.30	Adeq.	6	Gln, Gly	6.3	6.9
20.5	0.9	14.04	1.30	Adeq.	6	Gln, Gly	6.3	6.9
20.3	0.0	14.02	1.30	Adeq.	6	Gln, Gly	6.4	7.0

Fish meal, %	4.5	3.6	2.7	1.8	0.9	0.0
CP, %	21.1	20.9	20.8	20.6	20.5	20.3
Feed intake, g/d	528	517	537	525	531	546
ADG, g/d	376	372	389	378	380	380
G:F	0.713	0.720	0.730	0.719	0.715	0.698

Nemecek et al. (2014) ; JAS 92:1548-1561

Lowering dietary CP level directly impact N utilization and excretion in 12-27 kg pigs

Animals	32 pigs for growth trial (initial BW 12 kg; 22 d)
Treatments	4 CP levels (22.4, 20.4, 18.4, 16.9%); replacing SBM with CAA (6); 1.01g SID Lys/MJ NE
	Electrolyte balance, mEq/kg (250, 212, 173, 171); Performance + N balance (wk 1)

	Dietary CP, %				P-value
	22.4	20.4	18.4	16.9	
ADG, g/d	642	661	690	663	Linear **
FCR, g/g	1.50	1.58	1.54	1.58	ns
BW (d 22), kg	26.0	26.8	27.2	26.8	Linear **
Total N excretion (g/d)	10.7 ^a	9.4 ^a	6.8 ^b	5.1 ^c	Linear **
Water intake, g/d	1941	1887	1867	1645	Linear *
Urine, g/d	757	643	625	481	Linear *

ns: not significant; * P < 0.05; ** P < 0.01

1 %-pt CP reduction → - 9% N output; - 3% water need

Le Bellego and Noblet; 2002 (Liv Prod Sci)

Content

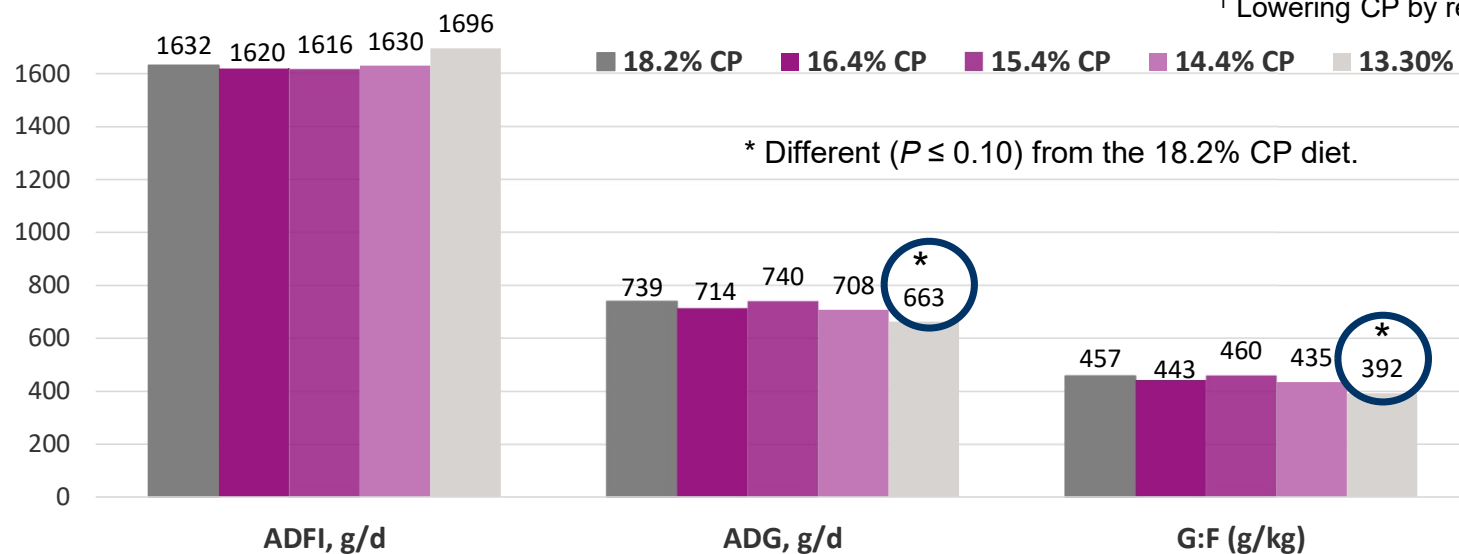
1. Introduction - development of low protein diet concept
2. Effects of lowering dietary CP level on piglet performance and N excretion
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4. Advanced low CP diet concept as a dietary strategy to enhance intestinal health
5. Sustainability?
6. Conclusions



Effect of reducing CP (using up to 4 CAA) on growth performance of 20-45 kg pigs

CP, % ¹	ME, Mcal/kg	SID Lys, %	CAA added	4 EAA level	Val:Lys, %	Ile:Lys, %	Lys:CP, %
18.2	3.24	0.83	0	Adeq.	89	80	5.3
16.4	3.24	0.83	3	Adeq.	79	70	5.7
15.4	3.24	0.83	4	Adeq.	73	64	6.1
14.4	3.24	0.83	4	Adeq.	67	58	6.5
13.3	3.24	0.83	4	Adeq.	61	52	6.9

¹ Lowering CP by replacing SBM with free AA.



- Reducing CP > 4%-pt reduced ADG and G:F.
- Due to deficient in next limiting AA.

Roux et al. (2011; JAS 89:2415–2424)

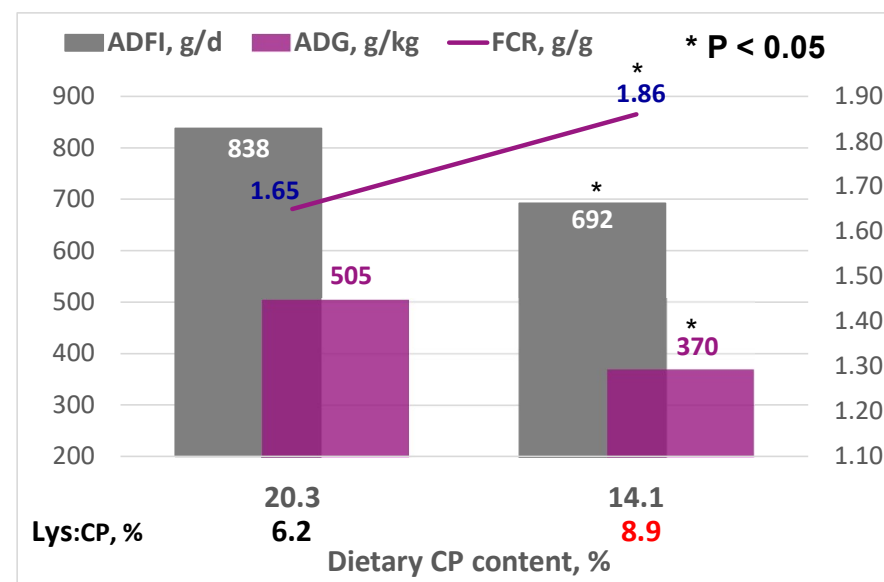
Reduction of dietary CP (6%-unit) on performance and plasma urea N and selected AA in 14-35 kg pigs

CP, %	14.14		20.27	
Lys:CP, %	8.9		6.2	
Lys	1.26	ratio, %	1.26	Ratio, %
M+C	0.63	50	0.63	50
Thr	0.76	60	0.76	60
Trp	0.2	16	0.2	16
Arg	0.71	56	1.09	87
His	0.30	24	0.44	35
Ile	0.46	37	0.71	56
Leu	1.11	88	1.52	121
Phe	0.56	44	0.81	64
Val	0.54	43	0.72	57

Nutrient composition[#] [% in dry matter]

Crude protein	14.14	20.27
Lysine	1.26	1.26
Methionine + cysteine	0.63	0.63
Threonine	0.76	0.76
Tryptophan	0.20	0.20
Arginine	0.71	1.09
Histidine	0.30	0.44
Isoleucine	0.46	0.71
Leucine	1.11	1.52
Phenylalanine	0.56	0.81
Valine	0.54	0.72

Animals	36 crossbred piglets (initial BW 10.1 kg; 45 d)
Treatments	2 CP levels (20.3, 14.1%) Lys:CP ratio (6.2, 8.9%)
Diets	Corn, SBM, whey, FM, 4 EAA
Formulation	Same SID AA, NE



Effect of reducing CP on performance and gut morphology of 7-10 kg pigs (14 d)

CP, %	Ingredient	ME, MJ/kg	SID Lys, %	EAA level	EAA	EAA:NEAA	SID Lys:CP**, %
23.1	Corn, SBM, whey	13.9	1.30	Adeq.	-	44:56	5.6 (6.3)
18.9	Corn, SBM, whey	13.9	1.30	Adeq.	4	44:56	6.9 (7.5)
17.2	Corn, SBM, whey	13.9	1.30	Adeq.	8	46:54	7.6 (8.1)

*Lowering CP by replacing of SBM with EAA; total Lys:CP in parenthesis.

	Dietary CP, %		
	23.1	18.9	17.2
Feed intake, g/d	357	342	307
ADG, g/d	266 ^a	252 ^{ab}	209^b
FCR, g/g	1.35 ^a	1.36 ^a	1.49^b
PUN (d 14), mg/L	139 ^a	80 ^b	61^c
Villus height, µm			
duodenum	454 ^a	457 ^a	433^b
jejunum	436 ^a	419 ^a	393^b
cecum	399	395	386
Proximal jejunum			
Protein, mg/g mucosa	55.9	54.2	51.8
Lactase, µmol/g protein	39.7 ^a	35.6 ^a	29.5^b
Sucrase, µmol/g protein	54.2 ^a	50.7 ^a	43.7^b

^{a,b,c} Within a row, values with different letters are different ($P < 0.05$).

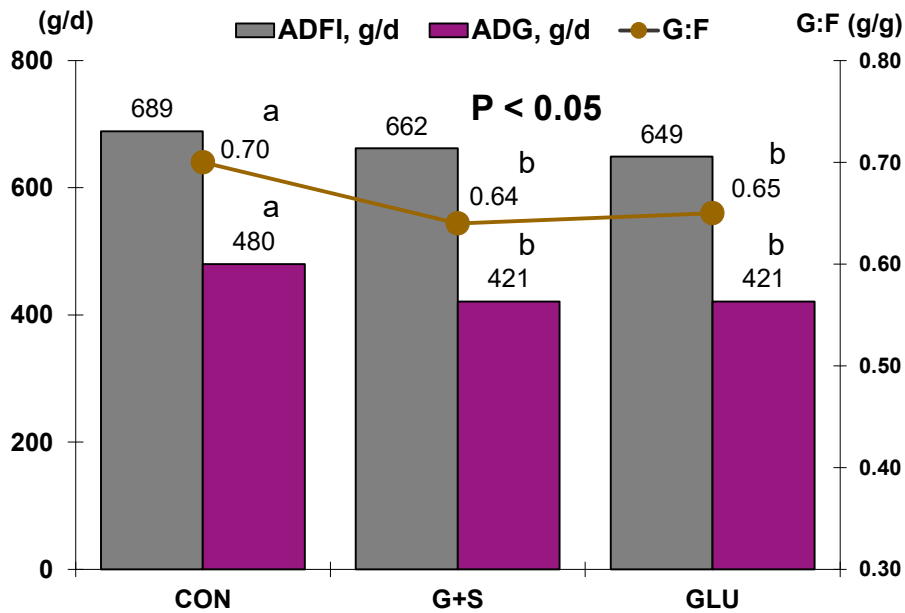
Yue and Qiao (2008)
Livestock Sci

Reducing dietary at > 8% Lys:CP may be deficient in NEAA affecting performance of 6-23 kg pigs (35 d)

104 weaned pigs (initial BW 6.3 kg; 35 d; 3 phases); 24 pigs were euthanized to collect skin samples (collagen)

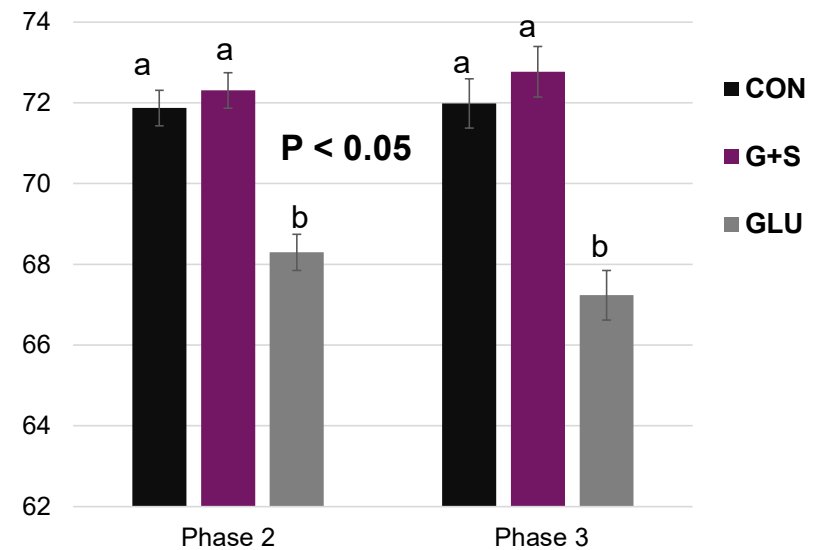
3 diets (CON high CP; Low CP¹ (12 CAA + Gly+Ser); Low CP¹ (12 CAA + Glu); similar NE and EAA N:total N (0.48)

¹ Lowering CP by replacing SBM with free AA.



CP, %	22.3	17.0	17.0
SID Lys:CP, %	6.3	8.3	8.3

Skin collagen abundance, %



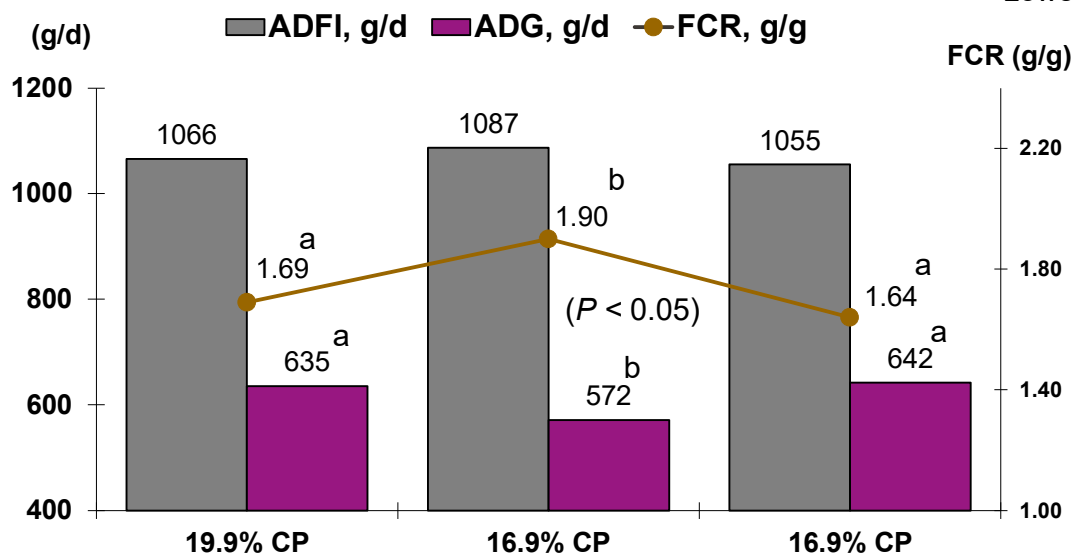
NEAA (N source) was insufficient in the Low CP diets)

Silva et al. (2020; JAS doi:10.1093/jas/skaa023)



Reducing dietary CP and SID Lys levels reduced performance of 9-32 kg pigs (45 d)*

*Lowering CP by replacing fishmeal with CAA.



SID Lys, %	1.24	1.04	1.24
SID Thr, SAA, Trp	----- Same ratios to Lys -----		
NE, kcal/kg	----- 2,500 -----		
Total Lys:CP, %	6.23	6.14	7.23

- Adequate Lys supply in low CP diet maintains pig performance.

Liu et al. (2019; Anim Nutri 5:115-129)

Effect of reducing dietary CP and supplemental AA on growth of 12-22 kg pigs (21-d)

CP, % ¹	NE, MJ/kg	SID Lys, %	EAA level	EAA added	NEAA added ²	EAA:NEAA	SID Lys:CP ³ , %	Elec. balance, mEq/kg
17.4	10.25	0.96	Adeq.	4	-	46:54	5.5 (6.2)	180
15.3	10.30	0.94	Adeq.	5	-	46:54	6.1 (6.7)	180
13.4	10.30	0.92	Adeq.	9	-	49:51	6.9 (7.4)	180
11.6	10.33	0.89	Adeq.	9	-	53:47	7.7 (8.2)	180
12.5	10.55	0.92	Adeq.	10	3	46:54	7.4 (7.8)	180
13.4	10.77	0.93	Adeq.	10	3	42:58	6.9 (7.3)	180

¹Diets 1-4: Grains-SBM-AA based; **Diets 5-6: Grains-AA diets**; ²**Glu, Gly, Pro**; ³Lys:CP in parenthesis.

CP, %	Grains-SBM-free AA diets				Grain-free AA diets	
	17.3	15.3	13.4	11.6	12.5	13.4
Final BW, kg	22.2 ^a	22.2 ^a	21.9 ^a	↓ 20.1 ^b	21.8 ^a	22.3 ^a
Feed intake, g/d	766	775	779	734	810	782
ADG, g/d	450 ^a	454 ^a	442 ^a	↓ 358 ^b	420 ^a	451 ^a
G:F	0.59 ^a	0.59 ^a	0.57 ^a	↓ 0.49 ^b	↓ 0.52 ^b	0.58 ^a

- NEAA (N) can limit >4% CP reduction
- SID Lys:CP ~ 6.9%**

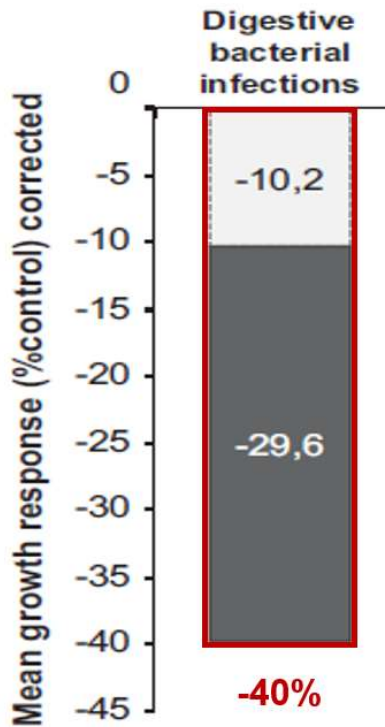
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Post-weaning diarrhea is a major gut health challenge for pigs



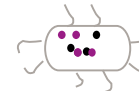
- fraction due to the change in feed efficiency
- fraction due to the change in the maintenance

Meta-analysis
(122 publications)



Bacterial infections of the gut causes 40% reduction in BW gain relative to healthy pigs

Pastorelli et al. (2012)

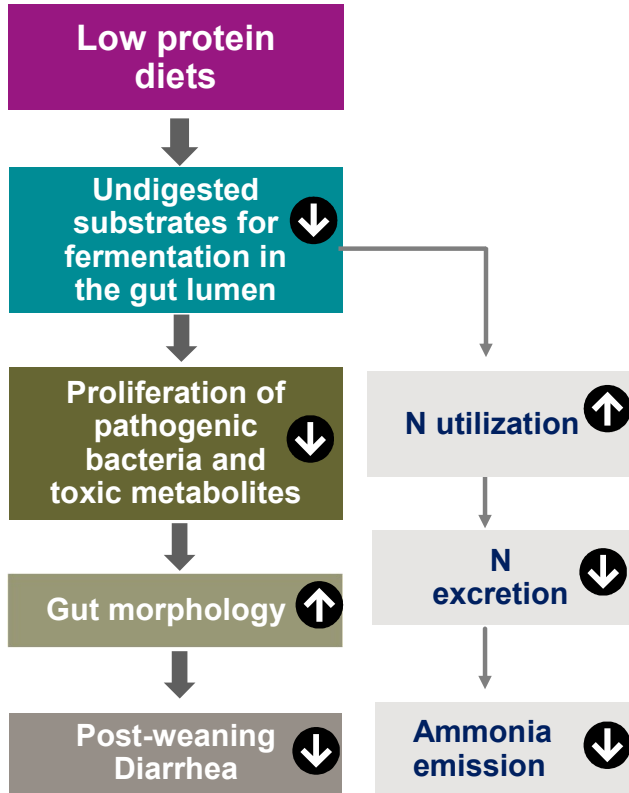


Enterotoxigenic *E. coli* (**F4**, **F18**): main cause of **PWD** in piglets

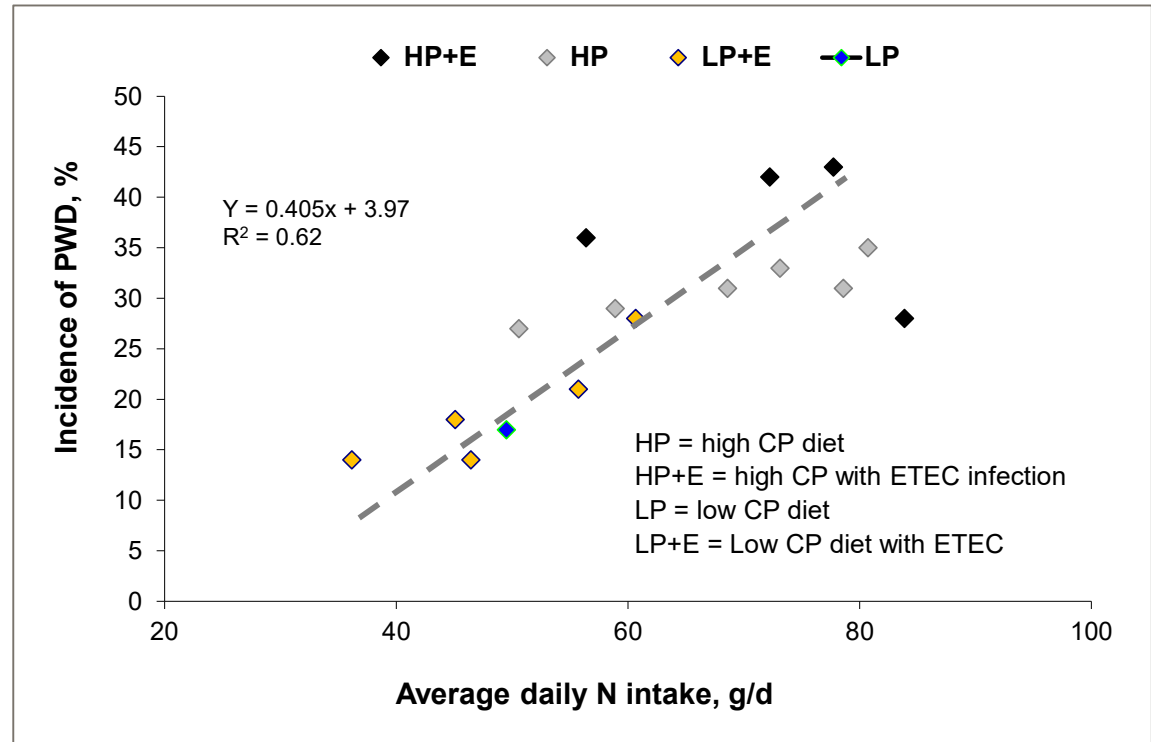
- due to F4: 65% (USA)
- due to F18: 34% (USA)

Zhang et al. (2007)

Lowering dietary CP level directly impact gut health and diarrhea incidence



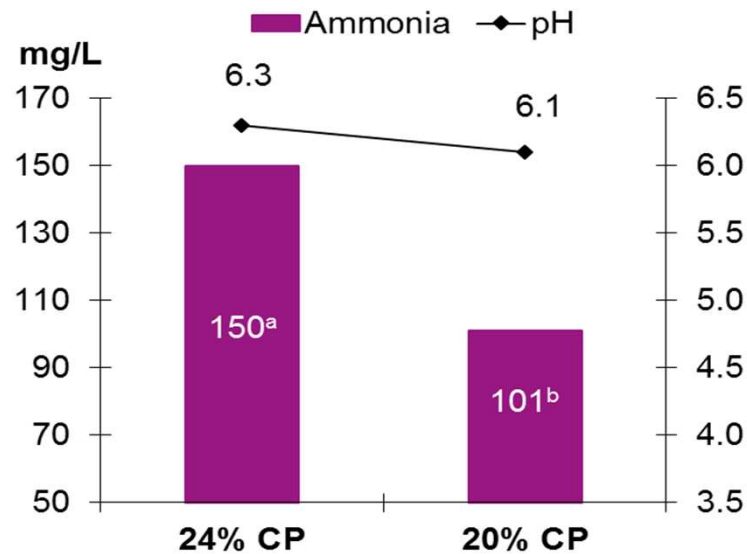
Lowering CP reduces post-weaning diarrhea incidence



Heo (2010)

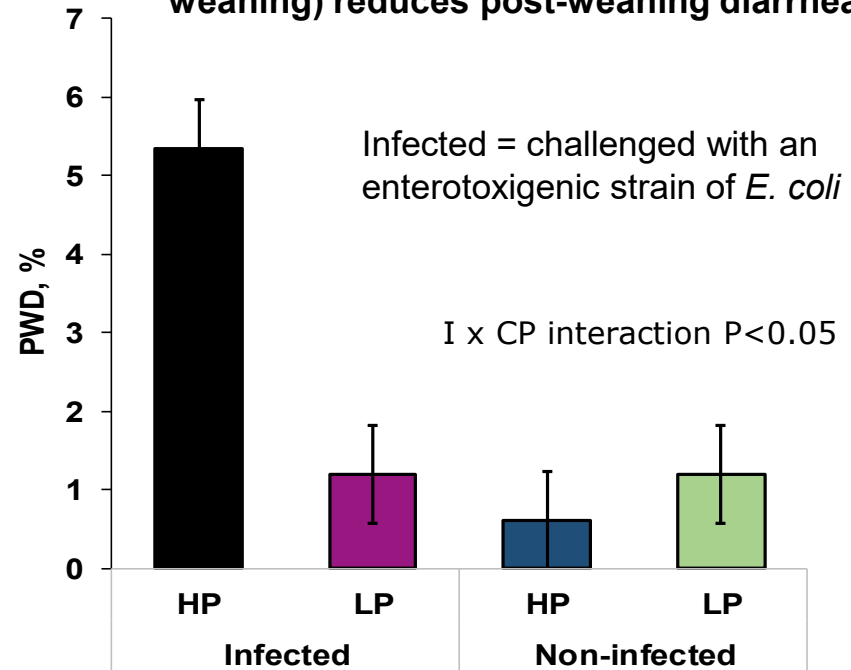
Lowering dietary CP reduces hindgut ammonia concentration and diarrhea incidence in piglets

Effect of diet CP content on ammonia and pH in cecum



Htoo et al. (2007)

Reducing dietary CP from 24 to 18% (2 wk post-weaning) reduces post-weaning diarrhea



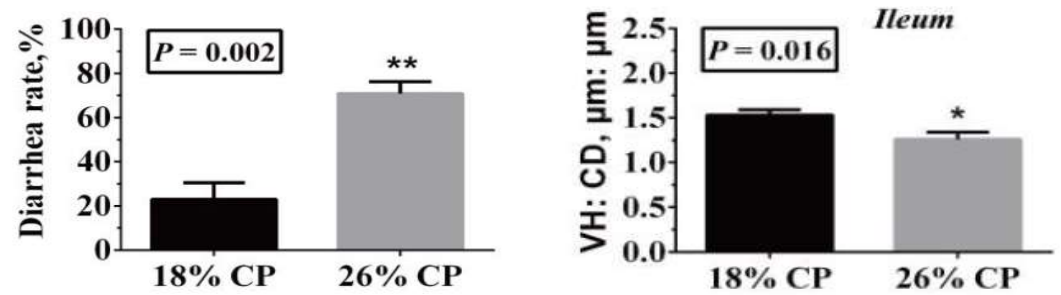
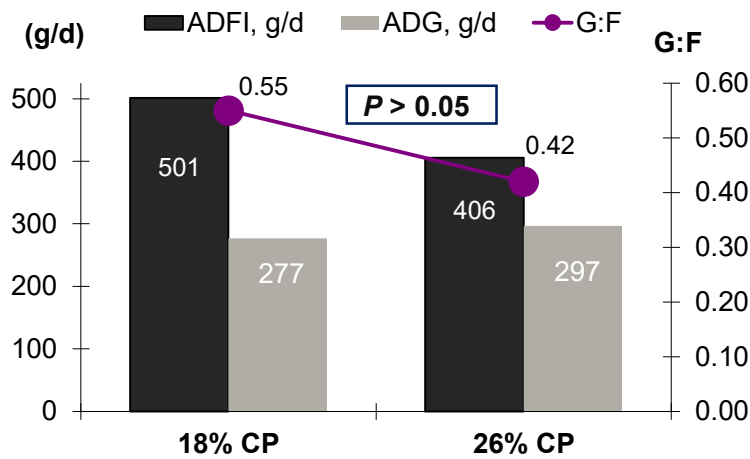
Heo et al. (2010)

Feeding a low CP diet improves intestinal function and reduces diarrhea and intestinal inflammation without affecting performance of weaned pigs (d 0-12)

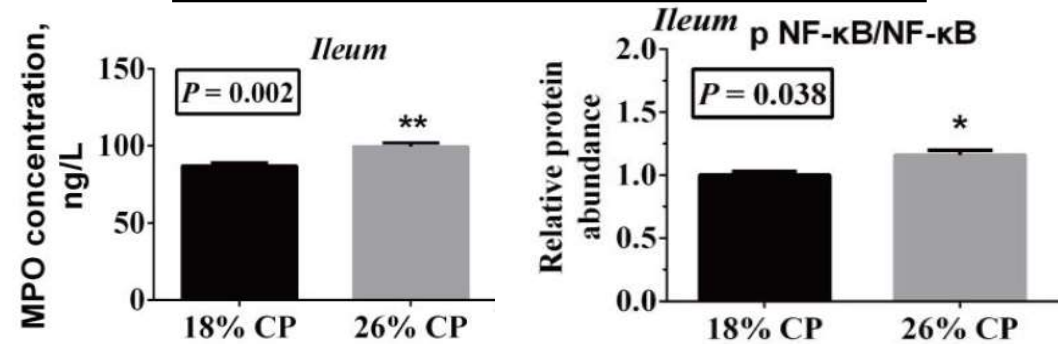
16 Duroc × (Landrace × Yorkshire) weaned pigs (9.7 ± 0.11 kg BW; 8 pigs/trt; fed 4 times/day; 12-d)

1) **26% CP** (corn, soy, 7% FM, whey; without CAA; AA-adeq); 2) **18% CP** (4 CAA; 3% FM; AA-adeq; same Lys, NE)

Yin et al. (2021)
Anim Nutr



Diarrhea rate and VH:CD in ileum



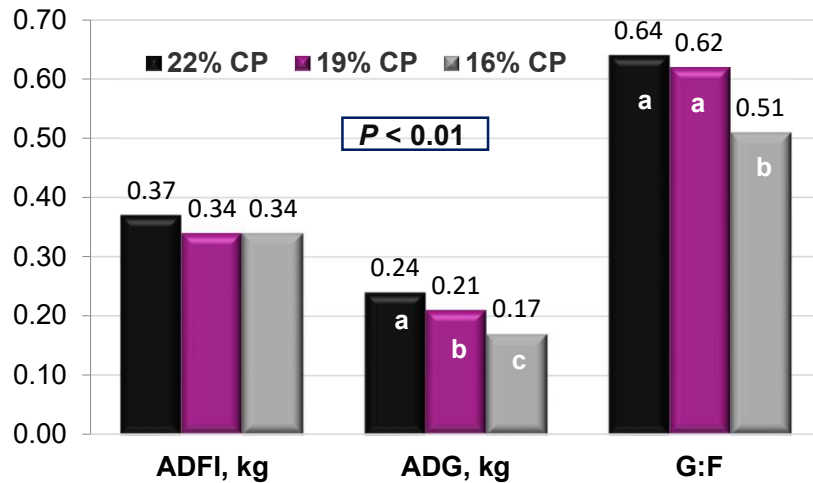
Myeloperoxidase (MPO) and phosphorylation of NF-κB:NF-κB ratio in ileum

Piglets fed low CP diet had lower diarrhea through enhanced morphology which may be due to decreased activation of NF-κB signaling to induce intestinal inflammation.

Effect of dietary CP level on growth performance and indicators of intestinal health in weaned pigs (d 0-28)

180 mixed-sex weaned pigs (5.5 ± 0.88 kg BW; 12 rep pens/trt; 5 pigs/pen; 28-d; 2 phase feeding)
 3 diets: **22% CP** (corn, soy, FM, whey; 3 CAA; AA-adeq); 2) **19% CP** (corn, soy, FM, whey; 7 CAA; AA-adeq; 3) **16% CP** (corn, soy, FM, whey; 3 CAA; AA-def (lower Lys); NRC (2012) basis.

Limbach et al. (2021)
 J Anim Sci



- Piglets fed low CP-AA adeq diet maintain performance and improve fecal score and gut morphology
- Piglets fed low CP-AA def diet reduces performance and impairs gut morphology

Fecal scores and blood characteristics of pigs affected by dietary CP levels

	22% CP	19% CP	16% CP	P-value
Blood urea N, mg/dL(d 27)	9.33 ^c	5.25 ^{ab}	4.83 ^a	0.01
IgG, mg/mL (d 27)	4.41	4.65	4.70	0.98
Haptoglobin, mg/mL (d 27)	0.70	0.37	0.88	0.43
Fecal score (d 1-28)	1.52 ^a	1.36 ^{ab}	1.19 ^b	0.02

Intestinal morphology of pigs affected by dietary CP levels

	22% CP	19% CP	16% CP	P-value
Ileum				
Villous height, μm	278 ^b	328 ^a	301 ^{ab}	**
Crypt depth, μm	213	192	210	ns
VH:CD	1.44 ^b	1.85 ^a	1.54 ^{ab}	**
Jejunum				
Villous height, μm	242	207	248	ns
Crypt depth, μm	200 ^a	161 ^b	172 ^b	***
VH:CD	1.22	1.30	1.37	ns

ns: not significant; ** P < 0.05; *** P < 0.001

Effect of dietary CP level on intestinal pH, ammonia, VFA and indicators of intestinal health in weaned pigs

pH, ammonia and VFA of pigs affected by dietary CP levels (d 12)

Limbach et al. (2021)
J Anim Sci

	22% CP	19% CP	16% CP	P-value
pH				
Stomach	2.90	2.97	3.43	ns
Ileum	7.07 ^a	6.99 ^{ab}	6.68 ^b	**
Colon	6.70 ^a	6.52 ^b	6.69 ^a	*
Ammonia, mg/g				
Cecum	0.20	0.17	0.19	ns
Colon	0.34	0.31	0.31	ns
Total volatile fatty acids, mg/g				
Cecum	131	118	137	ns
Colon	120	120	118	ns

ns: not significant; * P < 0.10; *** P < 0.05

- Piglets fed low CP-AA adeq diet maintain performance and improve fecal score and gut morphology
- Piglets fed low CP-AA def diet reduces performance and impairs gut morphology

Gene expressions of major cytokines in ileal mucosa of pigs (d 12)

	22% CP	19% CP	16% CP	P-value
Pro-inflammatory				
<i>TNF-α</i>	1.04	1.15	1.19	ns
IL-8	0.87 ^{ab}	1.07 ^a	0.69 ^b	**
<i>IFN-γ</i>	1.46 ^a	1.04 ^{ab}	0.69 ^b	**
Anti-inflammatory				
<i>IL-10</i>	0.84 ^a	0.94 ^b	0.98 ^b	*
<i>TGF-β</i>	0.86 ^a	0.92 ^{ab}	1.09 ^b	*
Gut-protective proteins				
OCLN	1.08 ^a	1.09 ^a	0.63 ^b	**
ZO-1	0.96 ^{ab}	1.01 ^a	0.88 ^b	*
MUC2	1.23 ^a	1.27 ^a	0.73 ^b	**

TGF-β, transforming growth factor-beta; *IFN-γ*, interferon gamma; *OCLN*, occludin; *ZO-1*, zonula occludens-1; MUC2, mucin 2.

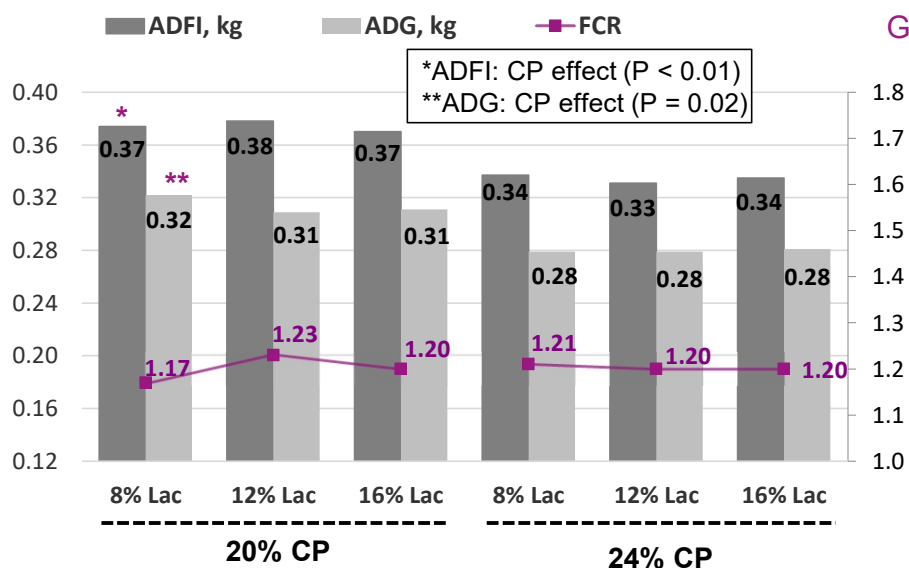
Effect of dietary CP and lactose levels on growth performance and intestinal morphology of weaned pigs (d 0-14)

144 mixed-sex weaned pigs (7.2± 0.97 kg BW)

2 x 3 factorial; 2 CP (20 vs 24%) and 3 lactose level (8, 12 and 16%); 8 rep/trt; 3 pigs/pen; 14-d)

[24% CP (without CAA); 20% CP (4 CAA); lactose was replaced with starch; same SID Lys and ME; 0.3% ZnO]

Soares et al. (2020)
Anim Sci



Gut morphology and tight junction proteins in jejunum of pigs at 14 d post-weaning)

Item	20% CP			24% CP			P-values		
	Lactose, %			Lactose, %			CP	LAC	CP x LAC
	8	12	16	8	12	16			
Villous height: crypt depth (VH:CD), μm									
Duodenum	1.99	2.38	2.40	1.94	2.41	2.37	ns	***	ns
Jejunum	2.56	2.71	2.86	2.24	2.64	2.74	**	***	ns
Ileum	2.74	2.43	2.55	2.23	2.62	2.69	ns	ns	***
Gene expression of tight junction proteins in the jejunum									
Occludin	2.29	3.46	3.04	2.33	3.10	2.77	ns	**	ns
ZO-1	1.74	2.15	2.13	1.21	2.27	1.79	ns	**	ns

ns: not significant; ** P < 0.05; *** P < 0.001

Feeding low CP diet increase feed intake and BW gain of weaned pigs.
Inclusion of lactose at 12% may improve intestinal morphology and integrity.

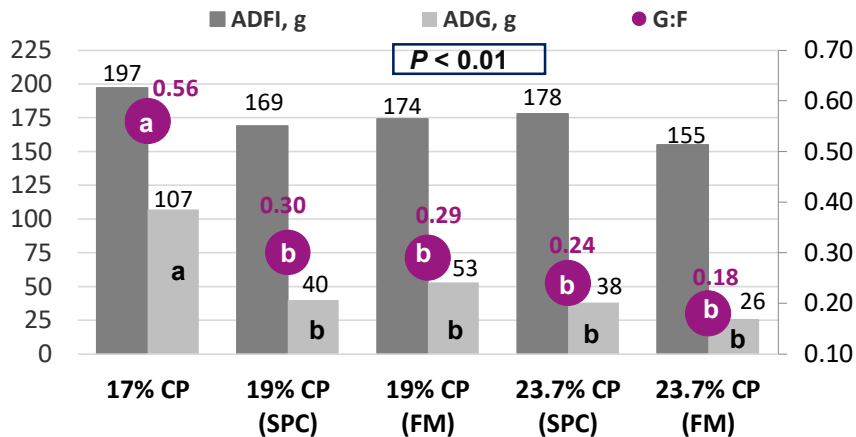
Effect of source and level of protein on performance and intestinal morphology of weaned pigs fed AGP-free diets (d 0-14)

150 barrow weaned pigs (6.0 ± 0.14 kg BW)

5 diets: 1) **LCP** (17% CP; corn, SBM, 3% FM, whey; 8 CAA; AA-adeq); 2) **19% CP** (adding 7.4% SPC*; 6 CAA); 3) **19% CP** (7.4% FM**; 6 CAA); 4) **24% CP** (adding 13.4% SPC; 1 CAA); 5) 24% CP (16.2% FM; no CAA)

Wu et al. (2015)
Anim Sci

*SPC, soy protein concentrate; **FM, Fish meal



Diarrhea rate, plasma urea N and gut morphology of pigs at 14 d post-weaned

Item	CP, %					P-values		
	17	19 (SPC)	19 (FM)	23.7 (SPC)	23.7 (FM)	Diets	Source	Level
Diarrhea rate, %	25 ^c	47 ^b	40 ^b	54 ^a	56 ^a	***	ns	***
PUN, mM	1.9 ^c	3.7 ^b	3.7 ^b	5.0 ^a	5.1 ^a	***	ns	***
Villous height: crypt depth								
Duodenum	2.01 ^a	1.32 ^c	1.59 ^b	1.21 ^c	1.31 ^c	***	***	***
Jejunum	2.25 ^a	1.39 ^{bc}	1.71 ^b	1.16 ^c	1.15 ^c	***	§	***
Ileum	1.73	1.46	1.58	1.40	1.41	ns	ns	ns

Pro-inflammatory cytokines and tight junction proteins in jejunum (d 14 d post-weaned)

Item	CP, %					P-values		
	17	19 (SPC)	19 (FM)	23.7 (SPC)	23.7 (FM)	Diets	Source	Level
IL-1β	1.00 ^a	1.36 ^{ab}	1.37 ^{ab}	2.28 ^a	2.19 ^a	**	ns	**
IFN-γ	1.00 ^c	3.01 ^{ab}	2.401 ^b	3.57 ^a	3.40 ^{ab}	***	§	**
ZO-1	1.00 ^a	0.46 ^c	0.77 ^{ab}	0.37 ^c	0.56 ^{bc}	***	***	*
Occludin	1.00 ^a	0.46 ^b	0.71 ^{ab}	0.32 ^b	0.41 ^b	***	§	*

AGP-free diets, feeding 17% CP diet increase performance and decrease incidence of diarrhea of weaned piglets compared to higher (19 or 23.7%) CP groups regardless of protein sources.

ns: not significant; * § = 0.07; * P < 0.05;
** P < 0.01; *** P < 0.001

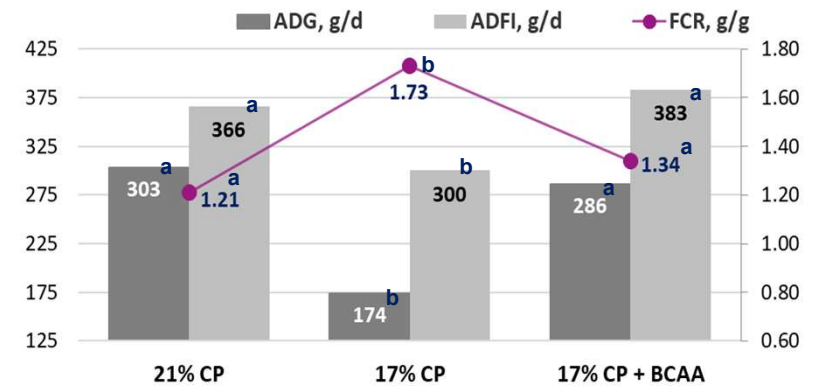
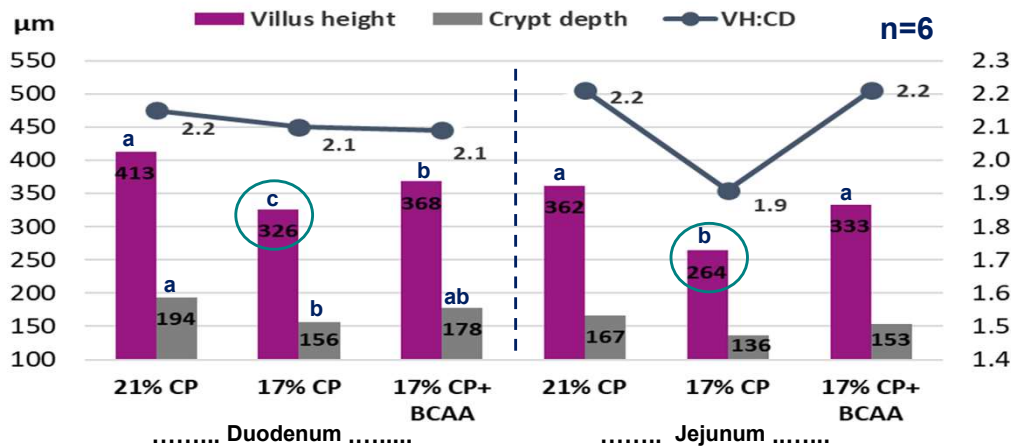
Supplementation with BCAA to a low CP diet improves intestinal function and regulates intestinal expression of AA transporters in weaned pigs

108 weaned pigs (8.0 kg BW; 6 pigs/pen; 6 pens/trt; 14-d)

Ile/Leu/Val:Lys ratios: 58/100/73%

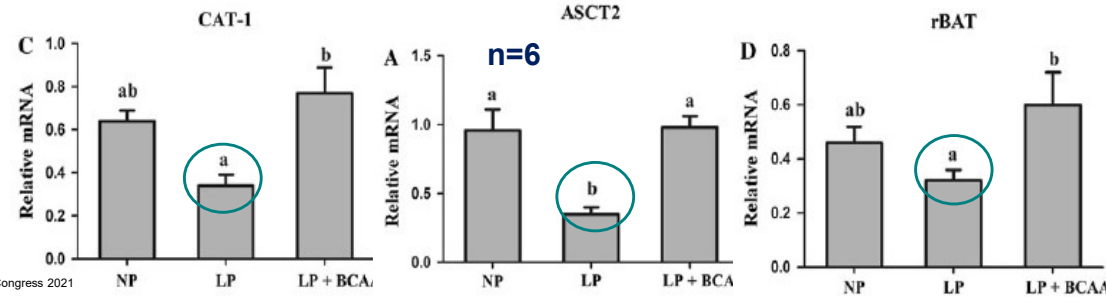
3 diets (21% CP (AA-adeq) ; 17% CP (BCAA-def) ; 17% CP + 0.19% L-Ile, 0.10% L-Leu, 0.34% L-Val (AA-adeq.)

Zhang et al. (2013)



Selected AA transporter mRNA expression in jejunum (after 14-d)

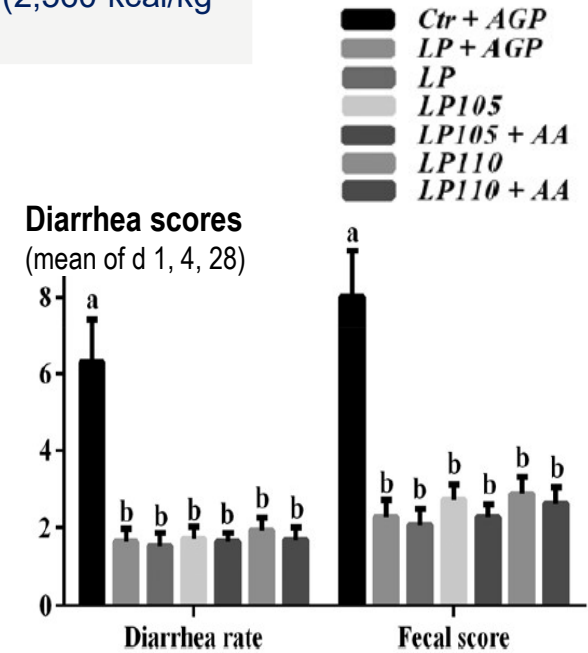
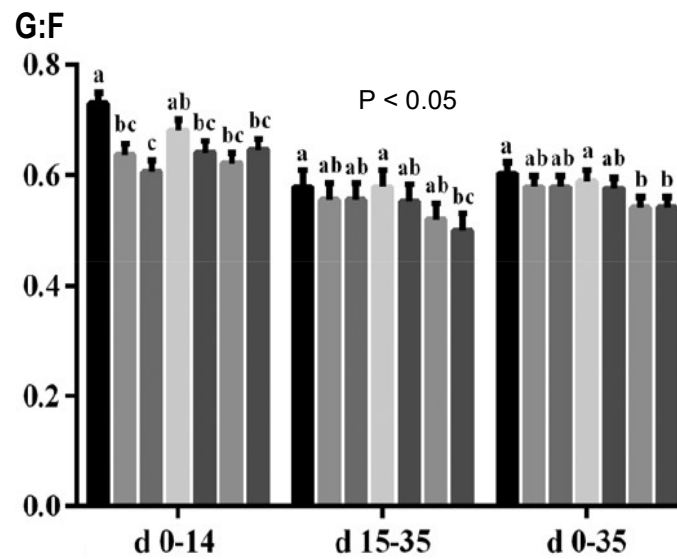
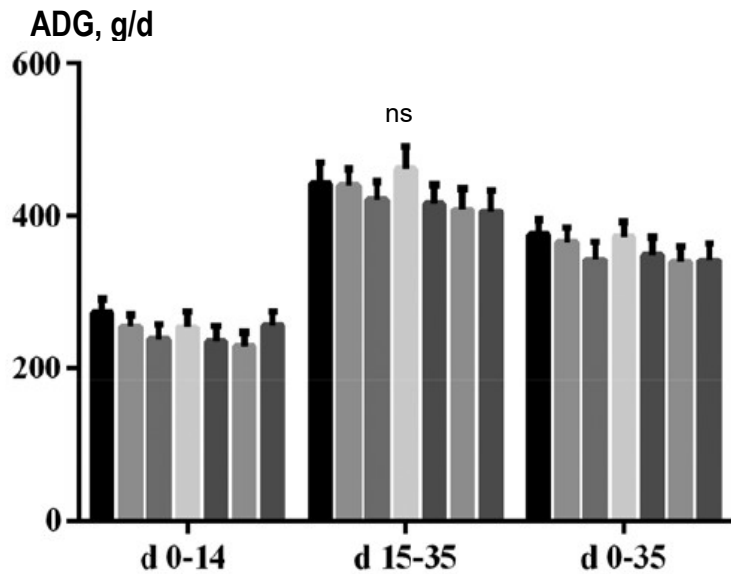
Combined supplementation of BCAA maintains intestinal development and AA absorption by regulating expression of intestinal AA transporters.



Effect of antibiotic-free, low-protein diets with specific AA levels on growth and fecal scores of weaned pigs

	Ctr+AGP	LP+AGP	LP	LP105	LP105+AA	LP110	LP110+AA
Essential AA				+5%	(+6% M+C, Thr, Trp)	+10%	(+6% M+C, Thr, Trp)
SID Lys (CP), %	1.29 (21)	1.30 (17)	1.30 (17)	1.37 (17)	1.37 (17)	1.43 (17)	1.43 (17)

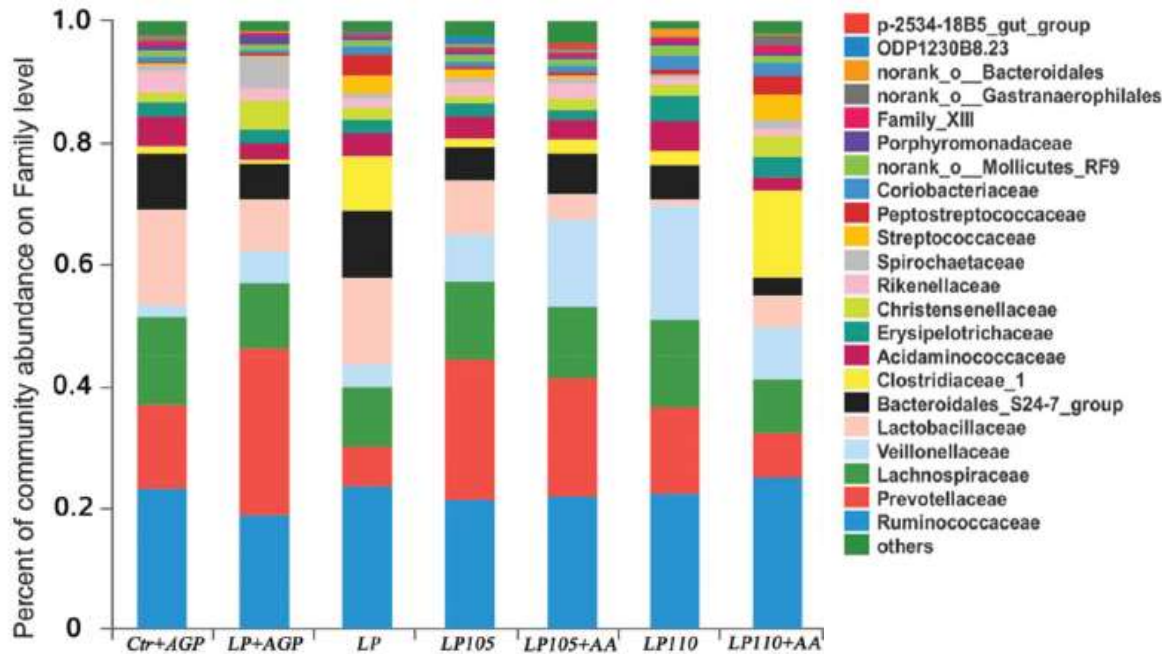
210 weaned pigs (7.2± 0.97 kg BW; 5 rep/trt; 6 pigs/pen; 35-d); SID AA (NRC, 2012); Isocaloric (2,560 kcal/kg NE)



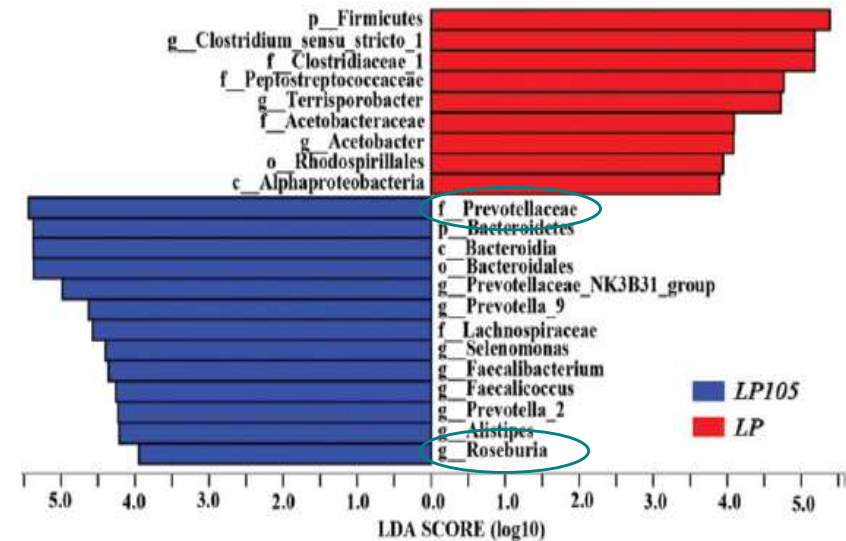
Effect of antibiotic-free, low-protein diets with specific AA levels on composition of fecal microbiota in weaned pigs (16S RNA sequencing)

Fecal bacterial community at the family levels in piglets

Zhou et al. (2020); Food Funct.



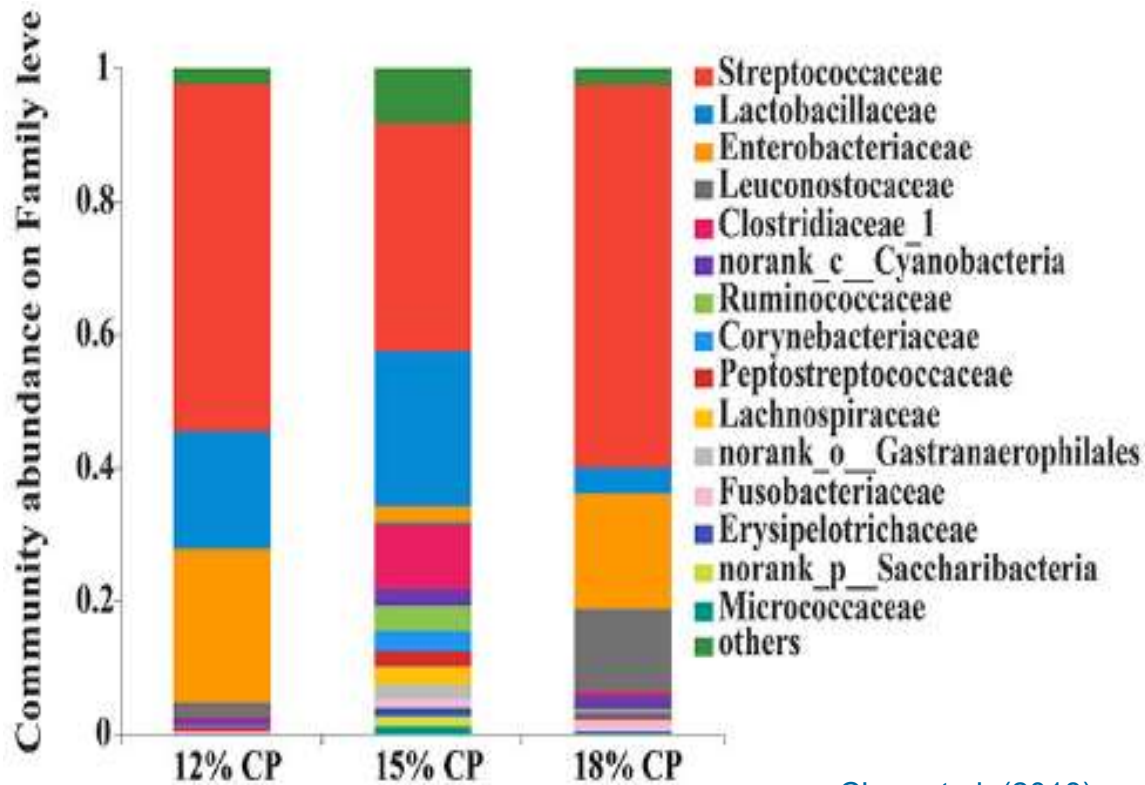
Linear discriminant analysis of fecal microbiota (LP vs LP105 treatment)



LP105 group had increased abundances of Prevotellaceae (positive effects growth performance and immune response; Amat et al. 2020) and Roseburia (butyrate-producing bacteria).

Dietary CP reduction (15% CP) optimizes the microbiota composition in the ileum of growing pigs

Effect of low-protein diets on the distribution of **ileal bacteria** at the family level in growing pigs



Chen et al. (2018)

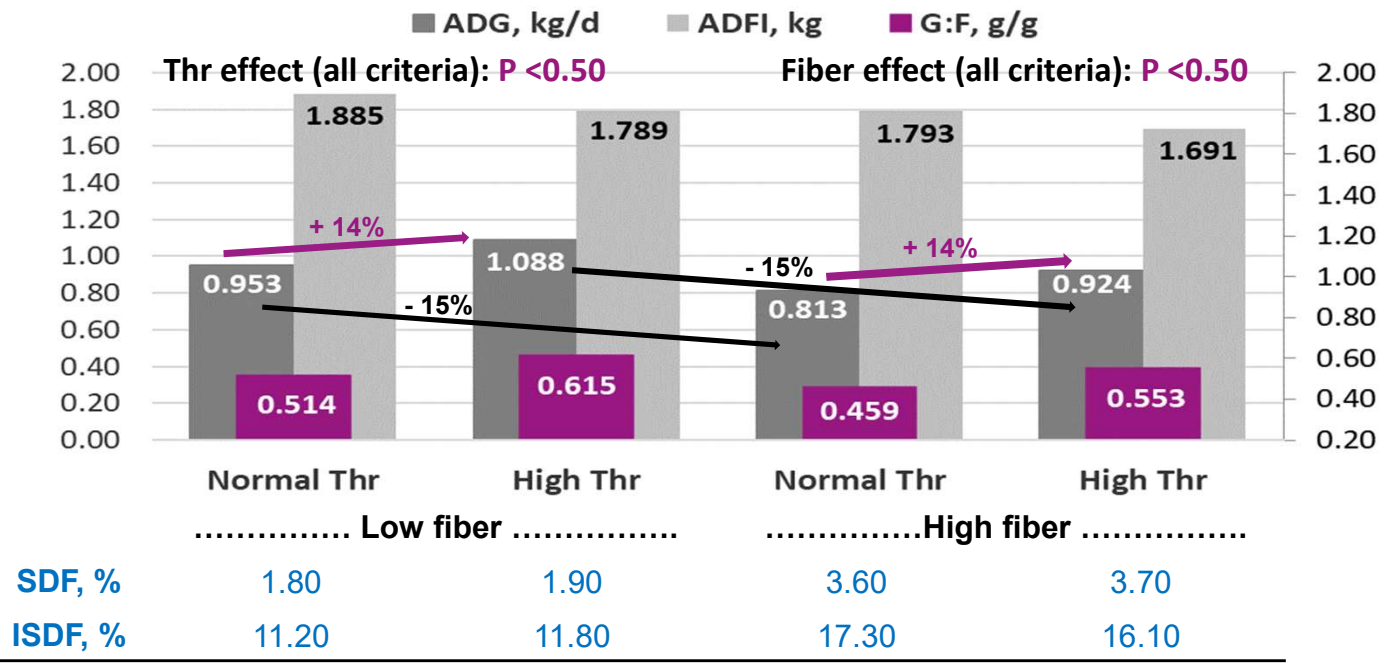
Pigs: 18 barrows (36.5 kg BW)
Diets: Corn-SBM-wheat bran based
 ○ 12% CP: Deficient in Ile, Val, Phe
 ○ 15% CP: adequate in all AA
 ○ 18% CP: adequate in all AA
 Ileum sample: **after 30 d**

Ileum

- **Streptococcaceae: 53, 35 and 58%** (12% and 15% to 18% CP diets)
- **Enterobacteriaceae** (*E. coli*, *Salmonella*) was lower in 15% CP diet.
- **Lactobacillaceae: 18, 24 and 4%** (12% and 15% to 18% CP diets)
- Reducing dietary CP increases **Latobacillus:Coliforms.**

Effect of increasing Thr supply in low CP diets above requirement on performance of *Salmonella typhimurium* challenged pigs fed high fiber diets

Animals	128 piglets (22.6 kg BW; 4 pigs/pen; 21-d study)
Diets	2 × 2 factorial (2 Thr (normal or + 20%) × 2 fiber level (Low or High (10% SBP + 5% wheat bran); 17.5% CP)



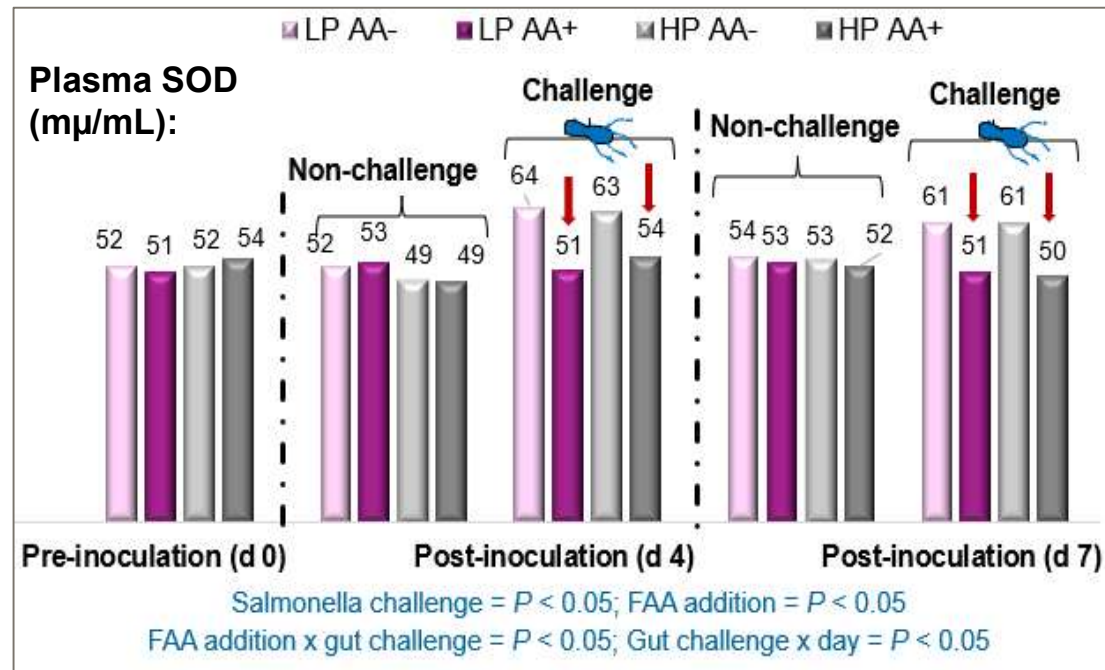
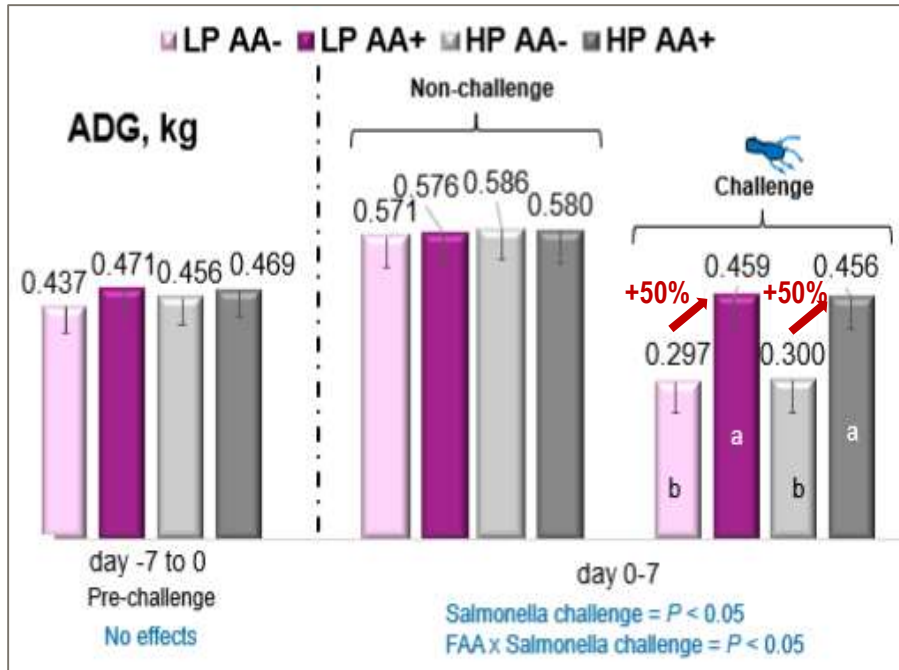
Wellington et al. (2019)
J Anim Sci

High DF reduced pig performance during an enteric disease challenge, however extra Thr (above requirement) improved pig performance regardless of the DF content.



Increasing FAA supply in both high and low CP diet reduces negative effect of *Salmonella* challenge on ADG and antioxidant capacity in weaned pigs

Animals	64 piglets (13.9 kg BW; 14-d study)
Diets	2 × 2 × 2 factorial; CP (LP (16%) vs. HP (20%)); AA (FAA- (NRC) vs. FAA+ (+20% Thr, Met, Trp)); enteric challenge (Non-challenge vs. Challenge (orally inoculation; <i>Salmonella typhimurium</i> after 7 d))

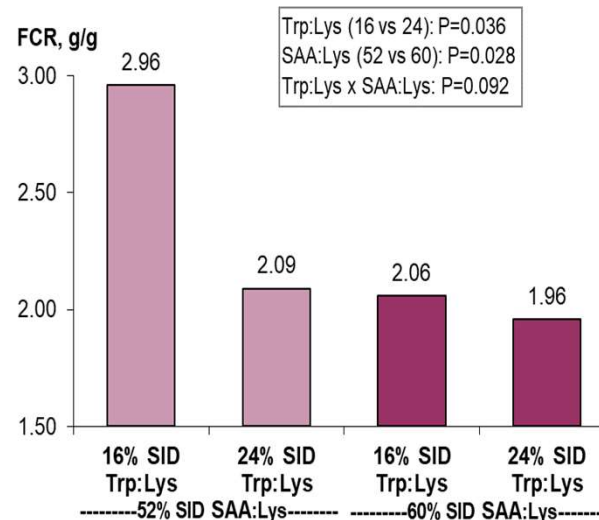
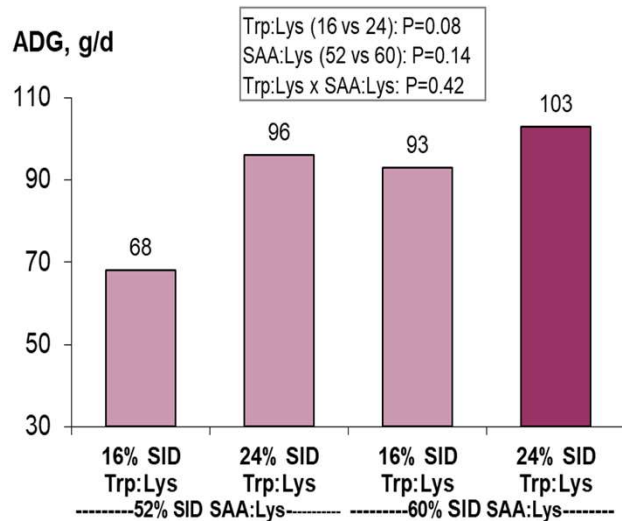


Effect of dietary Trp and SAA addition to moderate CP diets on performance and immune response of weaned pigs challenged with *E. coli* on day 5, 6 and 7 post-weaning

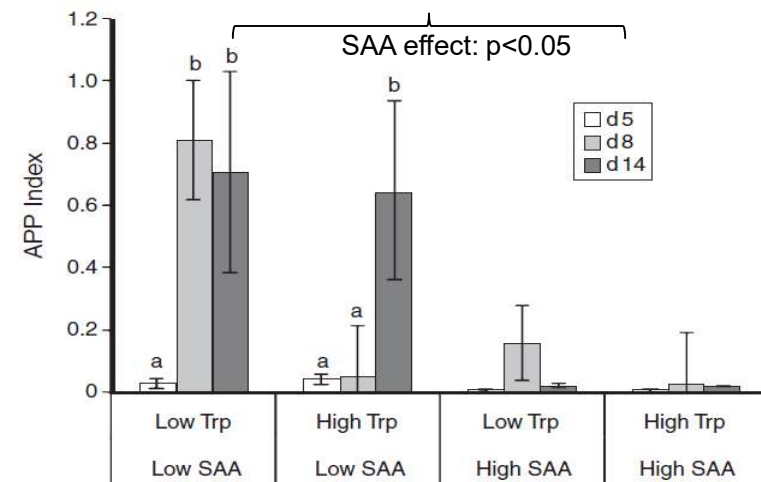
96 male pigs [6.2 ± 0.78 kg; weaned at 21 d; 12 replicate pigs (1 pigs/pen)/trt]

Wheat-barley-full-fat soybean (AGP-free) based diets (Lys was second limiting; SID Lys: 1.25%; 20% CP)

2 x 2 factorial [SID SAA:Lys (52 vs. 60% x SID Trp:Lys (16 vs. 24%)]; 14 d (*E. coli* challenge on d 5, 6 and 7 post- weaning)



Acute-phase index (positive APP:negative APP)*



ADG and FCR of *E. coli* challenged pigs were optimized at higher Trp:Lys and SAA:Lys ratios.

*APP Index = (C-RP · PigMAP)/ApoA1

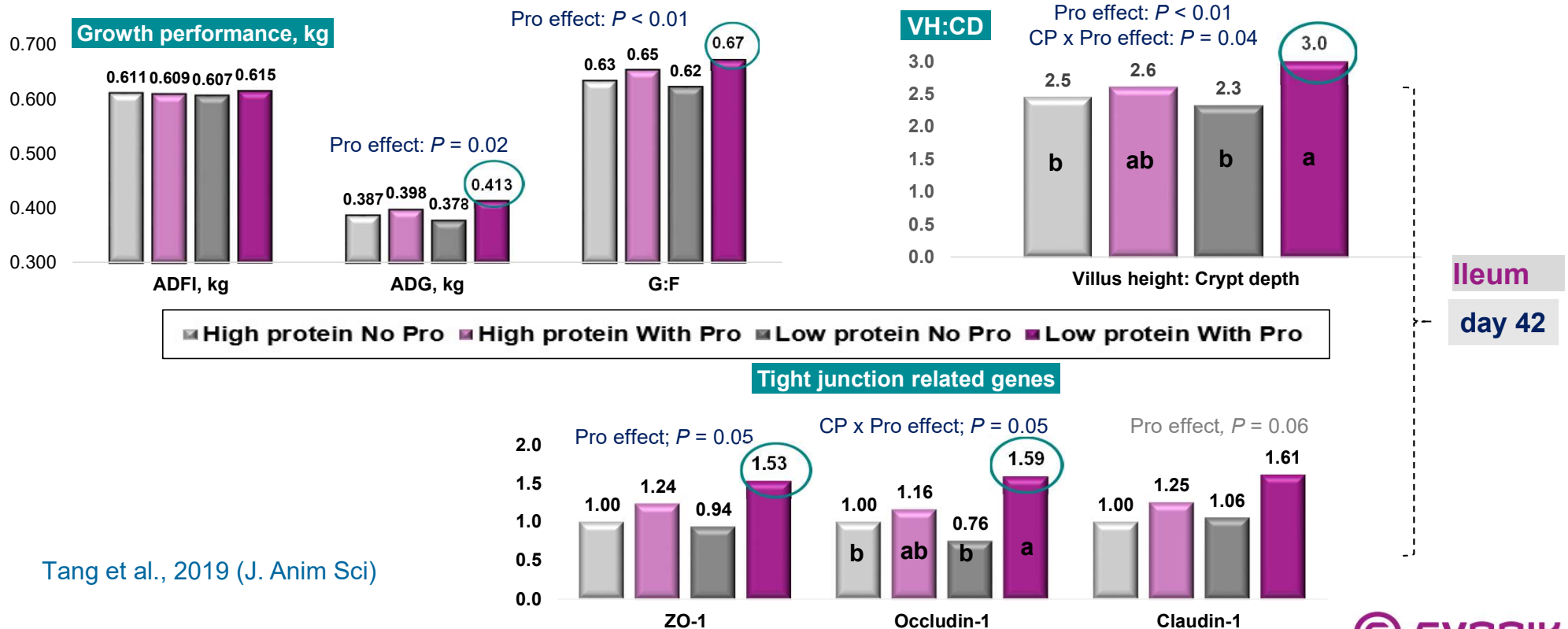
Suppl Trp of reduced inflammatory response in the acute phase, while SAA suppl reduced inflammatory response in the acute- and adaptive-phase.

Capozzalo et al. (2017) Animal Prod Sci

Dietary CP reduction combined with addition of *B. subtilis* probiotic (GutCare®) synergistically increase performance and maintain gut barrier function

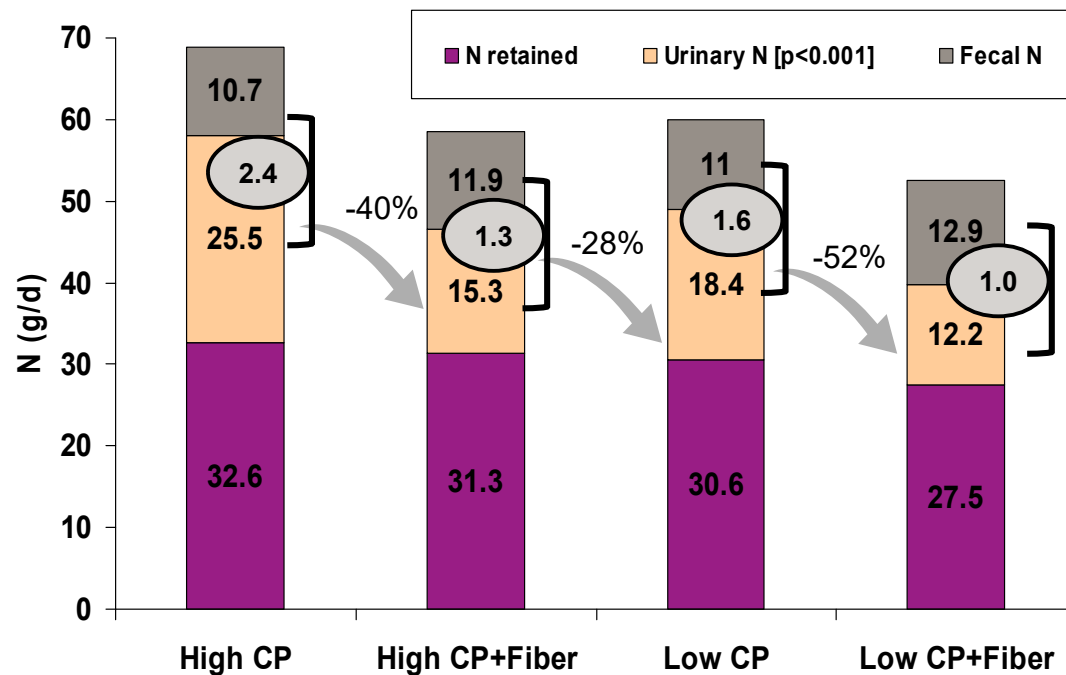
72 weaned piglets (25 d old; 7.6 ± 0.55 kg BW; 42 d trial; 2 phases)

4 diets: 2 × 2 factorial; **CP (High, 20%) vs. Low, (17.5%); Pro (0 or 500 g/t; *B. subtilis* DSM32315; 2 × 10⁹ CFU/g.**



Tang et al., 2019 (J. Anim Sci)

Reduction of dietary CP reduced urine N excretion and fermentable fiber shifted N excretion from urine to feces in 24 kg pigs



Diets:

High CP = 19% CP

High CP + Fiber = 18% CP + 15% soybean hulls*

Low CP = 16% CP

Low CP + Fiber = 15.6% CP + 15% soybean hulls

Design: N balance

*Soy hulls = Fermentable fiber source

Zervas and Zijlstra, 2002 (J. Anim Sci)

Fermentable protein (fCP) and fermentable fiber (fCHO) level on major bacterial groups, pH, SCFA and microbial metabolites in proximal colon of weaned pigs

32 mixed-sex weaned pigs (8.0± 0.22 kg BW; 4 rep/trt; 2 pigs/pen; 20-d)

Pieper et al. (2012); J Nutr

2 x 2 factorial; low of high fCP* (14.5 vs 20.0%) x low or high fCHO** (14.5 vs 17.2% TDF)

* 20% heat-damage SBM; ** 8% wheat bran + 5% SBP

	low fCP		high fCP		P-value		
	low fCHO	high fCHO	low fCHO	high fCHO	fCHO	fCP	fCHO x fCP
Bacterial counts	log cfu/g digesta						
Lactobacilli	8.6 ^a	8.1 ^b	8.3 ^{ab}	8.6 ^a	ns	ns	*
Bacteroides	10.2	10.9	10.6	10.7	*	ns	ns
<i>Cl. leptum</i>	9.8	10.1	9.9	10.2	**	*	ns
<i>Cl. coccooides</i>	10.2	10.8	10.4	10.8	**	0.22	ns
pH	6.1	6.0	6.0	5.8	ns	ns	ns
SCFA (mmol/kg digesta)	95.1	98.3	119.5	111.0	ns	**	ns
	molar ratio % of total SCFA						
Acetate	57.3	59.9	58.0	62.7	**	ns	ns
Propionate	27.1	25.1	25.7	23.0	*	§	ns
Butyrate	12.2	12.8	12.6	12.1	ns	ns	ns
	µmol/kg digesta						
Ammonia	7.3	3.2	13.8	9.1	**	**	ns
Putrescine	127	104	234	165	**	**	ns
Cadaverine	125	139	279	169	ns	ns	ns
Spermidine	211	225	274	267	ns	**	ns

Dietary fCHO can affect microbiota composition and ameliorate formation of toxic metabolites in the proximal colon of pigs fed diets with high level of fCP.

ns: not significant; § <0.10 * <0.05; ** <0.01

Effect of dietary CP (AA) and protease level on performance of weaned pigs

Effect of dietary CP and protease level on performance of 7-16 kg weaned pigs (d 0-28) Kim et al., 2021 (J Anim Sci Technol)

	CP level		Protease*			P-value		
	19%	17%	0%	0.3%	0.5%	CP	Protease	CP x Protease
BW (d28), kg	15.7	15.7	15.3 ^a	15.4 ^a	16.5 ^b	ns	**	ns
ADG, g	310	313	296 ^a	300 ^a	340 ^b	ns	**	ns
ADFI, g	435	428	420	421	453	ns	§	ns
G:F	0.71	0.73	0.70 ^a	0.71 ^a	0.75 ^b	ns	*	ns

*from a commercial Korean company

ns: not significant; § <0.10 * ≤0.05; ** ≤0.01

Effect of dietary CP and protease level on performance of 6-14 kg weaned pigs

Perez-Palencia et al., 2021 (J Anim Sci)

	CP level		Protease**		P-value		
	22%	19% (-15% AA)	0%	0.0125%	CP	Protease	CP x Protease
Phase 1 (d 0-14)#							
ADG, g	154	156	160	150	ns	§	ns
G:F	0.69	0.67	0.69	0.67	ns	ns	ns
Phase 2 (d 15-28) †							
ADG, g	455	396	431	420	**	ns	ns
G:F	0.77	0.69	0.73	0.73	**	ns	ns

**from a commercial Canadian company

ns: not significant; § <0.10 ** ≤0.01

Pigs received a common phase 1 diet. † Diets contained 2,500 FTU/kg phytase).

Content

1. Introduction - development of low protein diet concept
2. Effects of lowering dietary CP level on piglet performance and N excretion
3. Mistakes to be avoided when formulating low protein diets – how low we can go?
4. Advances on low protein diet concept to enhance intestinal health and microbiota
5. Conclusions



Conclusions

- For maintaining optimal growth of nursery pigs, low CP diets should be balanced for adequate level of both EAA and NEAA and net energy.
- How far we can lower CP level depends on its SID Lys level in the diet. Keeping ~ 6.9% SID Lys:CP) is useful to keep minimum NEAA level.
- On average, 1%-unit dietary CP ↓ » ~ 9% ↓ N excretion » ~ 3% ↓ drinking water
- Low CP-AA adequate diets is the first step to maintain gut health and lower incidences of diarrhea.
- Reducing dietary CP may act synergistically with functional AA, probiotics, functional fibers, enzymes.
- A holistic concept of combining “low CP diet with these functional feed additives” should be considered as part of “AGP-free” strategy towards sustainable pig production.



Thank you



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