Acute phase proteins as biomarkers of disease and stress in pigs

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PigCHAMP Pro Europa SL

Content

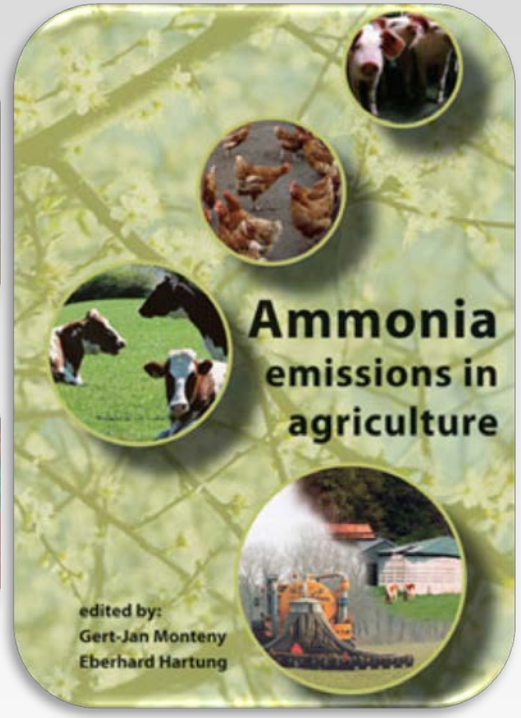
- Background.
  - Physiological basis of stress
  - The acute phase reaction
- Effects related
  - Health
  - Performance
  - Transport
  - Meat
  - Transversal through chain
  - Linkage between stress and acute phase response (non inflammatory effects)
- And from here, where to?

Background

Market price | Feed price | Regulations | Own business

Restrictive and getting worse

- Environment (IPPC-DEI, Gothemburg, Kyoto)
- Animal Welfare (UE 2013, States in USA, Canadá, Australia or companies –Macdonalds-)
- Antiinfectives use restriction
- Food safety



Trust the final product

Background

The potential of the current genetic lines often limited by stressors present in the productive system:

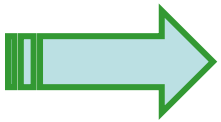
- Social stress
- Mixing
- Non adequate feeding
- Stocking density,
- Temperature
- Pathogens

Stress : biological response of an individual when a threat to homeostasis is perceived

(Moberg, 2000)

Background

- Stress
  - *Does not represent a threat*
  - *Represents a threat (distress)*
    - Welfare
    - Health
    - Productive performance
    - Carcass-meat quality
- Response (Baumann, 1994)
  - Behavior
  - Central Nervous System
  - Neuroendocrine System
  - Immune System



Acute Phase Response

Acute Phase Response (I)

TISSUE DAMAGE

Trauma, wounds, burns, surgery, infection, stress

↓

Release of pro-inflammatory cytokines (IL-1, IL-6, TNFα)

↓

Platelet activation, activation of complement and coagulation pathways, endotelium activation, increase of vasel permeability, release of inflammatory cells

↓

Enhancement of pro-inflammatory cytokines release

↓

Activation of hypothalamic-pituitary-adrenal (HPA) axis

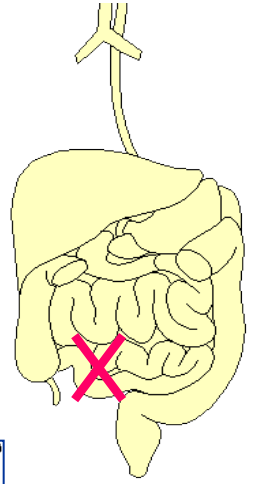
Systemic reaction

Acute Phase response (II)

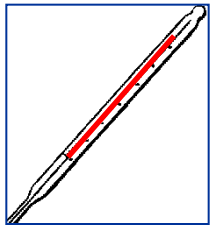
Proinflammatory Cytokines
TNFα
IL-1
IL-6

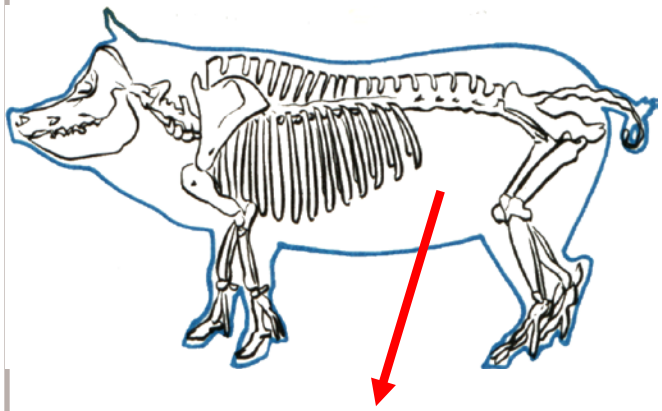
Decrease of food intake

Decrease of intestinal motility and higher mucus production



Fever





Decrease of Vit. A, Zn and Fe serum levels

Osteoclastes activity

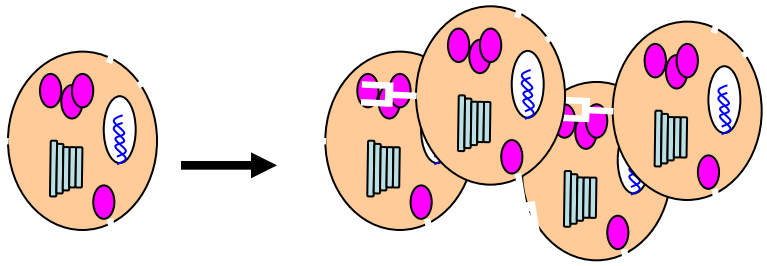
Acute phase response (III)

Proinflammatory cytokines

TNFα

IL-1

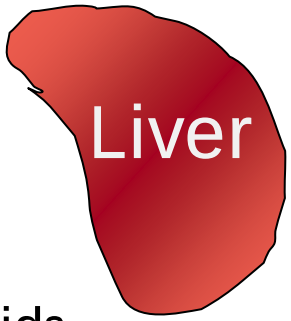
IL-6



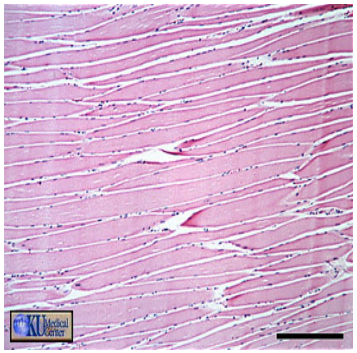
Proliferation and activation of immune cells

Alteration of nutrients metabolism:

- Lipids
- Carbohydrates
- Muscle catabolism



→ Fibrinogen
→ Haptoglobin
→ C-reactive Protein
→ Pig-MAP...



Free Aminoacids

Synthesis of Acute Phase Proteins

IMMUNOLOGICAL STRESS

Metabolic cost
(Hipocrates´s *'ponos'*)

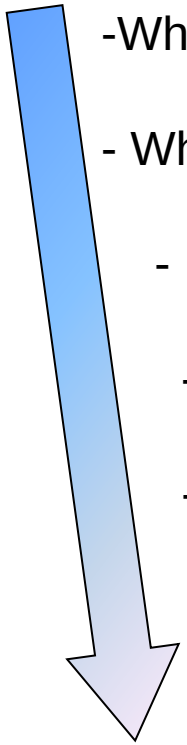
APP functions

- Defense mechanisms / Restoration of homeostasis

  - ✓ *Opsonization and complement activation*
  - ✓ *Neutralization of free radicals*
  - ✓ *Removal of released hemoglobin*
  - ✓ *Neutralization of proteolitic enzimes*
  - ✓ *Participation in regulatory processes*

APP in pigs, from basic knowledge to practical aplication.

Initial studies (mainly from late 90´s)



-Which are the main APPs in pigs

- What does elevate pig APPs?

- Basal levels/Acute phase levels

- Variability (animal/herd)

- Behaviour in commercial conditions

- Availability of assays

Concerted Action
AIR3-CT94-2255

Concerted Action
QLTR-1999-01531
Harmonization
of assays in Europe

Shared cost project
QLK5-CT-2001-02219
APP in pigs

PORK CHAINS

Application of APP in animal production

Measuring APP

- Availability of Assays:

A range of assays adequate for the different APP in different species have been developed.

- Assays should be validated:

Specifity

Accuracy

Precission

Sensibility…

- Importance of the standard:

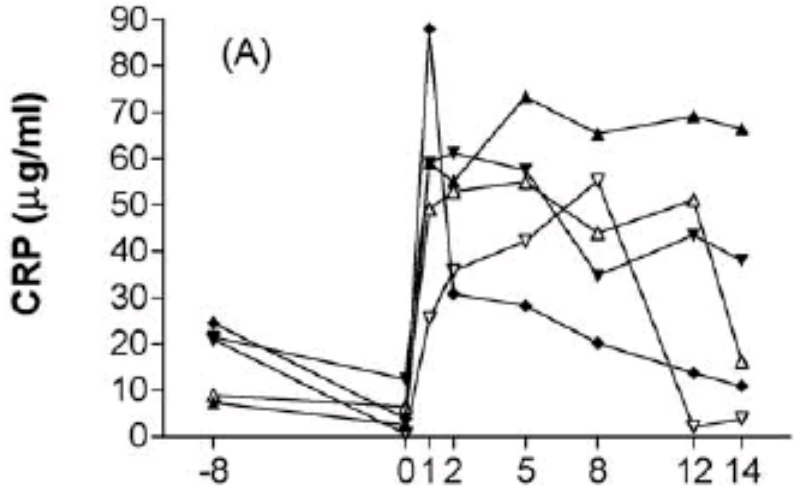
Use of common standards for comparing the results. Species specific.
European Reference Serum for pig APP (European concerted action QLTR-1999-01531)

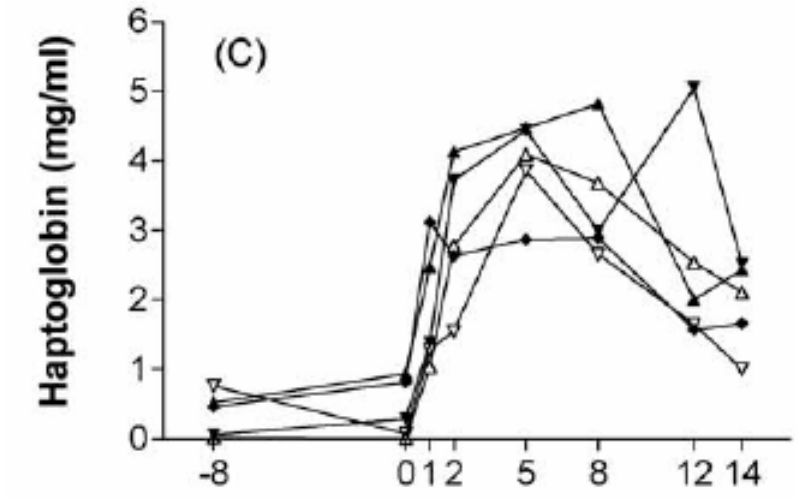
APP in swine health and production

- Reflects the presence of immunological stress
- **That has a cost!**
  - Looses of productive performance
  - Increased susceptibility to acute disease outbreaks

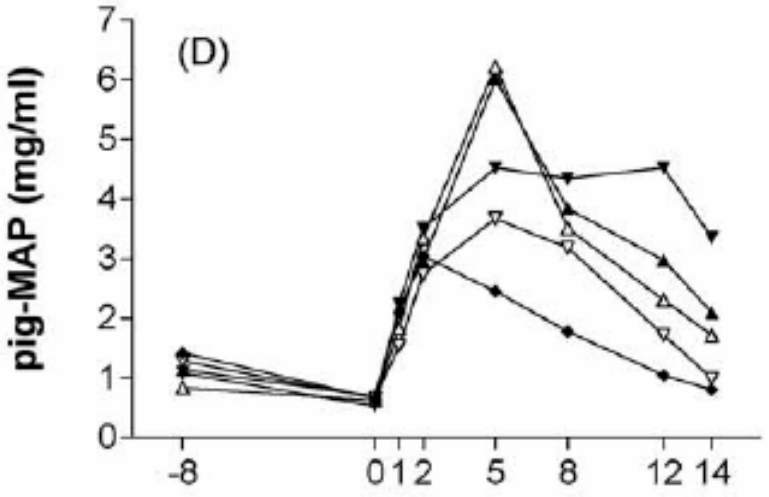
APP's in infectious diseases

APP during a bacterial infection: S. suis





- 5 weeks old SPF pigs.
- Infection with S suis serotype 2 (strain SS02-0119), 10^{10} CFU, on day 0



Sorensen et al. 2006, Vet Immunol Immunopathol 113:157- 68.

APP during a bacterial infection: H. parasuis

Martin AJ et al, Comp Immunol Microbiol Infec Dis 2008

Mean values ± SD of the serum concentration of pig MAP in the experimental groups after infection with a lethal dose of *H. parasuis*.

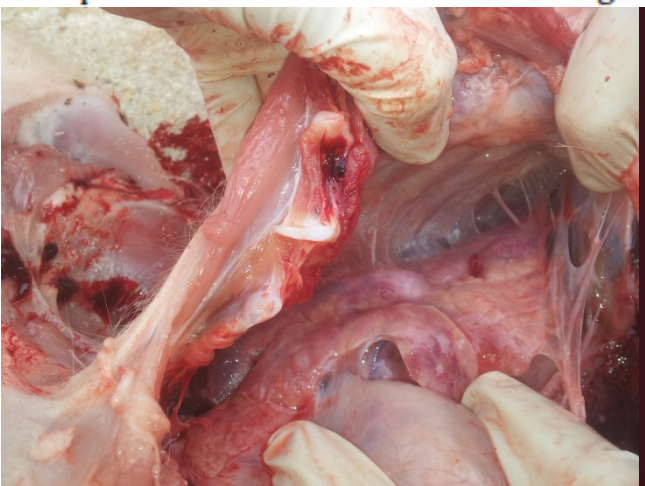
Treatment group[a]	Pig MAP concentration (mg/ml)					
	day 0	day 1	day 3	day 6	day 9	day 13
I	0.6 ± 0.1 $n = 4$	5.7 ± 0.4 $n = 2$ $(P < 0.0001)^b$	16.0 $n = 1$ $(P < 0.0001)^b$	16.0 $n = 1$ $(P < 0.0001)^b$	*	*
II	0.7 ± 0.3 $n = 5$	2.9 ± 2.1 $n = 5$ $(P = 0.0011)^b$	1.8 ± 0.9 $n = 5$ $(P = 0.0766)$	1.1 ± 0.3 $n = 5$ $(P = 0.5526)$	0.8 ± 0.2 $n = 5$ $(P = 0.8083)$	0.8 ± 0.2 $n = 5$ $(P = 0.7872)$
III	0.6 ± 0.1 $n = 4$	4.3 ± 1.7 $n = 4$ $(P < 0.0001)^b$	3.3 ± 0.2 $n = 2$ $(P = 0.0034)^b$	1.8 ± 0.2 $n = 2$ $(P = 0.2048)$	1.3 ± 0.3 $n = 2$ $(P = 0.4166)$	0.9 ± 0.0 $n = 2$ $(P = 0.7314)$
IV	0.6 ± 0.2 $n = 4$	3.1 ± 1.4 $n = 3$ $(P = 0.0006)^b$	1.6 ± 0.1 $n = 2$ $(P = 0.1066)$	1.2 ± 0.2 $n = 2$ $(P = 0.3801)$	0.8 ± 0.1 $n = 2$ $(P = 0.7147)$	0.7 ± 0.0 $n = 2$ $(P = 0.9076)$
V	0.6 ± 0.2 $n = 3$	1.6 ± 0.6 $n = 3$ $(P = 0.2422)$	1.3 ± 0.2 $n = 3$ $(P = 0.4864)$	1.1 ± 0.2 $n = 3$ $(P = 0.6443)$	0.8 ± 0.1 $n = 3$ $(P = 0.8663)$	0.7 ± 0.1 $n = 3$ $(P = 0.9313)$
VI	0.5 ± 0.1 $n = 3$	1.7 ± 0.2 $n = 3$ $(P = 0.1934)$	1.5 ± 0.3 $n = 3$ $(P = 0.2517)$	1.1 ± 0.2 $n = 3$ $(P = 0.5213)$	0.8 ± 0.1 $n = 3$ $(P = 0.7423)$	0.9 ± 0.2 $n = 3$ $(P = 0.6527)$

Pneumonia, differentiation between pleuritis (P-, P+) and cranio-ventral consolidation (M-,M+)

Saco et al, Res. Vet. Sci. (2010)

	Haptoglobin (g/L)		Pig-MAP (g/L)	
	Mean	Interval	Mean	Interval
P–M–	1.31[a]	±0.19	0.63[a]	±0.09
P+M–	1.59[a]	±0.25	0.81[b]	±0.11
P–M+	1.45[a]	±0.23	0.90[b]	±0.12
P+M+	1.91[b]	±0.23	1.04[c]	±0.13
Kruskal–Wallis test	14.99 ($P = 0.002$)		56.775 ($P = 0.000$)	
P–	1.38	±0.15	0.77	±0.08
P+	1.76	±0.17	0.93	±0.08
Mann–Whitney's U	22333.0 ($P = 0.001$)		20987.5 ($P = 0.000$)	
M–	1.45	±0.16	0.72	±0.07
M+	1.68	±0.16	0.97	±0.04
Mann–Whitney's U	23051.5 ($P = 0.033$)		15928.5 ($P = 0.000$)	

Groups with different letter showed significant differences (Kruskal–Wallis test).

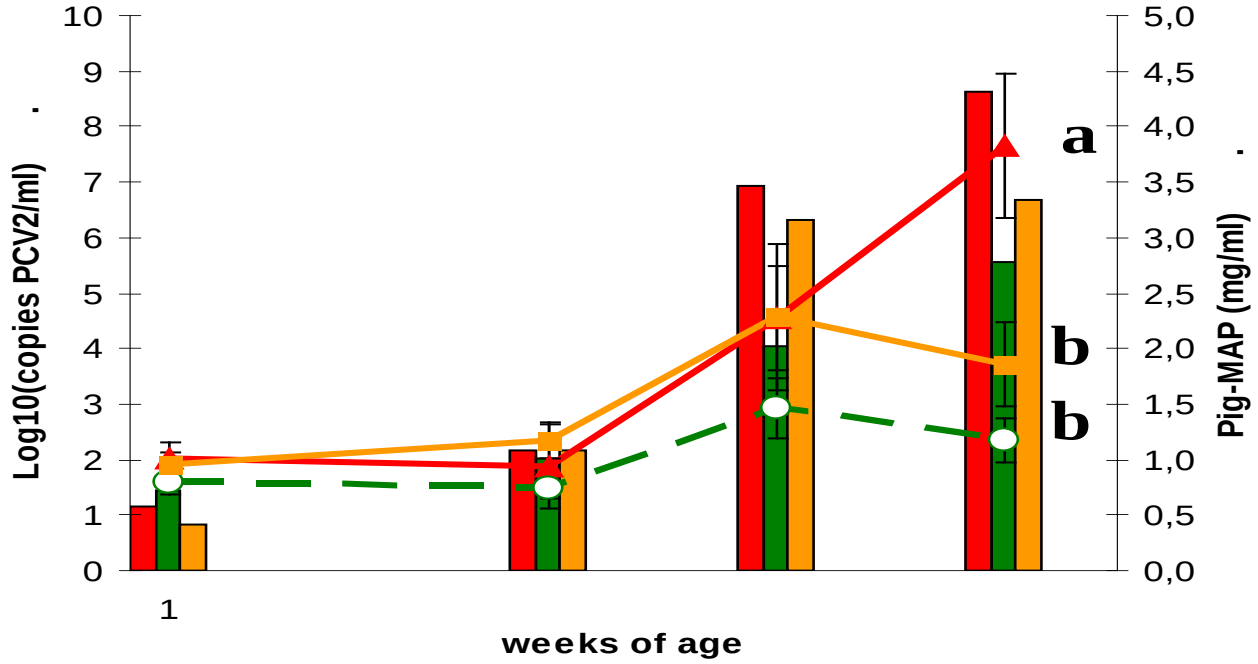


Pneumonia, differentiation between pleuritis (P-, P+) and cranio-ventral consolidation (M-,M+)

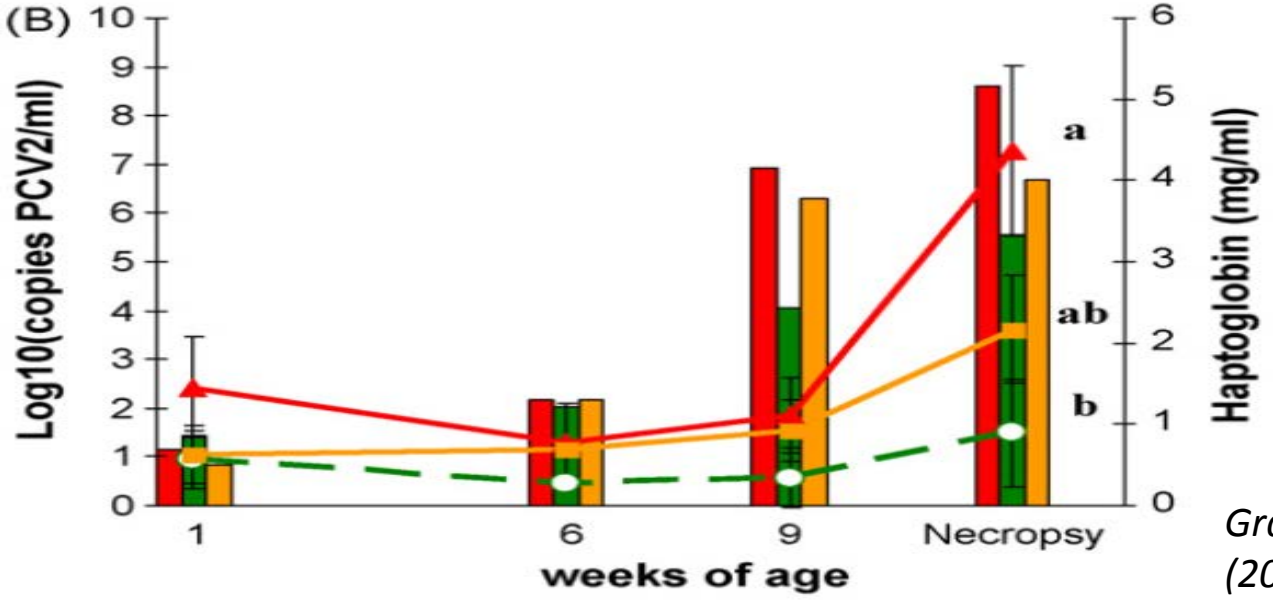
- Pigs with pleuritis (P+) showed higher concentration of APP's (PigMAP, Hp, CRP) than (P-).
- Pigs with higher incidence ov CVPC (M+) showed higher concentrations of PigMAP and Haptoglobin
- Pigs from farms (P+ M+) showed higher concentration of APP's (PigMAP, Hp, CRP) than (P-M-). PigMAP fwas the best biomarker to differenciate these farms.
- PigMAP was the only APP able to discriminate between farms P-M- and farms with prevalence of just one of the lessions (P-M+, P+M-).

Saco et al, Res. Vet. Sci. (2010)

Commercial farms affected by PMWS (PCV2)



7 farms, longitudinal study. Animals euthanised at the PMWS outbreak and classified as:



PMWS (35)
Healthy (29)
Wasted non-PMWS (43)

Grau-Roma et al, Vet. Microbiology (2009)

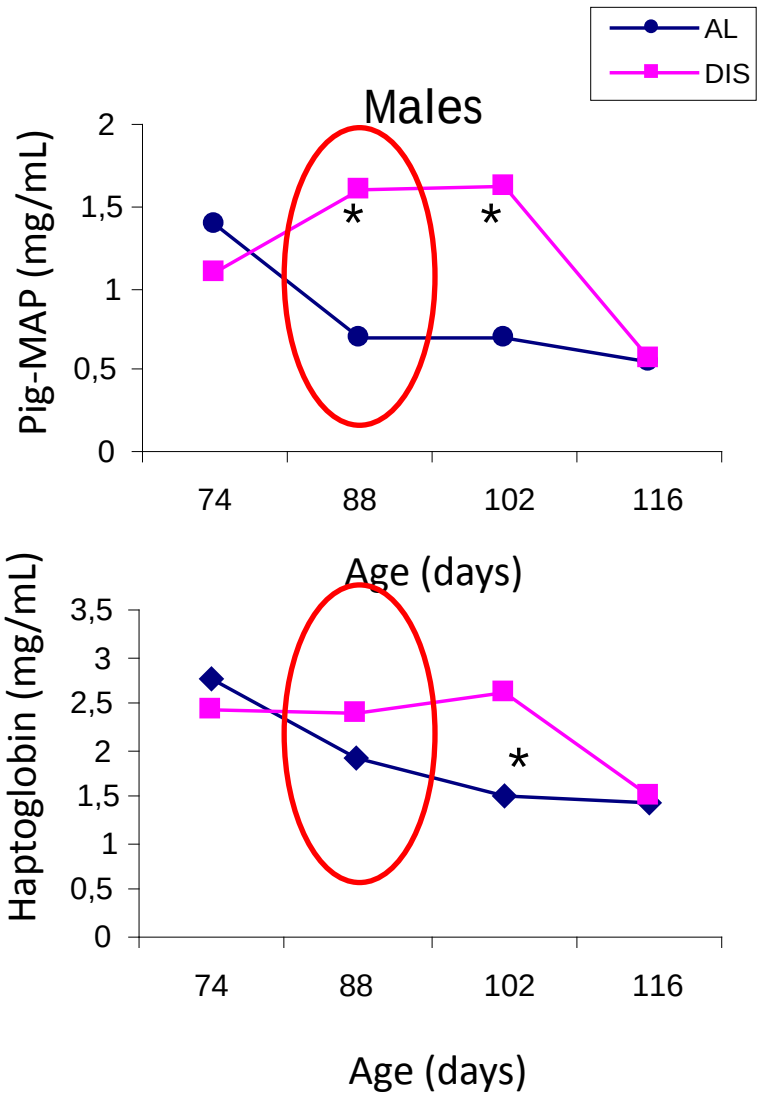
APP and productive performance

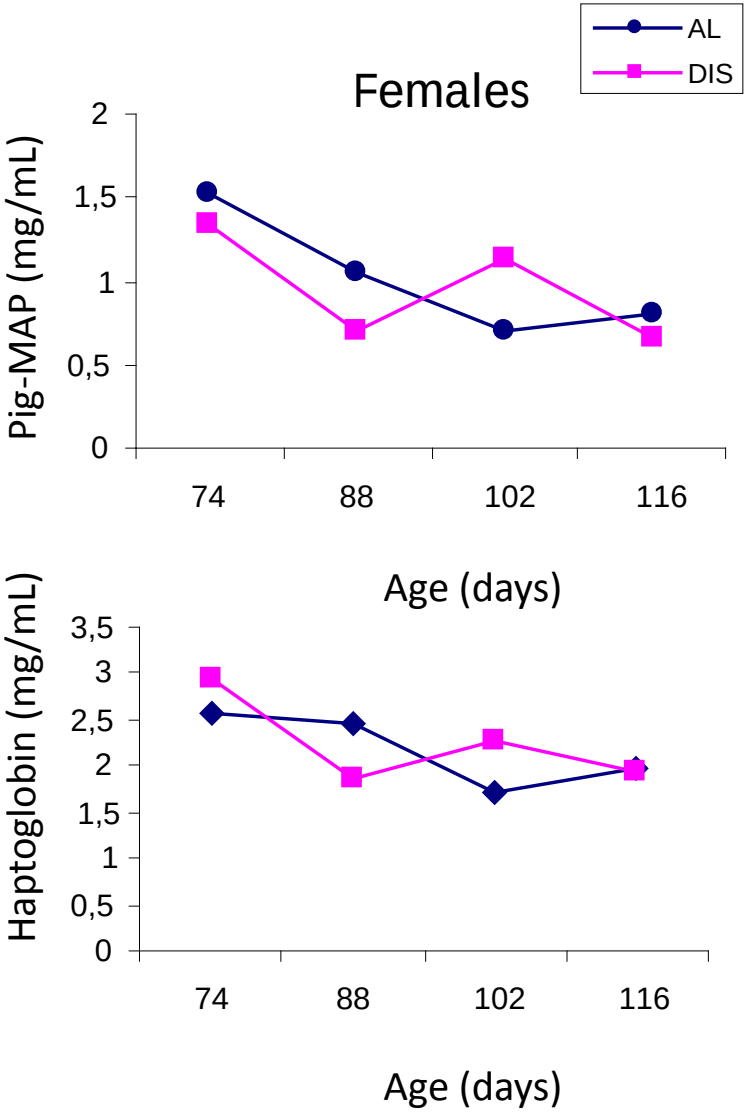
Changes in the pattern of feeding (Ad libitum -AL- vs Disordered –DIS-)

	74–88			88–102		
	ADG	FGR	FI	ADG	FGR	FI
AL group						
Male	523[a]	1.96	0.95[a]	577	1.91	1.05
Female	439[b]	2.36	0.99[a]	627	2.05	1.25
DIS group						
Male	398[b]	2.61	0.82[b]	513	2.37	1.09
Female	445[b]	2.11	0.91[ab]	574	2.03	1.13
s.e.	22.0	0.263	0.032	32.2	0.129	0.056
Significance						
Treatment	**		**	‡	‡	
Sex			‡	‡		*
Interaction	**				‡	

Piñeiro et al., 2007, Animal 1, 133-139

Changes in the pattern of feeding





Piñeiro et al., 2007, Animal 1, 133-139

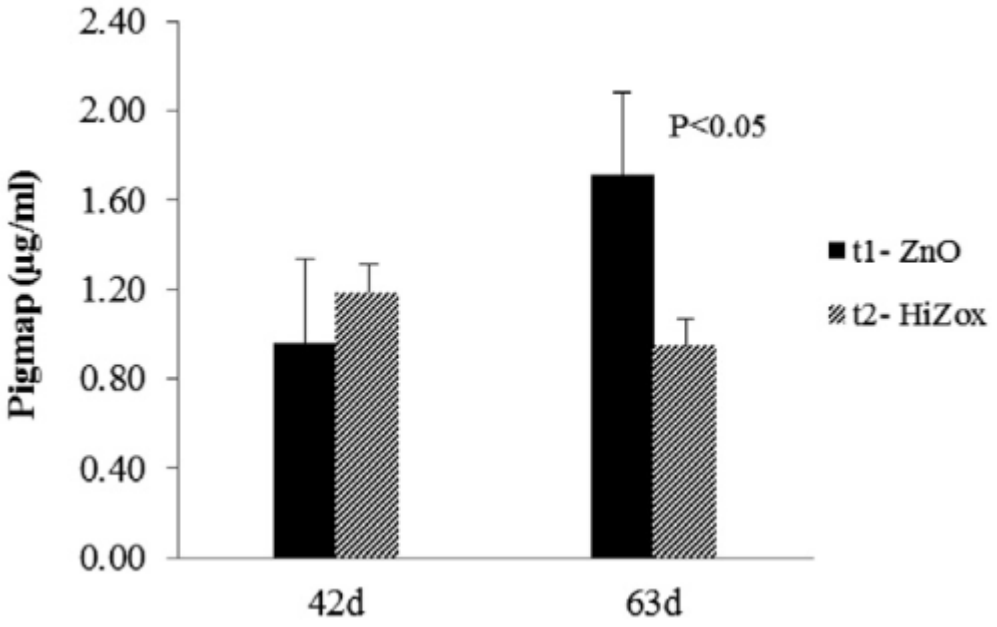
Feeding: AL: ad libitum, DIS: disorderly

E. Coli outbreak

- Nursery trial
- 2 different sources of Zn
- Comparison of productive performance and acute phase reaction

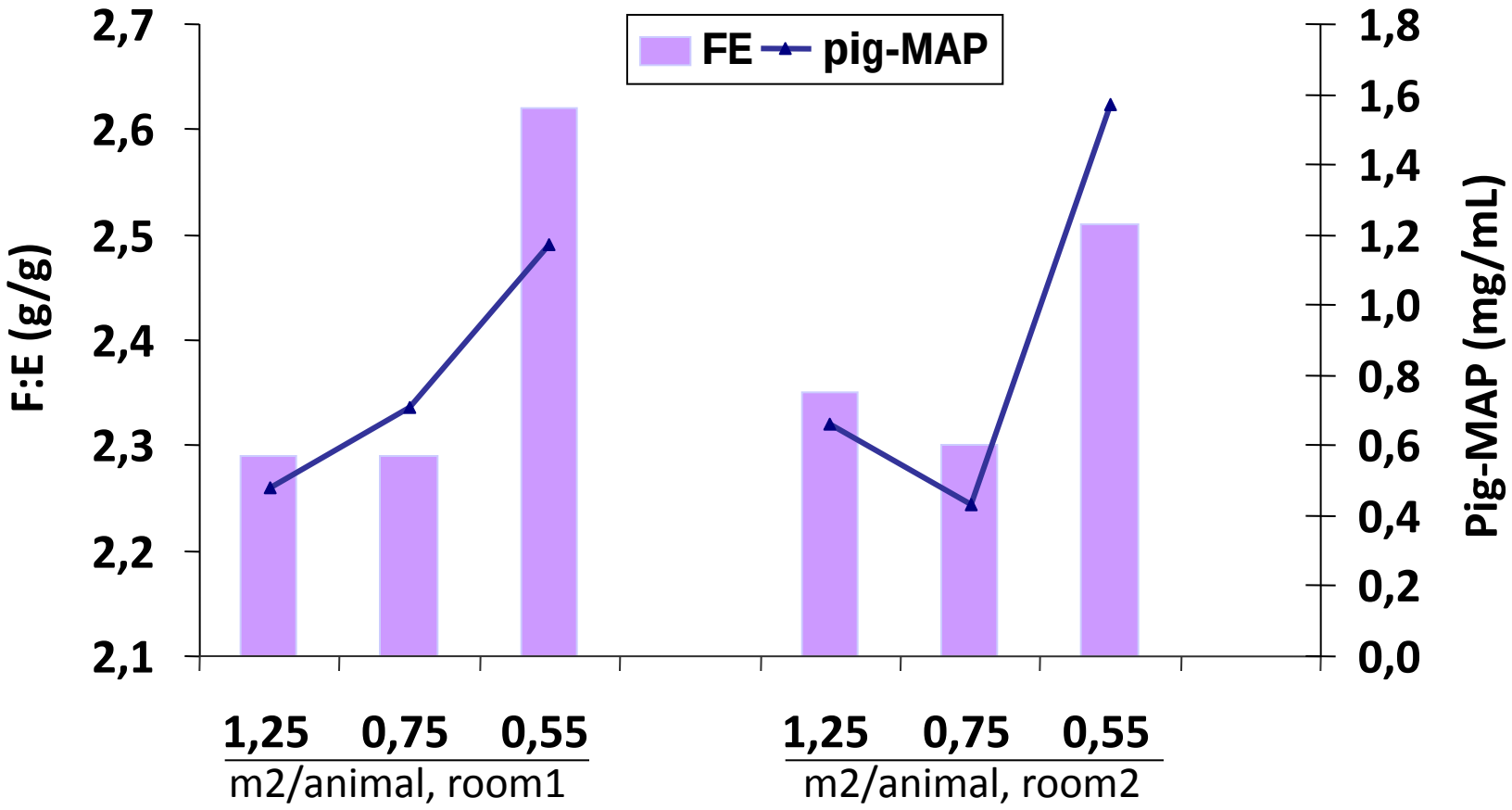
Table 1. Growth performance in the starter phase (42 to 63 d of age).

	Final BW, kg	ADG, g/d	G:F, g/g
T1-ZnO	16.51	358.8	0.586
T2-HiZox	17.93	424.3	0.694
SEM, n = 12	0.208	7.315	0.0069
Probability	<0.001	<0.001	<0.001



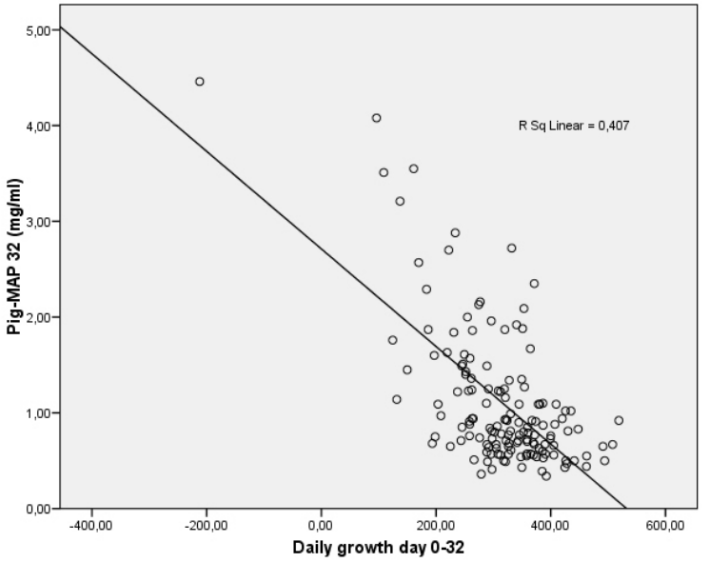
Morales et al, J. Anim. Sci 2012

Feed efficiency and APP in over stocking



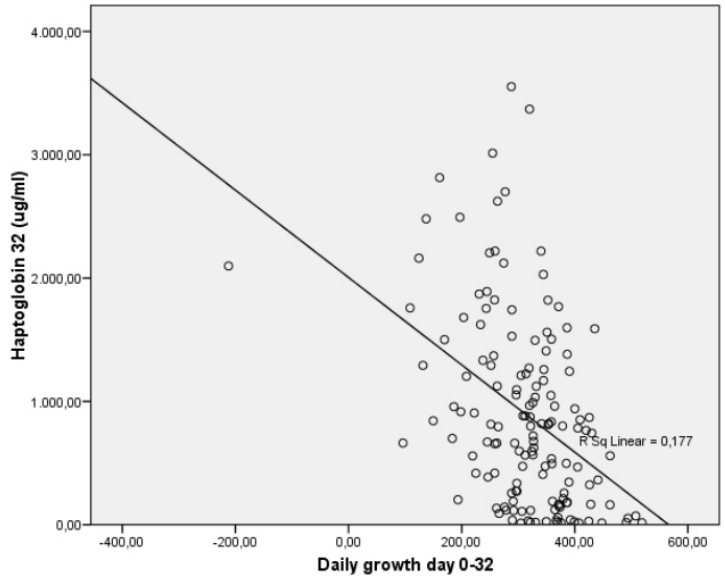
Own data, not published

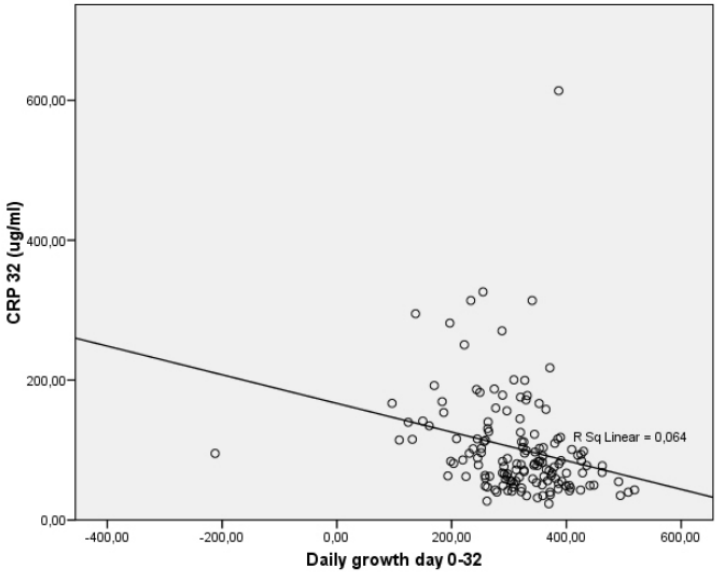
Effect of addition of butyrate in a weaned piglet diet on the acute phase response in nursery piglets



Thesis Vakdierenasts Varken, R. Jansen DVM, May 2013

- ADG : only significant correlation ($p<0.001$) for PigMAP with the highest $r2= 0.40$
- PigMAP was the best correlated with differences in mortality





APP and transport

APP and transport

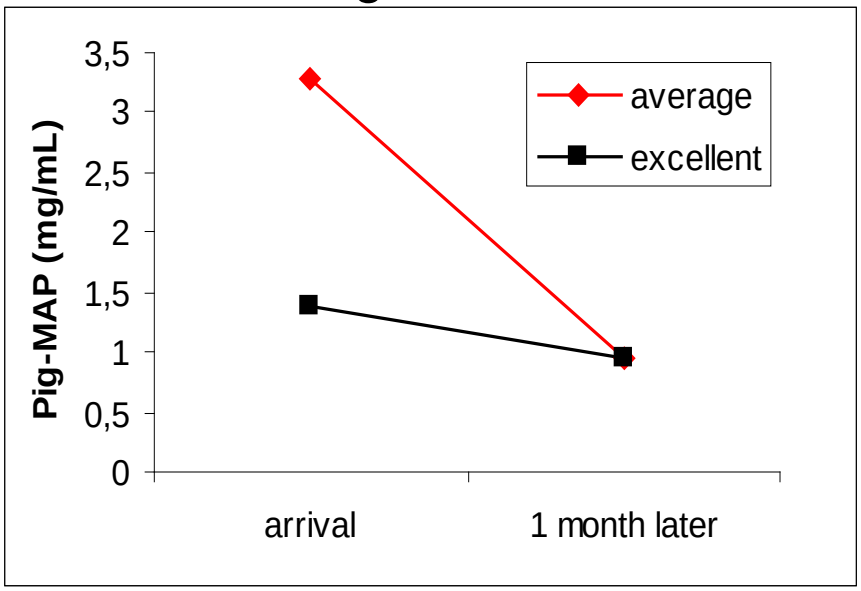
A) 24h, Average conditions

1.5 m2/boar, no sawdust, with no feed and water provided

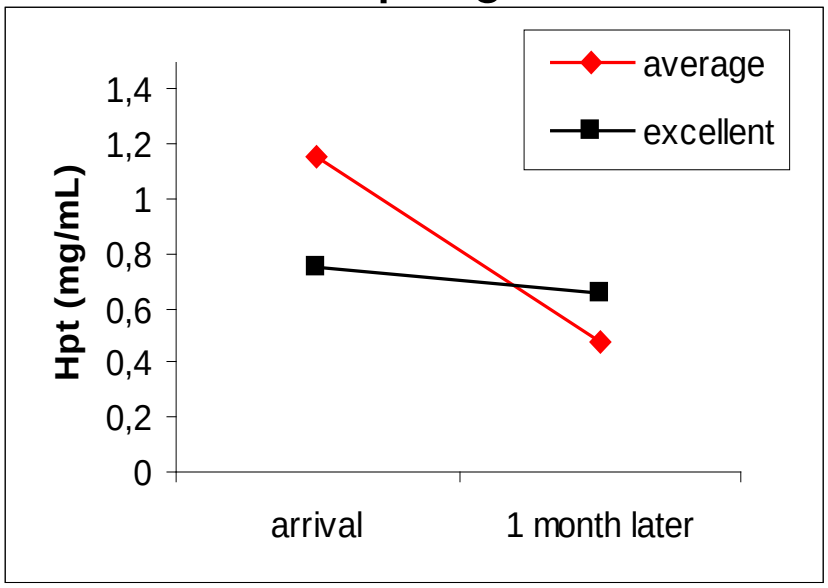
B) 48h, Excellent conditions

2 m2/boar, sawdust, feed and water provided

Pig-MAP



Haptoglobin



Piñeiro et al., 2007, Vet J 173,669-674

APP and transport

A) 24h, Average conditions

1.5 m2/boar, no sawdust, with no feed and water provided

B) 48h, Excellent conditions

2 m2/boar, sawdust, feed and water provided

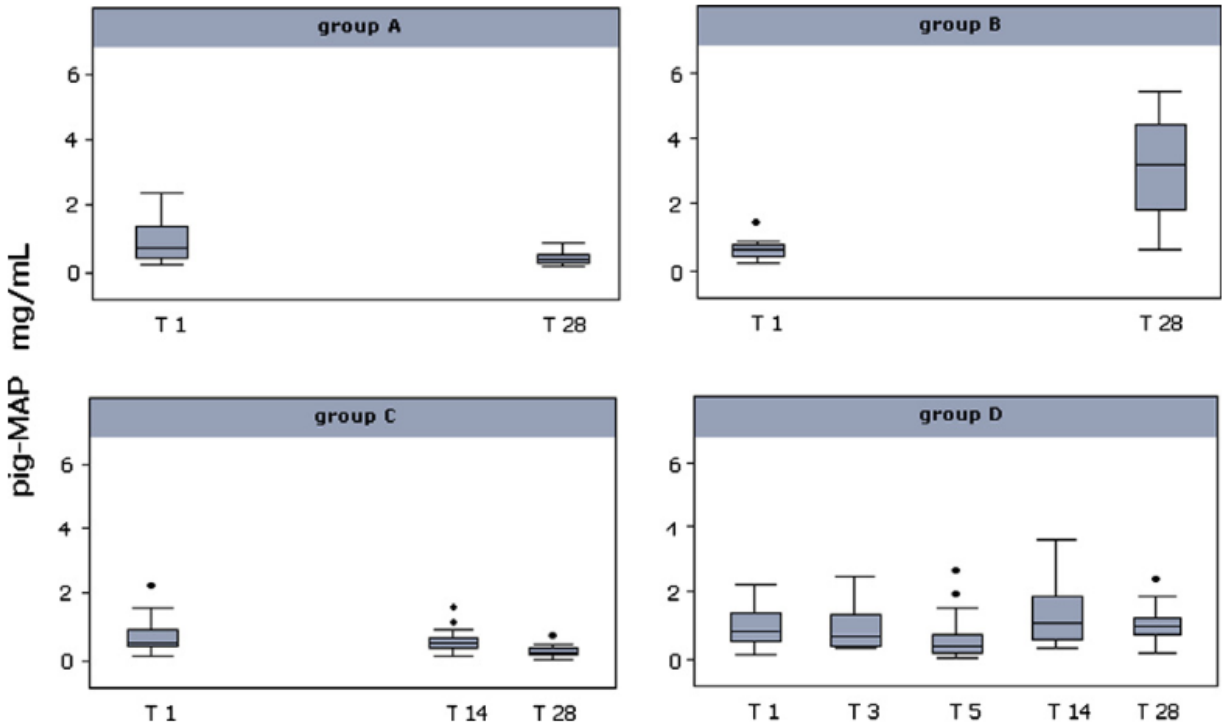
	Arrival	1 Month later	*P* value
Transport 1 ($n = 16$)			
Pig-MAP (mg/mL)	3.28 ± 1.50	0.94 ± 0.24	0.0004
Haptoglobin (mg/mL)	1.15 ± 0.54	0.48 ± 0.50	0.0011
CRP (μg/mL)	19.5 ± 12.8 (15)*	15.6 ± 9.0 (6)*	–
Albumin (mg/mL)	29.0 ± 2.2	25.2 ± 3.6	0.0858
Total protein (mg/mL)	91.5 ± 3.3	82.9 ± 7.4	0.0382
Cortisol (ng/mL)	29.0 ± 17.0	28.9 ± 11.3	1.0000
Transport 2 ($n = 32$)			
Pig-MAP (mg/mL)	1.39 ± 0.75	0.96 ± 0.14	0.001
Haptoglobin (mg/mL)	0.75 ± 0.61	0.65 ± 0.33	0.532
CRP (μg/mL)	22.5 ± 14.8 (12)*	17.0 ± 5.3 (5)*	–
Albumin (mg/mL)	29.1 ± 2.1	25.8 ± 2.5	0.000
Total protein (mg/mL)	88.8 ± 6.9	79.9 ± 6.2	0.0003
Cortisol (ng/mL)	26.2 ± 17.0	25.8 ± 11.0	0.857

Piñeiro et al., 2007, Vet J 173,669-674

APP and transport

4 groups, T1, T3, T5, T14, T28

Measuring transport effect and later adaptation



Conclusion suggest stress effect on APP besides of inflammatory classical explanatacion

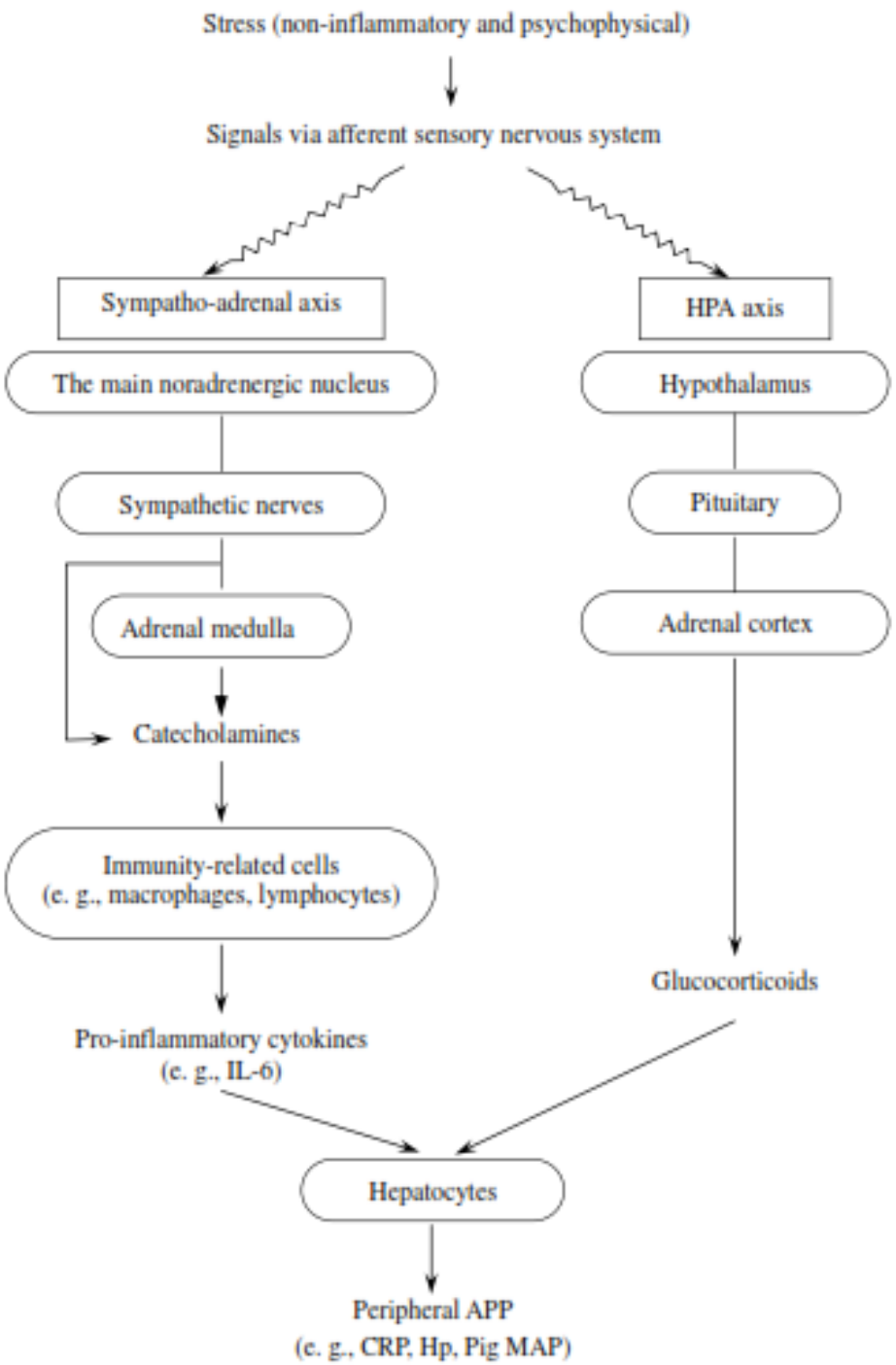
Salamano G et al., 2007, Vet J

Stress and acute phase response; an inconspicous but essential linkage

- *Strictly speaking, induction of APPs due to transportation stress has already been found in cattle (Murata and Miyamoto, 1993; Arthington et al., 2003) but the study of Pineiro and colleagues* ***is the first to confirm the stress APP linkage in pigs under commercial conditions, suggesting that the APP response is inducible to a considerable extent by stressful events to which domestic animals are ubiquitously exposed during daily management.***
- *In this editorial, I propose a hypothesis that could explain the nature of the stress-APP linkage.* ***The hypothesis is based on a neuroendocrine-immune network concept.*** *Briefly,*

  (1) signals originating in sensory organs in response to stress (in this case, non-inflammatory and psychophysical stress) are transmitted via afferent sensory nerve fibres to the brain and,
  (2) activate the neuroendocrine centres including the sympatho-adrenal axis and the hypothalamic-pituitary-adrenal (HPA) axis.
  (3) this activation leads to the release of catecholamines and glucocorticoids, which neurotransmitters
  4) directly and/or indirectly (through induction of pro-inflammatory cytokines in immunity-related cells) activate the production and release of APP in the liver, thereby
  5) augmenting peripheral APP levels in stressed animals.

Murata, H., 2007, Vet J. Guest editorial

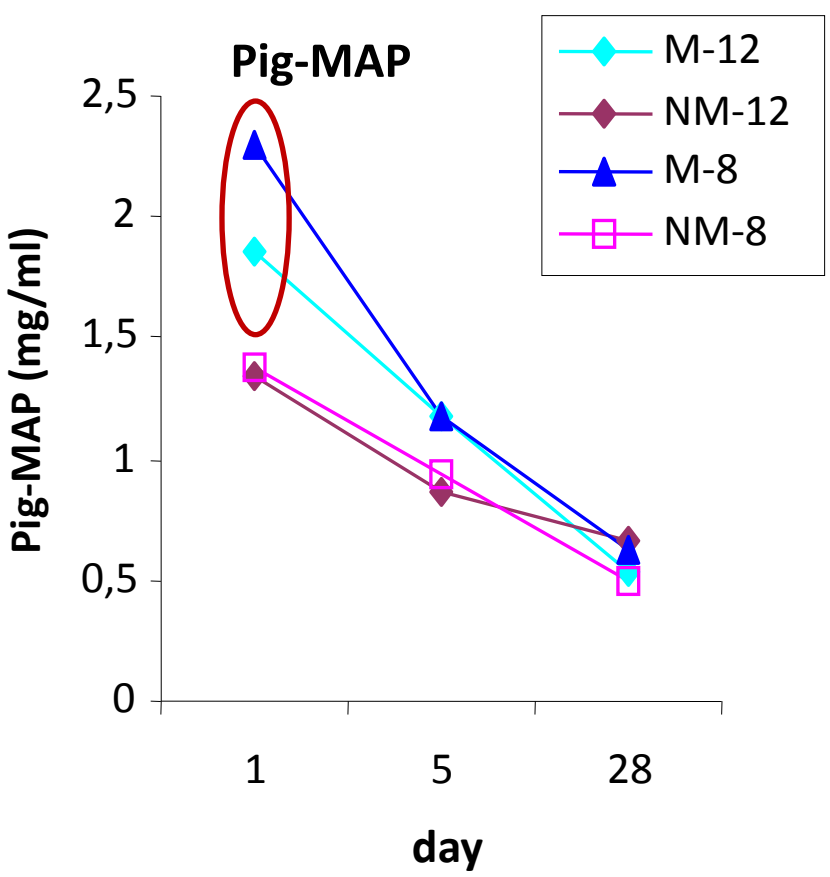


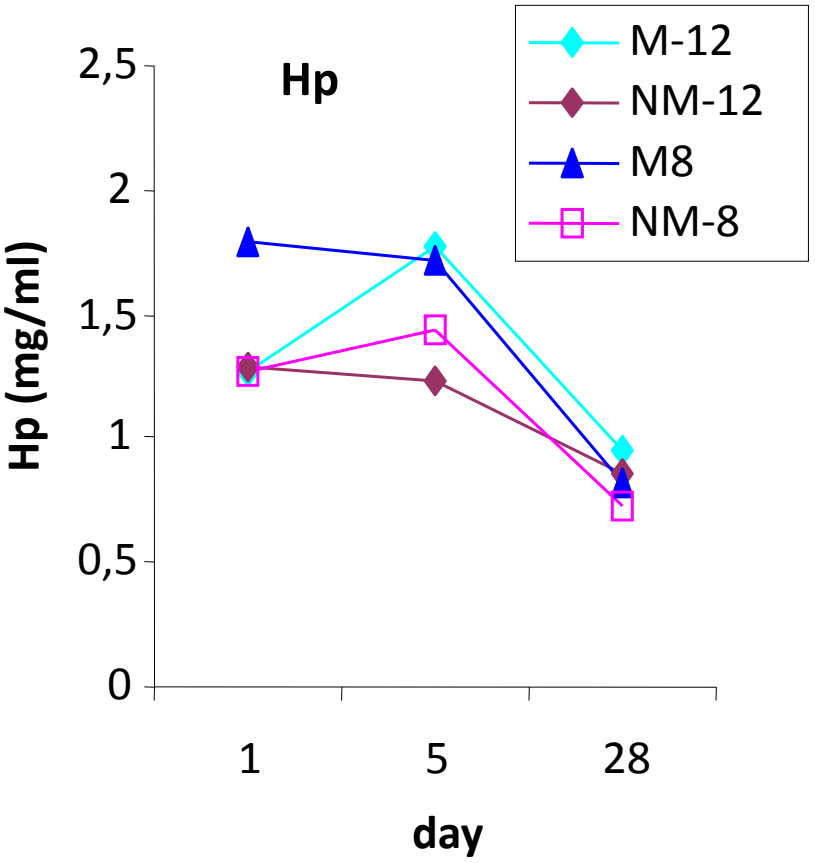
Stress and acute phase response; an inconspicous but essential linkage; *hypothesis for APP induction in stressed animals*

Murata, H., 2007, Vet J. Guest editorial

APP and management

Mixing at the entry of the fattening barn





Four experimental groups: M:mixed, NM:non mixed, 12 animals/pen, 8 animals/pen.

APP In pig production, QLK5-CT-2001-02219,

APP at abattoir

APP at abattoir

- Assesment of health status of pigs. Meat inspection.
- Posibility of measuring in blood or meat juice

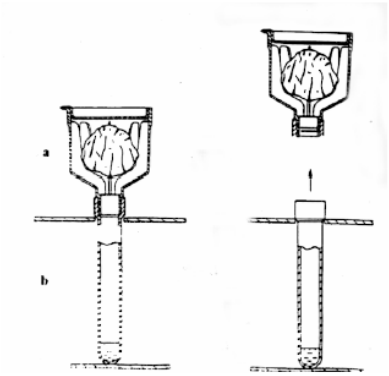


Figure 4.6: Meat juice container (Nielsen et al. 1998)

APP at slaughter: wasting pigs.

Yamane et al., 2006. **Increases in Pig Major Acute Phase Protein in Wasting Pigs Brought to the abattoir.** J. Vet Med. Sci. 68(5): 511-13

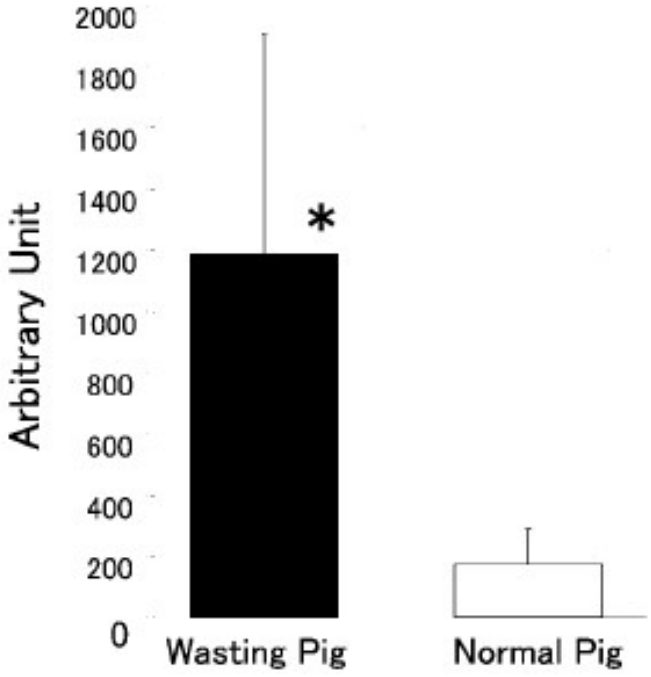
- Difficulties in stablishing a criteria to determine if these animals are adequate for human consumption

- Need of new diagnostic tools

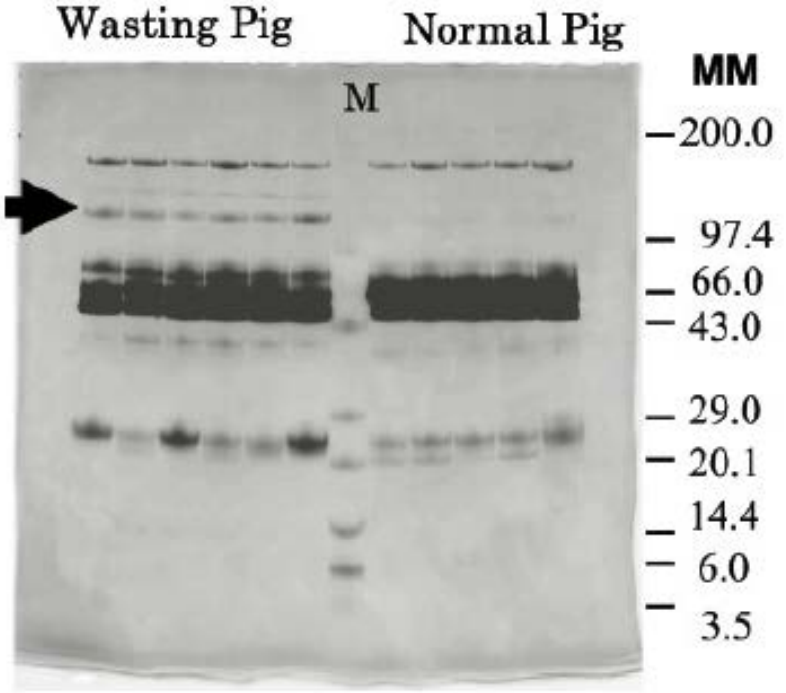
APP at slaughter: wasting pigs.

Proteomic analysis:

Identification of pig-MAP as a protein increasing significantly in wasting pigs.



SDS-PAGE:



Pig-MAP concentration 7 times higher in wasting pigs
(n=20 animals per group)

APP can be also determined in meat juice

- Obtaining of paired samples of slaughter blood and meat (*pars costalis diaphragmatis*)

- Meat juice collected after frozen (-20ºC) and thawing (24h, 4ºC) of the meat.

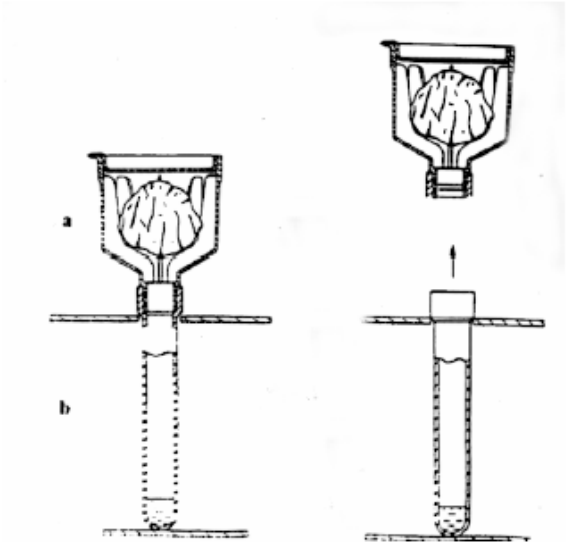
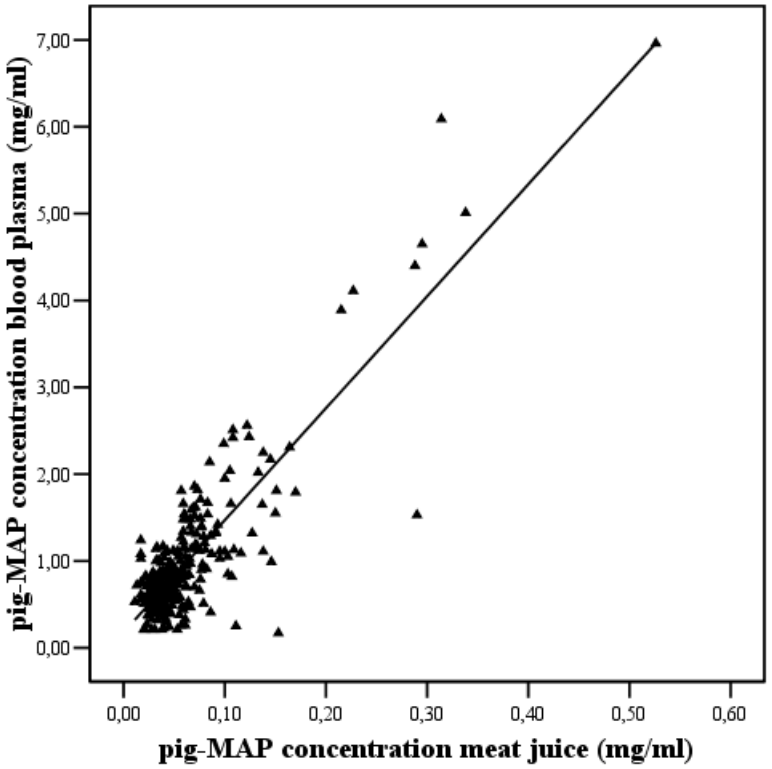


Figure 4.6: Meat juice container (Nielsen et al. 1998)

Piñeiro et al., 2009, Res Vet Sci 87, 273-276

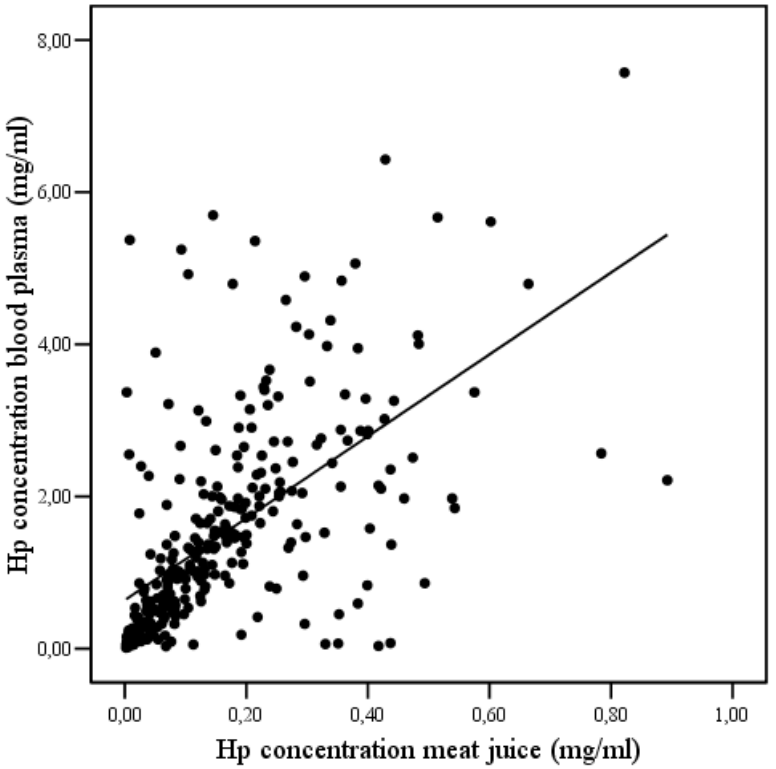
Correlation of APP concentration in blood and meat juice

Pig-MAP



r = 0.858, p < 0.001 (n = 292)

Haptoglobin



r = 0.695, p < 0.001 (n = 298)

Piñeiro et al., 2009, Res Vet Sci 87, 273-276

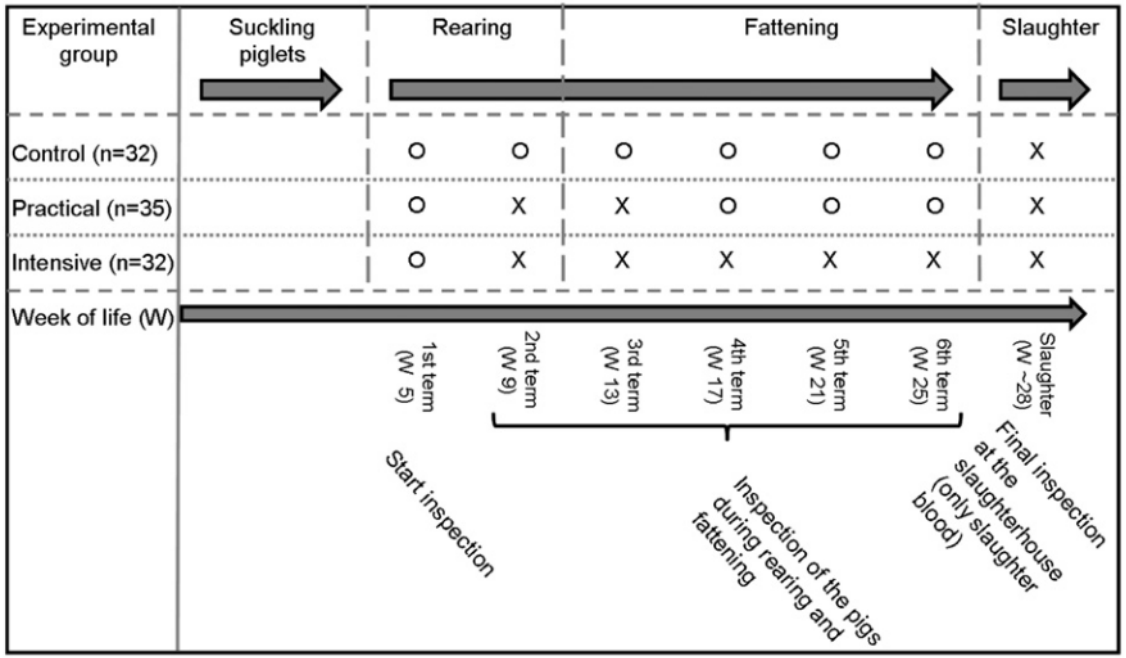
APP and production chain

Is everything coherent when combined?

Investigation of pig health and welfare, measured by APP concentration in serum-saliva (PigMAP, HP) with:

- **Pig performance data**
- **Carcass – meat quality attributes**
- **Organ findings**

through correlation coefficients



X = blood and saliva sampling
O = saliva sampling
W = week of life

Coherence of animal health, welfare and carcass quality in pork production chains.
Klauke et al. 2013, Meat Sci. 95(3):704-11.

Results

APP's and performance

- Correlations with feed efficiency were stronger for PigMAP and become more significant to the time point of slaughter

APP's and meat quality traits

- Positive significant correlation with IMF and negative correlation with water content in *L. dorsi*

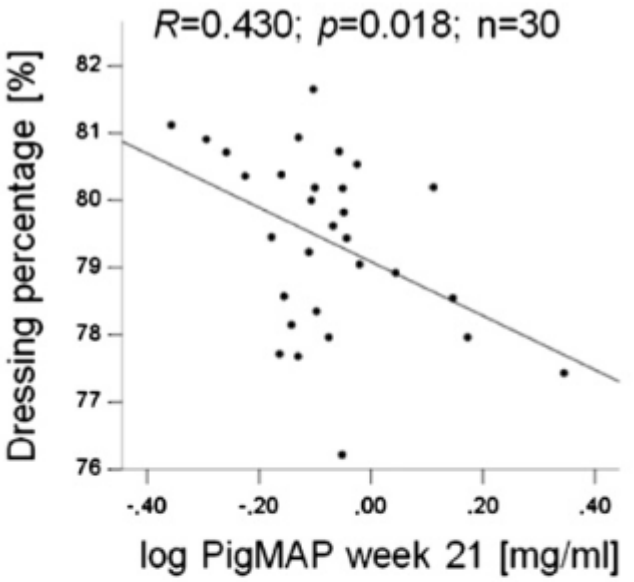
Coherence of animal health, welfare and carcass quality in pork production chains. Klauke et al. 2013, Meat Sci. 95(3):704-11.

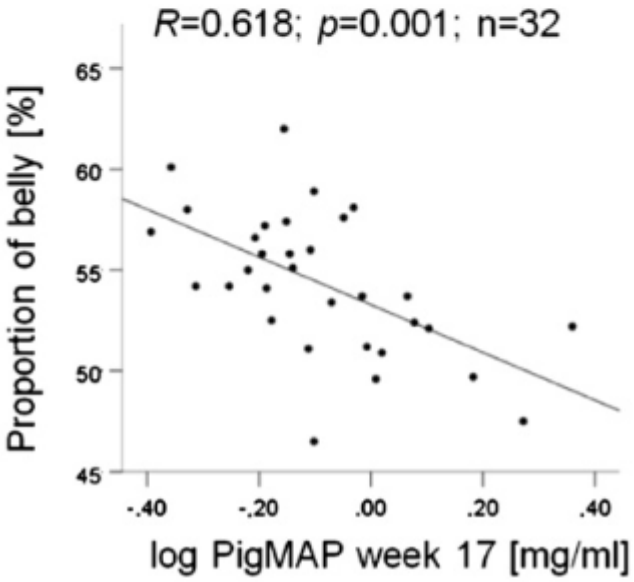
Results

Coherence of animal health, welfare and carcass quality in pork production chains. Klauke et al. 2013, Meat Sci. 95(3):704-11.

APP's and carcass composition

- Higher PigMAP concentrations resulted in lower weight of loin and reduced proportion of belly.
- Hp and PigMAP negatively correlated with lean meat carcass and belly content
- PigMAP positively correlated with fat / meat ratio



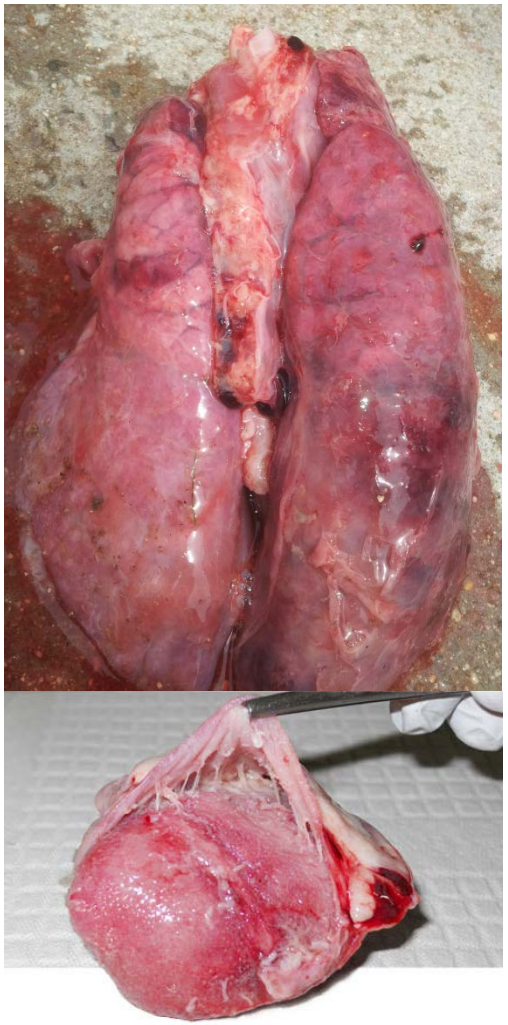


Results

Coherence of animal health, welfare and carcass quality in pork production chains. Klauke et al. 2013, Meat Sci. 95(3):704-11.

APP's as predictors of increased risk for organs findings

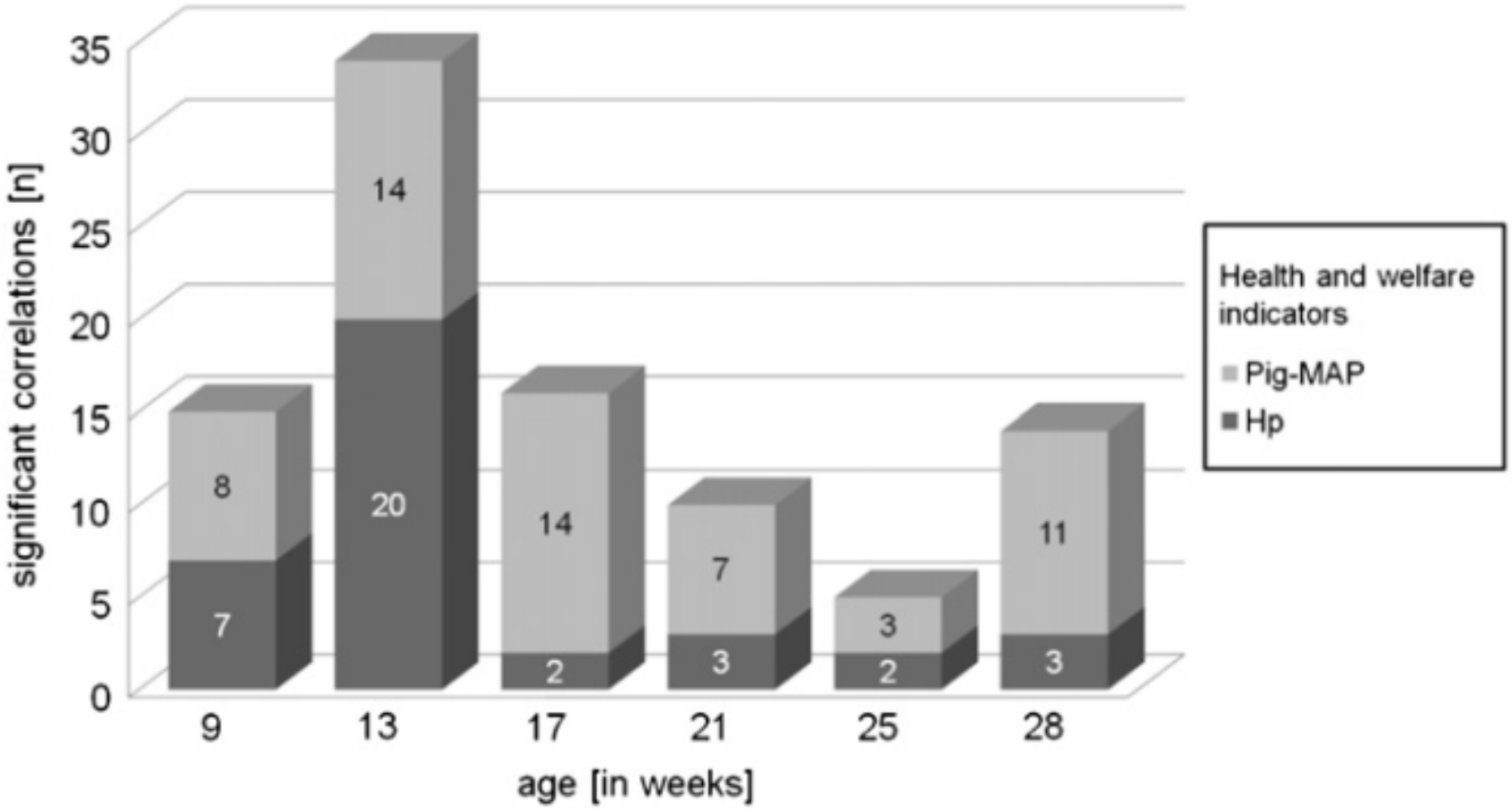
- No clinical symptoms during the study but 18 out of 99 showed organ findings (only one case with AB treat.)
  - 9 pneumonia
  - 2 pericarditis
  - 3 milk spots
  - 5 combinations of above
- PigMAP and Hp showed positive correlations (HP r=0.180 p=0.017 and PigMAP r=0.194 p=0.027)



Results

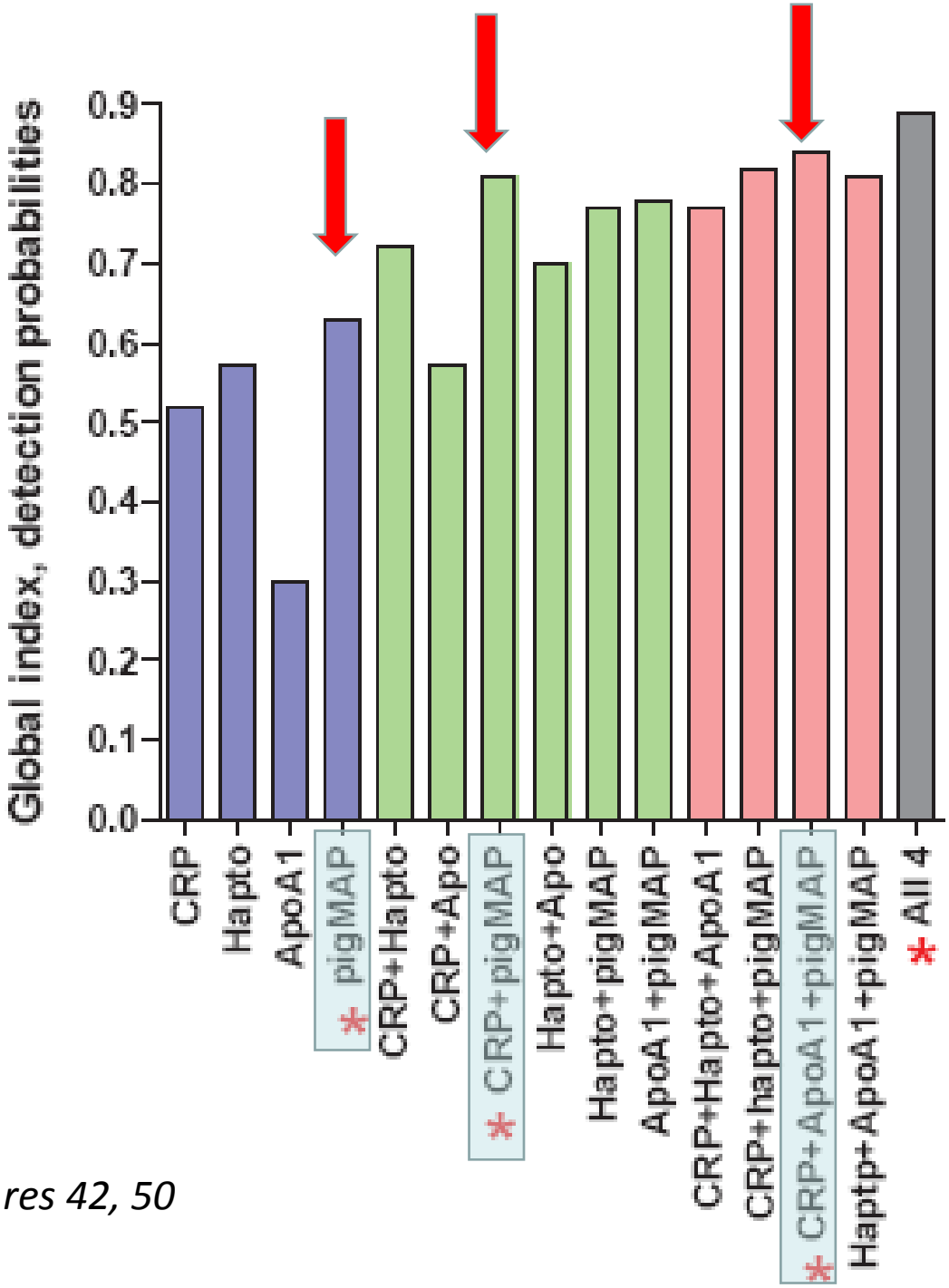
Coherence of animal health, welfare and carcass quality in pork production chains. Klauke et al. 2013, Meat Sci. 95(3):704-11.

Number of significant correlations identified through the age of the pigs (57 PigMAP, 37 Hp)



What APP should we determine?

Acute phase index (the best option using 1, 2 or 3 of them)



Heegaard et al., 2011, vet res 42, 50

APP economics

Association between APP concentration, hygienic status productive performance and medical cost.

- **Would anyone be surprised if the result is that this is costing a lot of money?**

We are putting our money in keeping the immune system active!

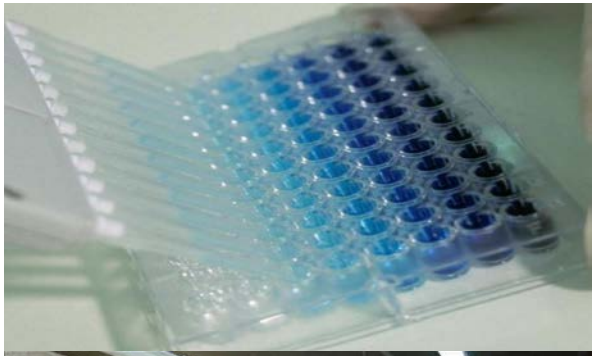
(energy, aminoacids,…)

Association between APP concentration, hygienic status productive performance and medical cost.

- **APP concentration** at the beginning of the rearing period was associated with **hygienic status** in the breeding farm.
- Pigs with **lower growth rate** had **higher APP concentration** at the end of the rearing period (> or < 450 g/day, APP measured 3 days before moving to the fattening).
- Animals with a **higher antibiotic treatment** cost during the rearing period, **had higher APP concentration** at the beginning of the rearing period.

And now what?
What is the practical use?

1. **Research** (immunity, diseases, health, welfare, …)
2. **Trials aiming the assessment of products** (AIF, vaccines), production systems or health plans
3. Quality of production evidences within **certification schemes**



Incorporation into **Quality Assurance Schemes**

Ensuring the health and management of animals delivered

Under a certain threshold of APP's, absence of clinical disease or poor management can be ensured.

Monitoring APP in pig production.

Incorporate into
quality assurance schemes

- supplier oriented receiving inspection.

Analysis of new animals entering the farm

- farm oriented in-process inspection.

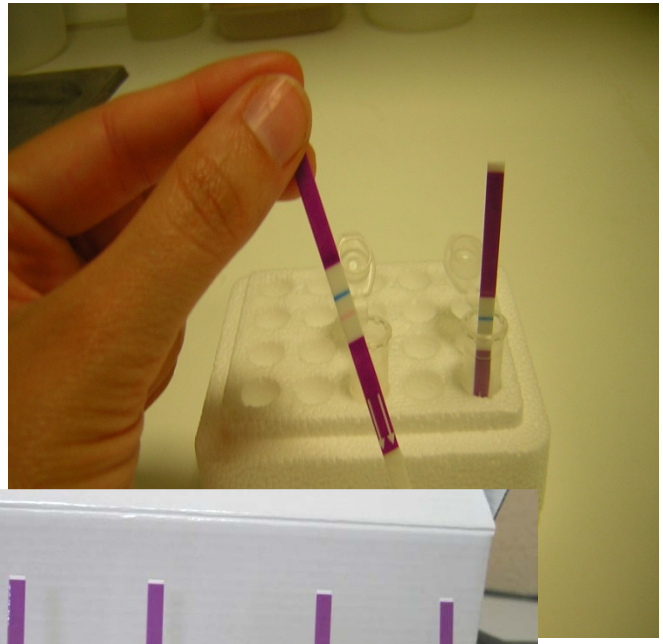
Evaluating health, management and welfare status of herds or farms

- customer oriented final inspection.

Animals to be delivered. End point analysis at slaughter line.

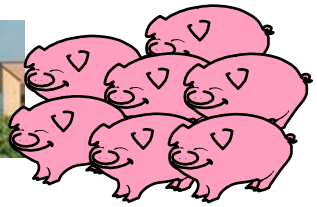
Rapid method
Stick PigMAP

- Immunocromatographic method (dipstick)
- Results in 15 minutes
- Positive with pig-MAP above 1.5 mg/mL
- No need of laboratory equipment
- For serum or whole blood

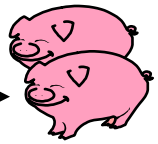


RISK ORIENTED INSPECTION SYSTEM

Option A) FARM



Group sample

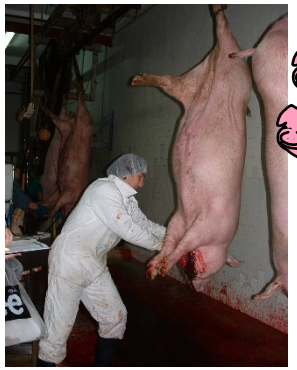


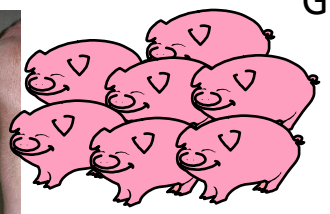
Blood sample

Finishing pigs to slaughter

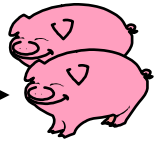
Pig-MAP analysis

Option B) ABATTOIR





Group sample



Blood sample

< 0.75 mg/mL low risk
> 0.75 mg/mL, high risk

Consider the effect of transport

- Categorization system for farms.
- Decission on final destination of the pigs/meat.
- Classification on a quality basis.
- Risk oriented meat inspection.
- Feedback of the health status of the farms

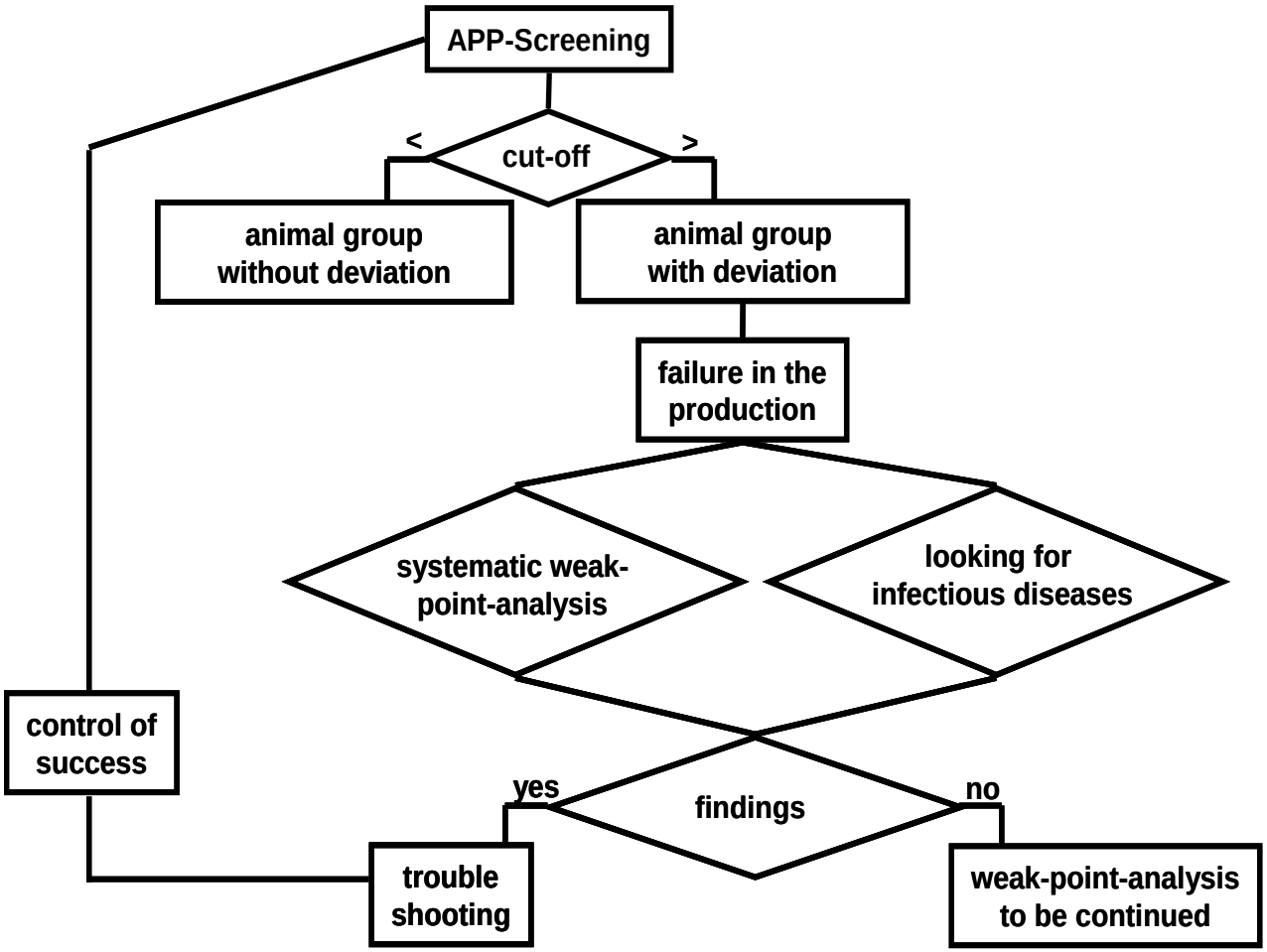
Conclusions APP

1. **General marker of health and welfare**
2. **Unespecific but sensitive, accurate enough to discriminate subtle situations and quantitative**
3. **Detects problems independently of its origin**
4. **Early detection**
5. **Increases with**
    1. Diseases (clinical or subclinical)
    2. Stress caused by poor management during raising period and transport
6. **Correlates with**
    1. Increased medicines costs
    2. Losses of productive performance
    3. Higher risk of organ findings in abattoir

Thank you!

carlos.pineiro@pigchamp-pro.com

APP monitoring



Questions to consider when Measuring APP

•How many animals to sample?

10 animals per age group will be enough for many aplications.

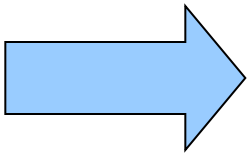
- Enough to detect the problem
- Not too much because of cost.

•Take samples randomly. Do not focus in runts or chronically sick animals.

•Record information about the animal (sex, age) and management and health conditions at the farm.
This will help you to interpretate the results

Questions to consider when Measuring APP

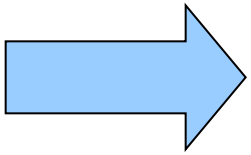
- Avoidable Changes



Detect problems in your productive system

  - Disease
  - Bad managment condition causing stress

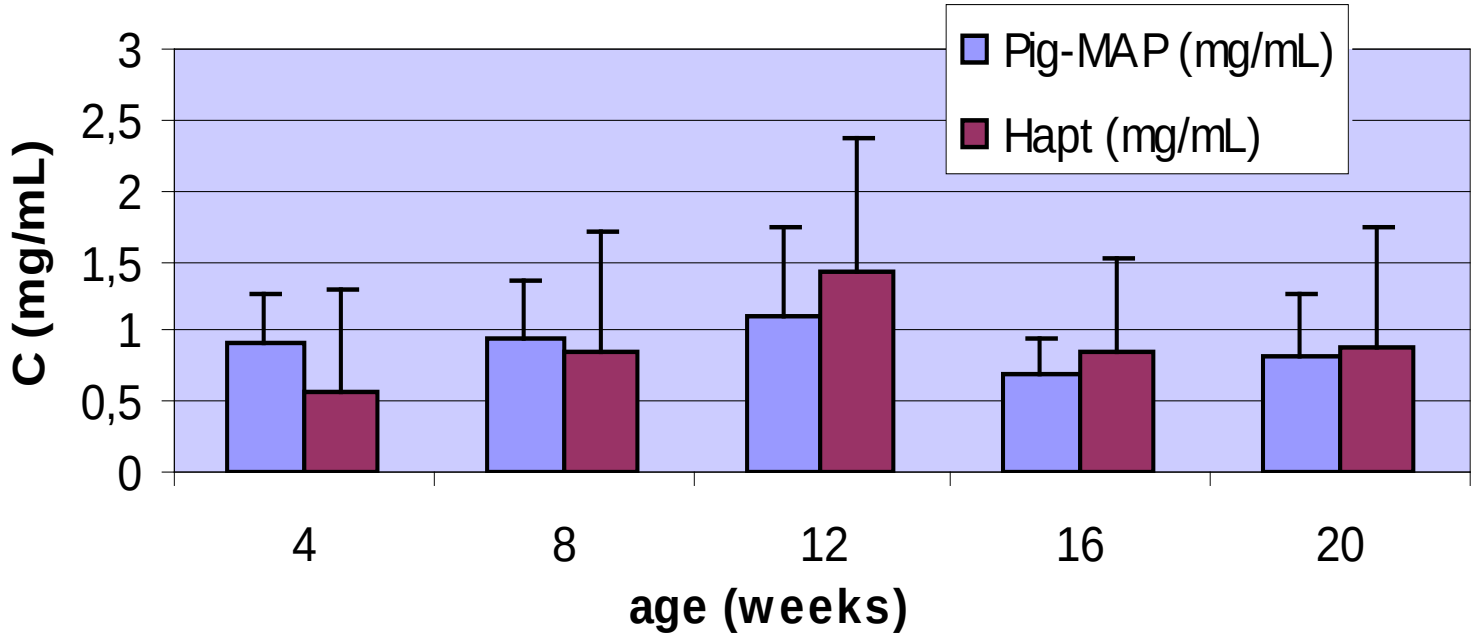
- Unavoidable Changes



Take into account when sampling

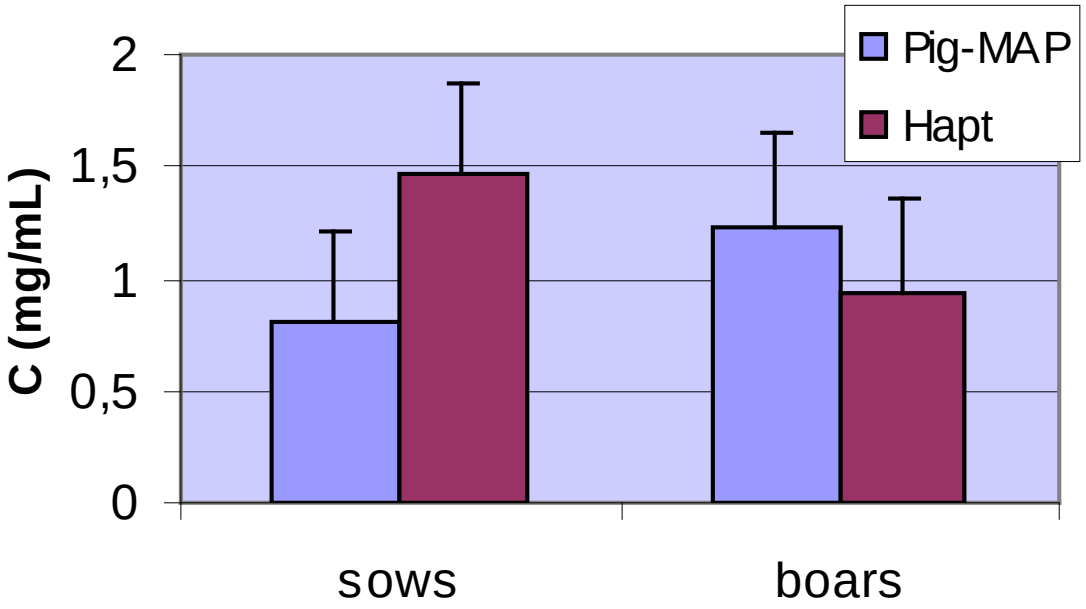
  - Parturition
  - Weaning
  - Transport
  - Vaccination..

Pig-MAP and Haptoglobin reference values in commercial farms.



Piñeiro et al. 2009, Vet J 179, 78-84

Pig-MAP and Haptoglobin reference values in commercial farms.

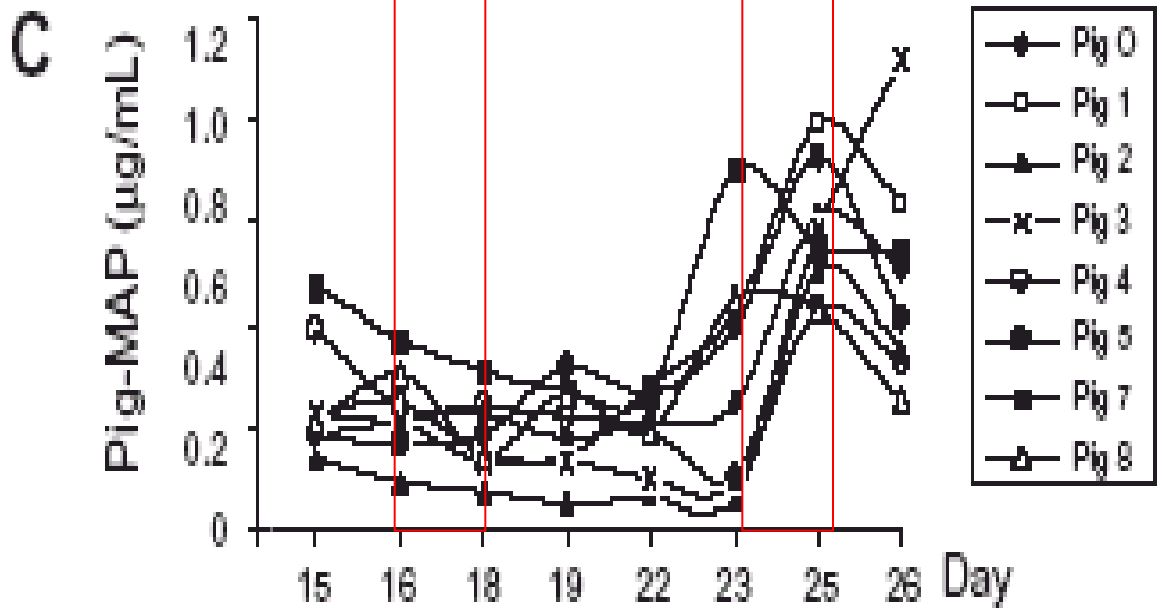


Piñeiro et al, 2009, Vet J 179, 78-84

APP during a crowding period

Crowding period:
0.25m^2/pig

No clinical signs



Eight Duroc × (Landrace × Large White) male pigs (18–20 kg bodyweight) were housed in a pen with a slatted floor at a density of 0.50 m^2/pig (100 kg/m^2, lower density, LD) at day 1, and the density was changed to 0.25 m^2/pig (200 kg/m^2, higher density, HD) for two 4-day periods over 26 days by moving the fence (Fig. 1). The

Marco-Ramell et al, 2011
Veterinary Journal.