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Innovation in Livestock production: From Ideas to Practice

Effect of SOD-rich melon in LPS challenged piglets on antioxidant status and growth performance

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INTRODUCTION

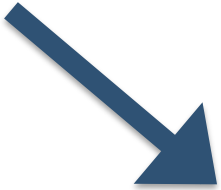
Piglets are subjected to stress at weaning

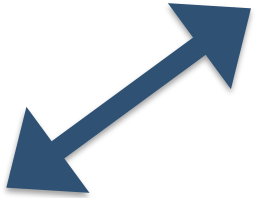
Negative effects on:

- Immune system (Kick et al., 2012)
- Intestinal functions (Wijtten et al., 2011)
- Endocrine system (Zhu et al., 2012)
- Free radical metabolism (Zhu et al., 2012)

Moreover:

Increased effects when a chronic immune stimulation happens (e.g. farm managment conditions)





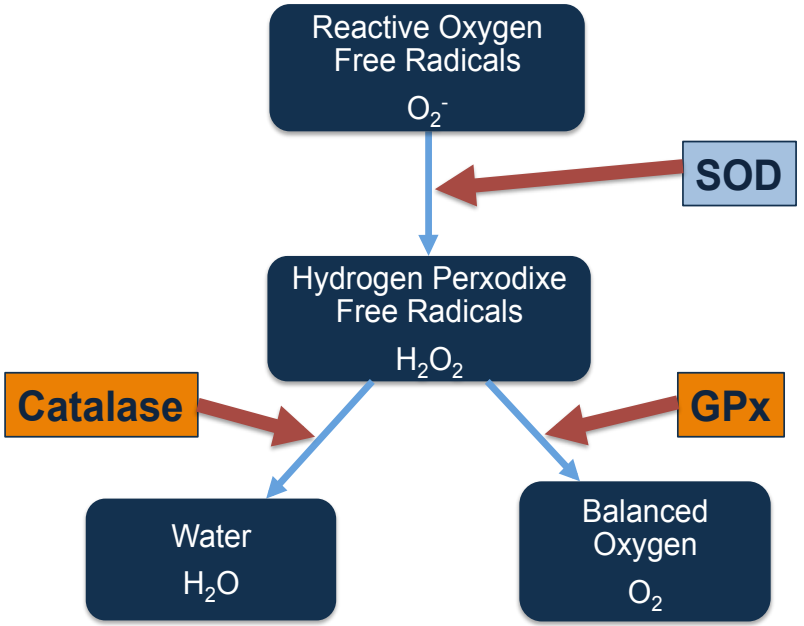
Results:

- Transient anorexia
- Impaired growth
- Increased inflammatory cytokines levels (Pié et al., 2004)
- Chronic immune system stimulation (Bontempo et al., 2014)
- Higher oxidative stress (Wang et al., 2008)
- Impaired antioxidant enzymes system (Han et al., 2011)

INTRODUCTION

- Superoxide dismutase (**SOD**) is a primary internal antioxidant
- SOD as anti-inflammatory substance regulating neutrophil apoptosis (Yasui et al., 2006).
- Improving the oxidative status, by both controlling the production of Reactive Oxygen Species (**ROS)** and increasing the antioxidant status by nutritional intervention.
- SOD-rich melon outlined positive results on the maintenance of cellular integrity in various experimental models. (Lallès et al., 2010; Notin et al., 2010; Carillon et al., 2013)

Figure 1. SOD Mechanism of action on oxydative metabolism



AIM OF THE TRIAL

The aim of the trial was to study the effects of a feed supplement (Melofeed®, Lallemand, France) that contains high levels of SOD as a primary antioxidant, on antioxidant status and growth performance of LPS challenged weaned piglets.

MATERIALS AND METHODS

Table 1. Trial design

Experimental subjects:	48 female piglets
Age at weaning	24 days
Body weight	7.79 kg ±0.17
Experimental groups	4
Duration of the trial	29 days
Replications/group	12 (individual pens)
Dietary phases:	1

Table 2. Experimental basal diet

Composition (Kg/100 kg as fed)	
Wheat meal	29.465
Barley meal	23.120
Wheat flaked	14.000
Soybean meal 48%	17.000
Sweet whey powder	6.000
Soybean oil	3.000
Corn gluten meal	2.500
Dextrose	1.500
Dicalcium phospate	1.300
L-Lysine	0.570
Calcium carbonate	0.500
Sodium chloride	0.300
Vit + trace elements*	0.250
L-Threonine	0.230
DL-Methionine	0.180
Flavour	0.050
Optisweet SD	0.015
Zinc oxide (HiZox)	0.010
Cu sulphate	0.010
Chemical composition (as fed)	
Moisture	11.30
Crude protein (%)	18.01
Ether Extract (%)	4.52
Crude fibre (%)	2.87
Ash (%)	5.08
DE (Mcal/kg)	3.44
NE (Mcal/kg)	2.45
Lys tot (mg)	1.25
Met+Cys tot (%)	0.80
Threonine tot (%)	0.85
Triptophan tot (%)	0.21
Starch (%)	41.43
Sugar (%)	7.54
Zn (mg/kg)	75.00
Cu (mg/kg)	24.70

MATERIALS AND METHODS

Table 3. Chronic challenge to reduce endotoxin tolerance (Rakhshandeh and de Lange, 2012).

LPS	**Intramuscular injection (E. *coli* serotype 055:B5).**
Performed on trial days	19, 21, 23 and 25.
Initial dosage	60 µg/Kg of BW
Subsequent dosage	+12% at each injection

MATERIALS AND METHODS

Table 4. Experimental groups and dietary treatments

- **Control (C-)**
  basal diet

- **Treatment (T-):**
  basal diet + 30g/ton Melofeed® (SOD=2.6 x 10^6 IU/kg)

- **Control + Challenge (C+)**
  basal diet + LPS challenge

- **Treatment + Challenge (T+)**
  basal diet + 30g/ton Melofeed® (SOD=2.6 x 10^6 IU/kg) + LPS challenge

MATERIALS AND METHODS

Table 5. Growth performance, oxidative and immune parameters timetable

		Days on trial									
Item	**Method**	**0**	**8**	**15**	**19**	**21**	**23**	**25**	**26**	**27**	**29**
Body Weight (BW)	Electronic scale (Ohaus ES100L)	x	x	x	x	x	x	x	x	x	x
Feed Intake (FI)			x	x	x	x					x
Body Temperature (BT)	Rectal				x	x	x	x			x
Total Antioxidant Oxidative Capacity (TAOC)	Sigma Aldrich, Cat n. CS0790	x			x	x	x	x		x	x
Reactive oxygen species (ROS)	Satorelli et al., 2002	x			x	x	x	x		x	x
Super oxide dismutase (SOD)	Sigma Aldrich, Cat n. C19160	x			x	x	x	x		x	x
8-oxo-dGuo	ELISA				x			x			x
Kit Radicaux Libres (KRL)	Pastorelli et al., 2013				x			x			x
Haptoglobin (Hp)	Cooke and Arthington, 2013	x			x	x	x	x			x
IL-1β, IL-6, TNFα (pro-inflammatory cytokines)	ELISA	x			x	x	x	x			x

LPS challenge period

STATISTICAL ANALYSIS

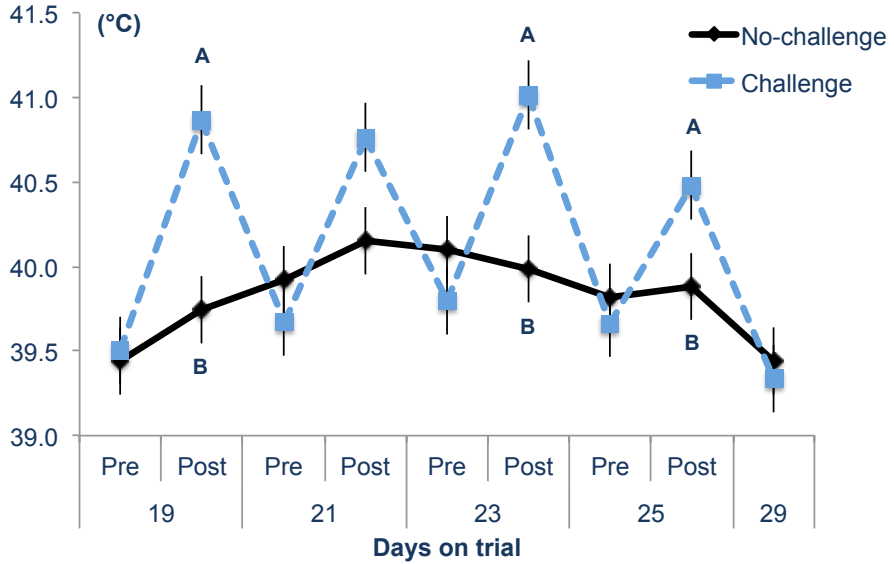
- BW, BT, oxidative and immune response parameters were analyzed by a MIXED procedure of SAS.
- ADG, FI, G:F considered two different trial periods corresponding to day 0-19 (pre-challenge) and 19-29 (challenge and post challenge) using a GLM procedure of SAS with period 0-19 as covariate over challenge and post-challenge period.
- Final BW was also analysed by a GLM procedure using day 19 as covariate.
- Piglet was the experimental unit nested within the diet*challenge group.
- Significance level was fixed for $^{A,B}P \leq 0.01$ and $^{a,b}P \leq 0.05$,
- $0.05 < P \leq 0.1$ was considered as a trend.

RESULTS

Validation of the LPS challenge model

Figure 2. Effect of LPS challenge on body temperature of weaned piglets pre and post (2hrs) intramuscular injection

2



A, B P<0.01

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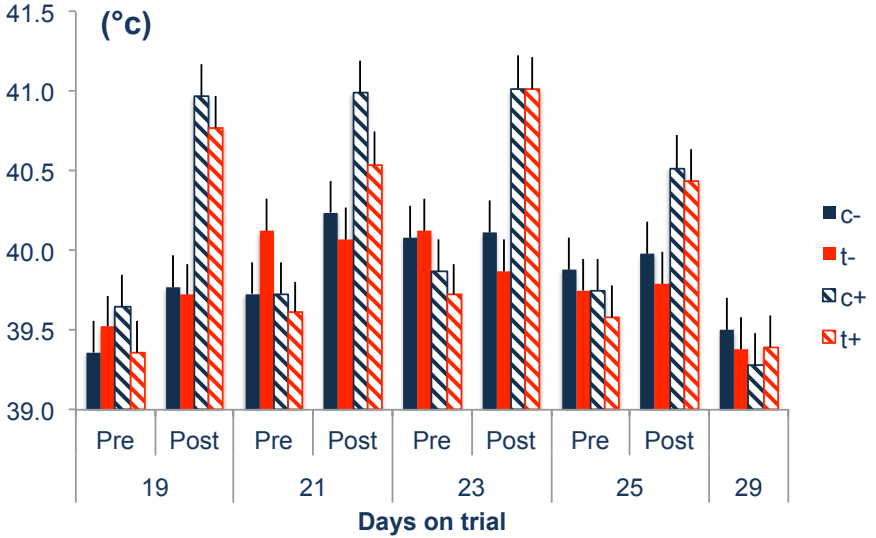


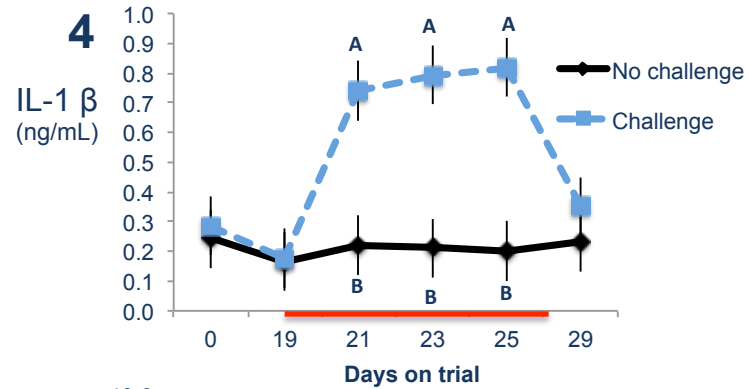
Figure 3. Effect of LPS challenge on body temperature in the four experimental groups pre and post (2hrs) intramuscular injerction)

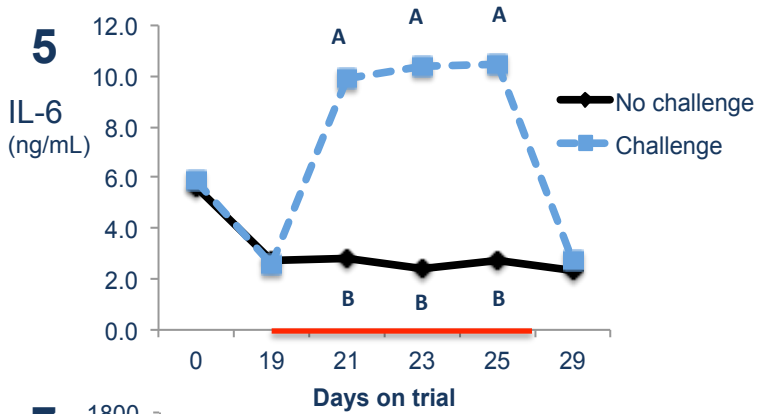
Day 19:	Diet x Challenge	P=0.05
Day 21:	Diet x Time	P=0.04
	Diet x Challenge	P=0.06
Day 25:	Diet	P=0.02

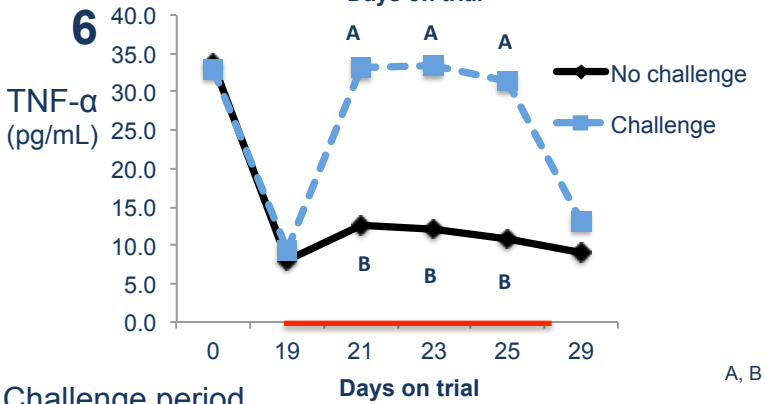
RESULTS

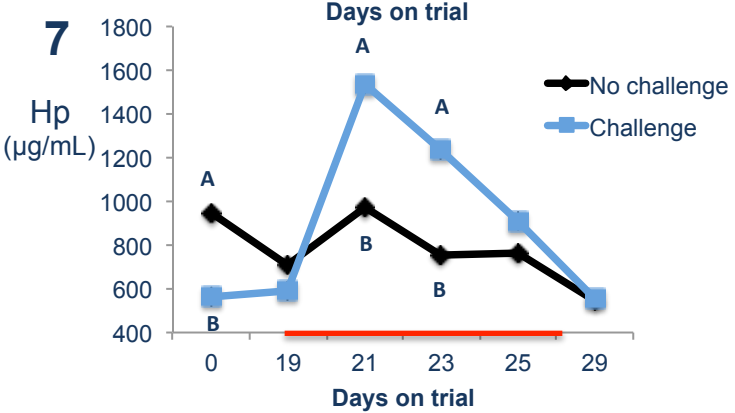
Validation of the LPS challenge model

Figures 4,5,6,7. Effects of LPS challenge on IL-1 β, IL-6, TNFα and Hp levels in blood of weaned piglets









Challenge period

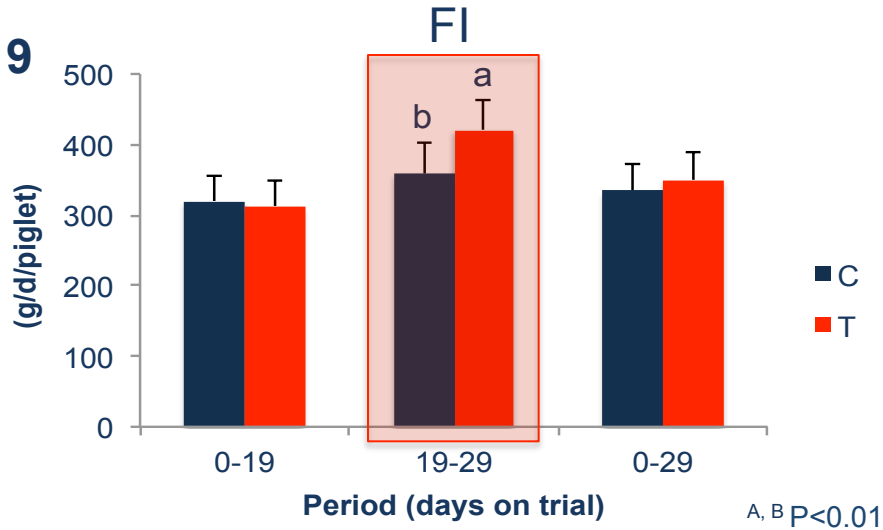
A, B P<0.01

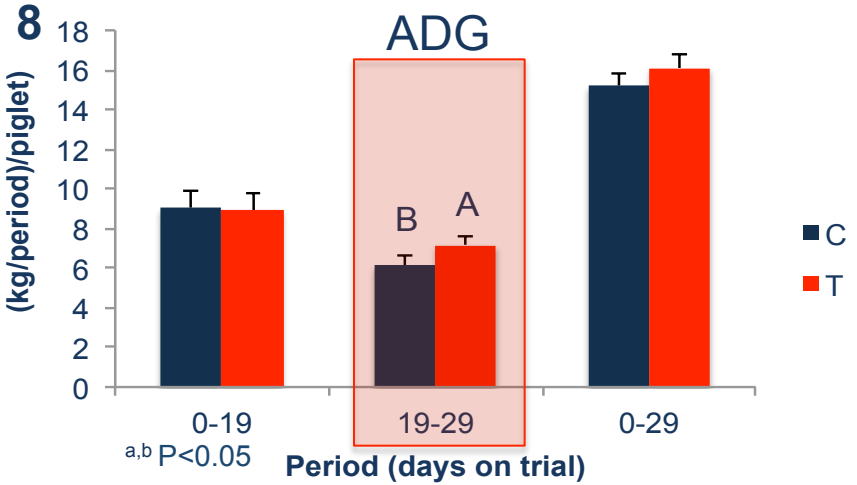
RESULTS

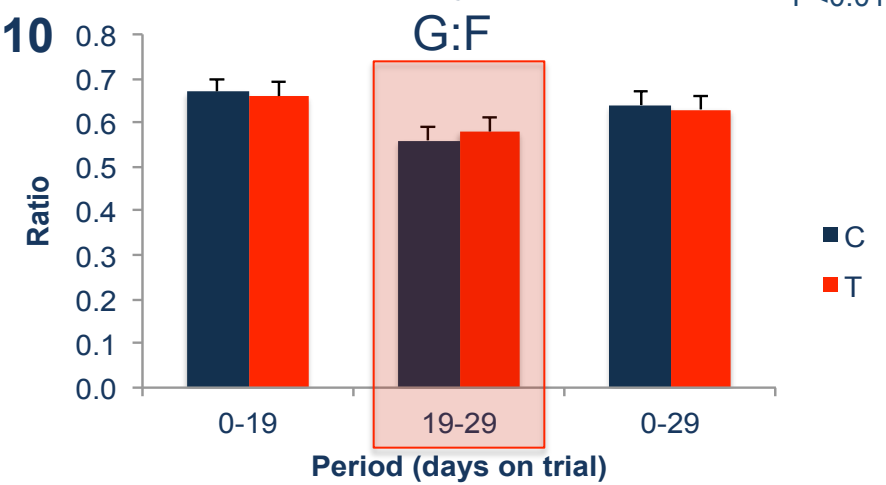
Effect of Melofeed® on growth performance

Figures 8,9,10. Effect of Melofeed® on growing performance of weaned piglets

Challenge period







Significant challenge effect on all the parameters from 19 to 29 days on trial (P<0.01)

RESULTS

Figures 11,12,13,14,15. Effects of Melofeed® on oxidative markers of weaned piglets

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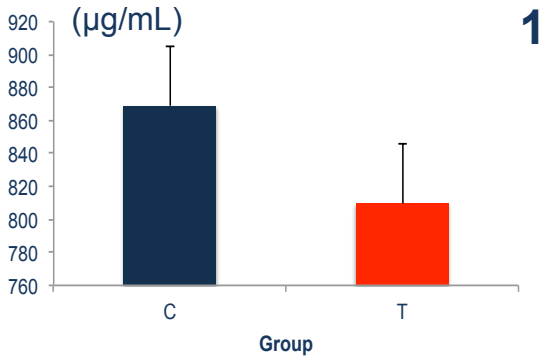

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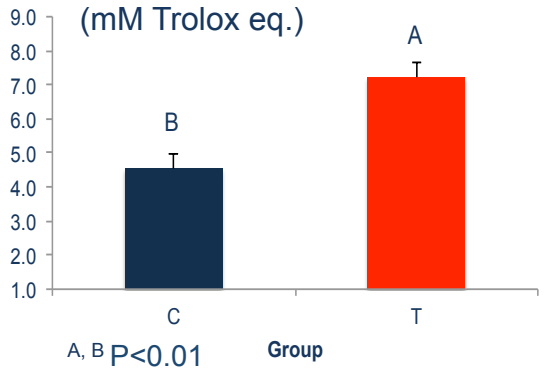

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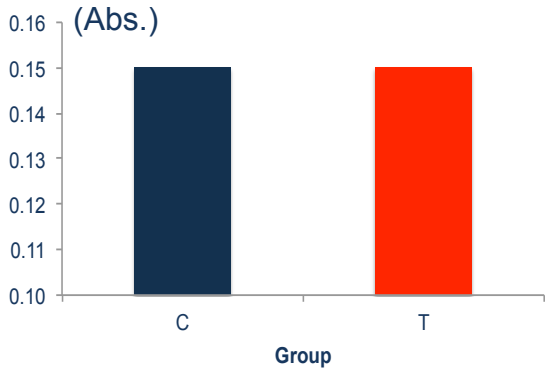

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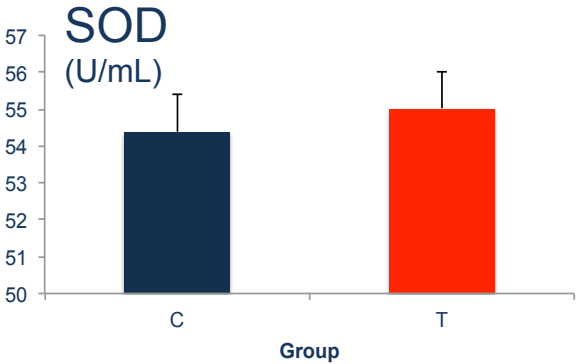

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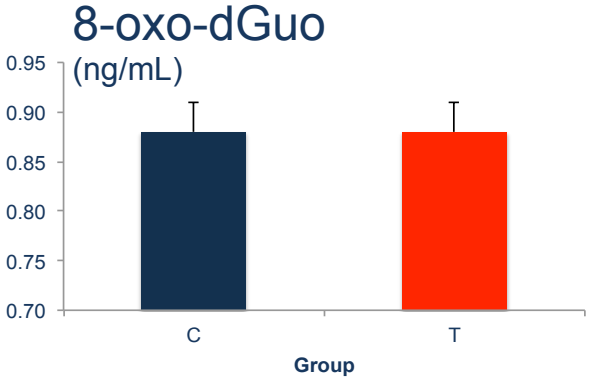
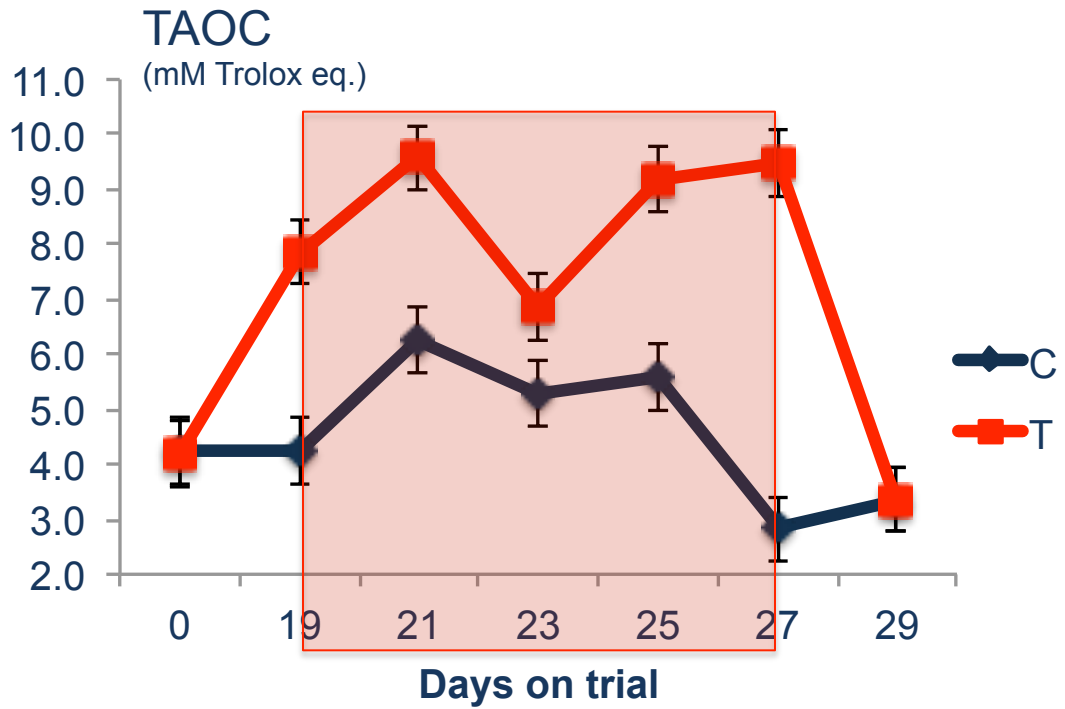


Significant challenge effect on Hp (P<0.03) and trend on TAOC (P=0.08)

RESULTS

Figure 16. Effect of Melofeed® on Total Antioxidant Oxidative Capacity (TAOC) of weaned piglets

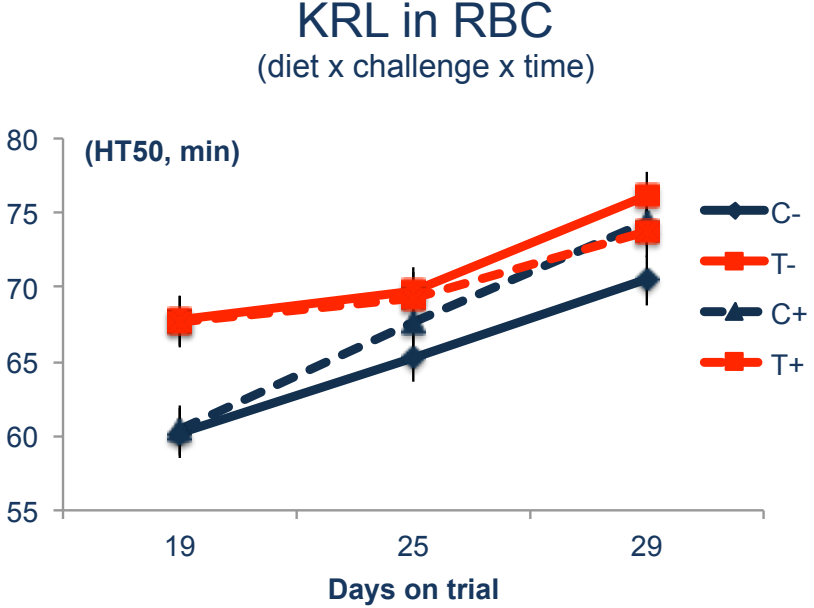


Diet	P<0.01
Challenge	P=0.08
Time	P<0.01
Diet x Time	P=0.08

RESULTS

Figures 17,18. Effect of Melofeed® and LPS challenge on Kit Radicaux Libres (KRL) in RBC of weaned piglets

17



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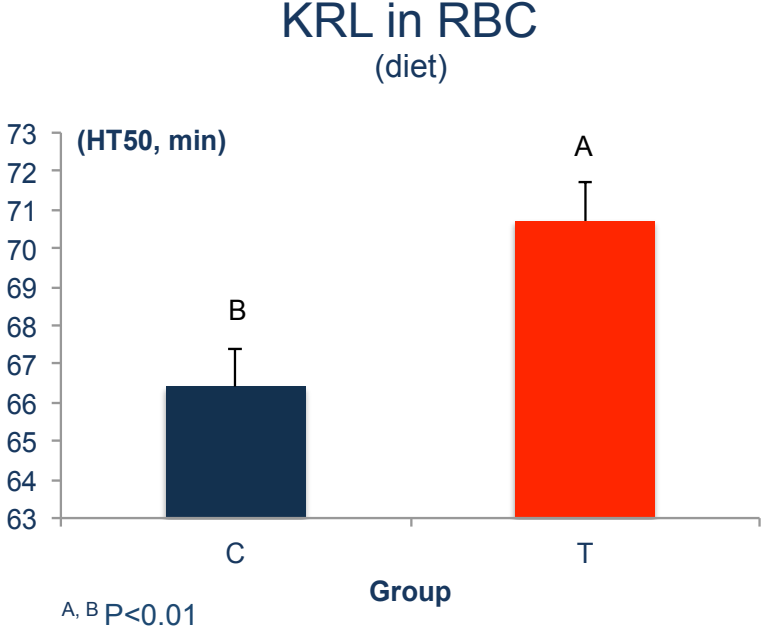

DISCUSSIONS

Melon concentrate administration to postweaning piglets leads to:

- Improved growth performance (differently from Lallès et al., 2010), independently from the LPS challenge
- Higher antioxidant plasma levels (TAOC), as reported by Vouldoukis et al. (2004) *in vitro*
- Increased RBC resistance to haemolysis, according with Vouldoukis et al. (2004) *in vitro* and Notin et al. (2010) in horses

- No effects on proinflammatory ILs, SOD and ROS

DISCUSSIONS

What is the mechanism of action of exogenous SOD?

Beside the chelating effect that can lower the oxidative stress, the question is still debated:

- Does it pass the intestinal barrier?
- Does it work as an antigen in the intestine, leading to an increase of the local immune response and regulating some nuclear factors? (e.g.: NFR2) (Carillon et al., 2013)

- NEXT STEP: Pending analyses for gene expression in liver for SOD, CAT and NFR2….

CONCLUSIONS

The administration of SOD-rich melon concentrate was able to improve some oxidative markers and growth performance of LPS challenged weaning piglets.

Thank you for your attention!

Any questions?