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The effects of superoxide dismutase-rich melon pulp concentrate on inflammation, antioxidant status and growth performance of challenged post-weaning piglets

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Short title: Melon pulp concentrate fed to challenged piglets

Abstract

Piglets can often suffer impaired antioxidant status and poor immune response during post-weaning, especially when chronic inflammation takes place, leading to lower growth rates than expected. Oral administration of dietary antioxidant compounds during this period could be a feasible way to balance oxidation processes and increase health and growth performance. The aim of the trial was to study the effects of an antioxidant feed supplement (melon pulp concentrate) that contains high concentration of the antioxidant superoxide dismutase (**SOD**) on inflammation, antioxidant status and growth performance of lipopolysaccharide (**LPS**) challenged weaned piglets. Forty-eight weaned piglets were individually allocated to four experimental groups in a 2 x 2 factorial design for 29 days. Two different dietary treatments were adopted: a) Control (**CTR**), fed a basal diet, b) Treatment (**MPC**), fed the basal diet plus 30g/ton of melon pulp concentrate. On days 19, 21, 23 and 25 half of the animals within CTR and MPC groups were subjected to a challenge with intramuscular injections of an increasing dosage of LPS from *E. coli* (serotype 0.55:B5) (**+**) or were injected with an equal amount of PBS solution (**-**). Blood samples were collected at the beginning of the trial and under the challenge period for interleukin 1β, interleukin 6, tumour necrosis factor α, haptoglobin**,** plasma SOD activity, total antioxidant capacity, reactive oxygen species, red blood cells and plasma resistance to haemolysis, and 8-oxo-7, 8-dihydro-2'-deoxyguanosine. Growth performance was evaluated weekly. A positive effect of melon pulp concentrate was evidenced on total antioxidant capacity, half-haemolysis time of red blood cells, average daily gain and feed intake, while LPS challenge increased proinflammatory cytokines and haptoglobin serum concentrations, with a reduced feed intake and gain:feed. The obtained results show that oral SOD supplementation with melon pulp concentrate ameliorates the total antioxidant capacity

and the half-haemolysis time in red blood cell of post-weaning piglets, with positive results on growing performance.

Keywords: Cucumis melo, total antioxidant capacity, growth, lipopolysaccharide, pig.

Implications

Piglets can show impaired antioxidant status and inflammation processes during post-weaning, leading to lower growth rates than expected. This is especially true when occasional presence of pathogens in the farm causes a chronic inflammation status. In this context superoxide dismutase administration could be a feasible way to overcome inflammation, impaired antioxidant status, and low performance. The present study demonstrates that oral supplementation with melon pulp concentrate, rich in superoxide dismutase, is able to increase the total antioxidant capacity of weaned pigs, contributing to sustain the health status and the growth rate of these animals.

Introduction

Inflammation and oxidative stress are closely linked together (Kick *et al.*, 2012; Carillon *et al.*, 2013a) and lead to decreased growth rates and feed efficiency in weaned piglets. This is mainly due to the imbalance in oxidant/antioxidant equilibrium, the decreased activity of major antioxidant enzymes, and the increase in radical-mediated lipid peroxidation and protein and DNA oxidation (Campbell *et al.*, 2013; Gessner *et al.*, 2017). In this context, the use of dietary antioxidant compounds after weaning seems to be a feasible way to overcome impaired antioxidant status and poor immune response in piglets (Bontempo *et al.*, 2014; Jiang *et al.*, 2015a,b). Nowadays

there is great interest in the therapeutic application of dietary superoxide dismutase (**SOD**) (Carillon *et al.*, 2013a,b), as a primary antioxidant molecule. A specific cantaloupe melon (*Cucumis melo L.*) from the *Cucurbitaceae* family, known to be characterized by high SOD activity, showed antioxidant and anti-inflammatory properties in *in vitro* and *in vivo* animal models such as rodents and horses (Vouldoukis *et al.*, 2004a,b; Notin *et al.*, 2010).

Although some trials have been conducted on orally melon concentrate administration in different species, at the present moment the effects of feeding SOD-rich melon pulp concentrate on piglets subjected to stress are only reported in two studies by Lallès *et al.* (2011) and Royer *et al*. (2016) on stress proteins along the gastrointestinal tract and changes in blood oxidative stress biomarkers, respectively. At experimental level, oxidative and inflammatory stress conditions can be mimed by chronic immune system stimulation with an increasing dose of lipopolysaccharide (**LPS**) challenge (Rakhshandeh and de Lange, 2012). The aim of the present study is to evaluate the effects of oral supplementation of a SOD-rich melon pulp concentrate in increasing antioxidant status, inflammation response, and growth performance in LPS-chronically challenged weaning piglets.

Material and methods

Animals housing and experimental design

The present trial was performed at the Centro Clinico-Veterinario e Zootecnico-Sperimentale d'Ateneo di Lodi, Università degli Studi di Milano. In total, 48 crossbred female piglets (Topigs 40 x Topdelta) from the same herd were weaned at 24 ± 1 days of age (BW 7.79 ± 0.17 kg) and divided in four homogeneous experimental groups of

97 twelve animals each in a 2 x 2 factorial arrangement. The piglets were placed in
98 individual pens (0.47 m^2) and allocated in the same environmentally-controlled post-
99 weaning room on slatted floor. Each pen was equipped with one standard nursery pig
100 bite-style nipple drinker and a self-feeder to allow for *ad libitum* access to water and
101 feed. Room temperature and ventilation were electronically controlled over a 24 hours
102 period. Starting room temperature was 28°C with a ventilation of 10 m^3/hour/piglet and
103 was decreased by 1°C/week until 25°C at the end of the trial.
104 The first factorial arrangement consisted of the administration of a basal diet or
105 the same basal diet plus melon pulp concentrate (Melofeed, Lallemand SAS, Blagnac,
106 France). The second factorial arrangement consisted on a LPS challenge, with
107 repeated increasing intramuscular injections of LPS from *E. coli* (serotype 055:B5,
108 Sigma-Aldrich Canada Ltd, Oakville, ON, Canada; cat. no. L2880) (**+**) to mimic chronic
109 inflammation, or the injection of an equivalent amount of PBS solution (**-**). The
110 challenge was performed starting on day 19 of the trial and subsequent injections were
111 performed on days 21, 23 and 25. The initial LPS dosage of 60 µg/kg of BW was
112 increased by 12% at each subsequent injection to reduce endotoxin tolerance
113 (Rakhshandeh and de Lange, 2012). Specifically, the applied concentrations of LPS
114 from the second to the fourth injection day were 67.2, 75.26, and 84.30 µg/kg of BW.
115 Individual body weight was determined prior to each LPS injection to calculate
116 individual total LPS amount to be injected.
117

Experimental diets

To avoid any other influencing stress factor besides the weaning and the challenge, in the present trial pigs were fed with a mash wheat-based basal diet (Table 1) for all the trial period with (**MPC**) or without (**CTR**) the inclusion of 30 g/ton of melon pulp concentrate. Melon pulp concentrate was provided in powder form and incorporated through the premix at 1.5 kg/ton of feed to get the final content of 30g of Melofeed/ton of feed (Lalleman SAS, Blagnac, France). The applied dosage of melon pulp concentrate was based on the previous publication of Lallès *et al.* (2001) where Promutase (former name of Melofeed) was supplemented at 5 and 20 g/ton feed. Because a decrease of feed intake was expected due to the LPS challenge, in the present trial the dosage was increased to 30 g/ton feed during the whole experimental period.

The experimental diets were formulated to be isocaloric and isoproteic on net energy and CP basis, and were produced with the same batches of feeds by Tracciaverde S.R.L. (Bonemerse, Italy). Both CTR and MPC diets did not contain any antimicrobials or growth promoters, being designed to meet or exceed the nutrient requirements of weaned piglets recommended by National Research Council (NRC, 2012), with the exception of suboptimal concentration of tryptophan. In the present trial no inclusion of tryptophan was performed besides its content in the feeds in order to amplify the inflammation effect of the LPS challenge performed in the experimental animals (Le Floc'h and Seve, 2007).

Samples and data collection

Blood samples for pro-inflammatory interleukin 1β (**IL-1β**), interleukin 6 (**IL-6**) and tumour necrosis factor α (**TNF-α**) and haptoglobin were collected on days 0, 19,

21, 23, 25, and 29, while blood samples for plasma SOD activity, total antioxidant capacity (**TAOC**), and reactive oxygen species (**ROS**) serum content were obtained on days 0, 19, 21, 23, 25, 27, and 29. Each blood sample under the challenge period was collected prior the LPS injection. For sampling procedures a 10 mL clot activator vacutainer tube (VF-109SP, Venoject®, Terumo Europe N.V., Leuven, Belgium) was used to yield serum from the cranial vena cava. Blood sample for plasma SOD activity was collected separately using a 4 mL vacutainer tube containing EDTA (VF054STK, Venoject®, Terumo Europe N.V., Leuven, Belgium). The serum was obtained by allowing the whole blood to clot at room temperature for 30 min. The tubes were then centrifuged at 1500 x g, 10 min at 4°C, the supernatant was placed in 2 mL Eppendorf tubes (Eppendorf AG, Amburg, Germany) and subsequently stored at -80°C to prevent changes in antioxidant and inflammation biomarkers. Blood samples for the whole blood and red blood cells (**RBC**) resistance to haemolysis and plasma contribution by Kit Radicaux Libres (**KRL**), and 8-oxo-7, 8-dihydro-2'-deoxyguanosine (**8-oxodGuo**) were collected on days 19, 25, and 29 of the trial. Blood samples for KRL test were collected by 4 mL vacutainer tubes containing EDTA (VF054STK, Venoject®, Terumo Europe N.V., Leuven, Belgium) (Rossi *et al.*, 2013), immediately stored at 4°C, processed within three hours from sampling, and analysed in the next 24 hours after collection. Blood samples for 8-oxodGuo were collected with a 10 mL clot activator vacutainer tube (VF-109SP, Venoject®, Terumo Europe N.V., Leuven, Belgium) and serum was obtained by centrifugation at 1500 x g, 10 min at 4°C. Serum was then placed in a 2 mL Eppendorf tube (Eppendorf AG, Amburg, Germany), and stored at -80°C for pending analysis.

A sample of the basal diet was collected at the beginning of the trial and stored at -20°C for pending analyses. Individual piglet BW was recorded on days 0, 8, 15, 19,

21, 23, 25, 26 and 29 with an electronic scale (ES100L, Ohaus, Switzerland), and individual feed intake **(FI)** for 0-19 and 19-29 days periods was calculated by subtracting the relative orts to the total daily-administered amount of feed. Subsequently average daily gain (**ADG**) and gain:feed (**G:F**) were calculated. The health status of the piglets was daily checked and any sanitary treatment was recorded. Morbidity and mortality were recorded. Rectal temperature was manually measured before each LPS injection as a baseline measurement, after two hours from each LPS injection, and on the last day of trial.

Chemical analysis

Serum pro-inflammatory cytokines IL-1β, IL-6 and TNF-α were measured by porcine-specific ELISA kit according to the recommendations of the manufacturer (R and D Systems Inc., Abingdon Science Park, UK). The serum concentrations of haptoglobin were determined by colorimetric assay (Tridelta Phaserange serum haptoglobin assay, Cat. No. TP-801) and expressed on the basis of a standard curve (Cooke and Arthington, 2013). All samples were assayed in duplicate. Intra-assay coefficient of variation were 5.71%, 6.33%, 5.83%, and 7.41%, while inter-assay coefficient of variation were 5.59% 6.38%, 5.34%, and 6.18% respectively for IL-1β, IL-6, TNF-α, and haptoglobin. All the intra- and inter-assay coefficients of variations were within the range of values declared by the commercial product datasheet. Plasma SOD activity and serum TAOC were measured using commercial available kits according to the manufacturer's instructions (Sigma-Aldrich, Cat. No. 19160 and Cat. No. CS0790, respectively), while ROS were evaluated by Cyt C reduction assay (Sigma Aldrich, cat. No. C2506), as reported by Sartorelli *et al.* (2000). The total antiradical activity of whole blood, RBC and the plasma contribution was determined

using the KRL biological test (Rossi *et al.*, 2013). Results were expressed as the time (min) that is required to reach 50% of maximal haemolysis. Half haemolysis time for total blood cells (**HT_{50}WB**) and for red blood cells (**HT_{50}RBC**) refers to the whole blood and the red cell resistance to free radical attack, respectively. Lastly, the plasma resistance to haemolysis (**HT_{50}PC**) was calculated by subtracting HT_{50}RBC from HT_{50}WB. Serum concentration of 8-oxodGuo were determined using a competitive ELISA method based on monoclonal antibody highly specific to 8-OHdG (Japan Institute for Control Aging Fukuroi, Japan) following the manufacturer's instructions. For 8-oxodGuo assays six different standard dilutions were used in triplicate for each ELISA plate. Intra- and inter -assay coefficients of variation were respectively 5.56% and 8.86%.

The chemical composition of the basal diet was analysed at the beginning of the trial to determine DM (method 930.15), CP (method 984.13), ether extract (method 920.39A), ash (method 942.05), Ca (method 968.08) and P (method 946.06) content following the relative Association of Official Analytical Chemists methods of analysis (AOAC, 2005) (Table 1). Neutral detergent fibre content in the diet was determined as reported by Van Soest *et al.* (1991).

Statistical analyses

Data relative to inflammatory and oxidative biomarkers, BW, and rectal temperature were performed by ANOVA using the Proc MIXED for repeated measurements in SAS (SAS Inst. Inc., Cary, NC), with the piglet as the experimental unit. The statistical model included the effects of LPS challenge, dietary treatment (CTR, MPC), time and their interaction as fixed effects. Average daily gain, FI and G:F considered two different trial periods corresponding to days 0-19 (pre-challenge) and

19-29 (challenge and post-challenge) of the trial. Statistical analyses for these parameters were performed by a GLM procedure in SAS with the piglet as the experimental unit and the corresponding data relative to the pre-challenge period (0-19 days) as covariate for 19-29 days period. Differences were considered significant for $P<0.05$. A tendency toward a significant difference between treatment means was also considered at $P<0.10$.

Results

Inflammatory and oxidative biomarkers status

In the present trial LPS challenge induced a strong effect on inflammatory status with increased serum concentration of IL-1β (0.53 ng/mL vs. 0.21 ng/mL, $P<0.01$), IL-6 (6.99 x 10^3 ng/mL vs. 3.10 x 10^3 ng/mL, $P<0.01$), TNF-α (2.56 x 10^{-2} ng/mL vs. 1.44 x 10^{-2} ng/mL, $P<0.01$), and haptoglobin (8.96 x 10^5 ng/mL vs. 7.82 x 10^5 ng/mL, $P<0.05$) (Figure 1), while melon pulp concentrate supplementation did not induce any significant change in these parameters. Time effect and the interaction between LPS challenge and time were always significant ($P<0.01$) (Figure 1). No significant differences between challenged or not challenged piglets were found at the beginning of the trial for IL-1β, IL-6, and TNF-α, while a higher haptoglobin content was found in piglets not subjected to LPS on day 0 (9.50 x 10^5 ng/mL vs. 5.62 x 10^5 ng/mL; $P<0.01$). Starting form day 21 challenged groups showed increased concentration of all inflammation biomarkers until day 23 for haptoglobin or day 25 for interleukins and TNF-α ($P<0.01$).

Oxidative biomarkers were not affected by LPS challenge (Table 2), although some trends were found for decreased TAOC (5.30 mM Trolox equivalent vs. 6.46 mM

243 Trolox, *P*=0.08), and increased HT_{50}WB (113.78 min vs. 109.14 min, *P*=0.09) and
244 HT_{50}PC (44.90 min vs. 40.89 min, *P*=0.08). Melon pulp concentrate supplementation
245 increased serum TAOC (7.22 mM Trolox equivalent vs. 4.54 mM Trolox equivalent,
246 *P*<0.01) and HT_{50}RBC (70.71 min vs. 66.41 min, *P*<0.01), but did not induce significant
247 effects on HT_{50}WB, HT_{50}PC, 8-oxo-dGuo concentration, SOD activity, and ROS.
248 Sampling time was always significant (*P*<0.01), with the exception of SOD activity and
249 HT_{50}PC, but no difference was detected considering the interaction between dietary
250 treatment, challenge and time.
251
252 *Growth performance and rectal temperature*
253 No significant effects of LPS challenge (*P*=0.47) or melon pulp concentrate
254 supplementation (*P*=0.70) and their interactions (*P*=0.73) were found on BW. The LPS
255 challenge impaired ADG (291 g/d vs. 490 g/d, *P*<0.01), FI (5.57 kg vs. 7.76 kg, *P*<0.01)
256 and G:F (0.52 vs. 0.62, *P*<0.01) from days 19 to 29. Average daily gain and FI were
257 increased in pigs fed melon pulp concentrate under the challenge and post-challenge
258 period (19-29 days on trial) (422 g/d vs. 359 g/d for ADG, P<0.05; 7.18 kg vs. 6.15 kg
259 for FI, *P*<0.01) (Table 3), but no differences were found during the first 19 days of the
260 trial and overall the experimental period. No significant differences were outlined for
261 G:F among the dietary treatments, and the diet x challenge interaction was found not
262 to affect growing performance.
263 Rectal temperature was always increased by LPS challenge at two hours after
264 injection (*P*<0.01, data not shown), while no effect of dietary melon pulp concentrate
265 supplementation was evidenced, with the exception of a significant reduction at two
266 hours after the fourth LPS injection (day 25 of the trial) (CTR= 40.25 ± 0.09 °C vs.
267 MPC= 40.12 ± 0.08 °C, *P*=0.02). No differences in piglet's rectal temperature at the

end of the trial (day 29) were found between CTR and MPC groups, also considering the challenge effect or the diet x challenge interaction. During the trial no antibiotic treatments were performed on experimental animals and no deaths occurred within CTR and MPC groups.

Discussion

Weaning is a critical stress period for piglets and it is characterized by decreased immune response, FI, and nutrient absorption (Niekamp *et al.*, 2007). During this period, pigs are subjected to a number of stressors such as a sudden separation from the sow, transportation and handling, different environment, liquid to solid feed shift, establishment of a new social hierarchy, and increased exposure to pathogens. All these factors lead to metabolism, immune system, and intestinal functions alterations. Several studies report how serum pro-inflammatory cytokines and their gene expression are increased at weaning (Pié *et al.*, 2004; Lallès *et al.*, 2011). In accordance with this, in the present trial initial high concentrations of IL-6 and TNF-α were found in all the experimental subjects, although they were decreased just before the LPS challenge (day 19). At the starting of the trial IL-6 showed half of the concentration further evidenced during the challenge, while TNF-α concentration detected on day 0 and during the LPS injection were comparable, outlining how weaning is able to strongly affect the immune response of piglets.

Pro-inflammatory cytokines IL-1β, IL-6, and TNF-α are reported to be important inducers of the synthesis of acute phase proteins, such as haptoglobin, among others (Carroll *et al.*, 2004). In the present trial a higher concentration of haptoglobin in both no-challenge groups compared to challenge groups was found at the beginning of melon pulp concentrate supplementation. Although all the experimental animals were

293 selected, grouped and placed in the experimental facilities at the same time, this initial
294 difference seems to be unfortunately related to some un-accounted (not estimable)
295 stress factors (Salamano *et al.*, 2008; Cray *et al.*, 2009) rather than a direct effect of
296 high concentration of interleukins that could lead to increased levels of haptoglobin.

297 Besides the strong effect of weaning, occasional presence of pathogens in the
298 farm can causes a chronic inflammation status that can further impairs the immune
299 response and the antioxidant status of piglets. This last scenario can be efficiently
300 obtained from an experimental point of view applying a chronic challenge in the post-
301 weaning period. With this purpose, in the present trial a chronic challenge procedure
302 with LPS was adopted to mimic subclinical or mild clinical disease conditions that
303 frequently occur in the field due to the presence of pathogens that lead to immune
304 system stimulation (Rakhshandeh and de Lange 2012; de Ridder *et al.*, 2012). As a
305 result, in the present trial LPS was found to act on the immune system through
306 increased concentration of serum pro-inflammatory cytokines IL-1β, IL-6, and TNF-α
307 and haptoglobin according to Carroll *et al.* (2001) and Frank *et al.* (2005), while the
308 administration of melon pulp concentrate did not lead to any significant variation on
309 immune response.

310 Inflammation and oxidative stress have been reported to be closely linked to
311 each other, since the pathways generating the mediators of inflammation are all
312 induced by oxidative stress. (Carillon *et al.*, 2013a). Oxidative stress decreases
313 antioxidant defences and induces elevated radical-mediated lipid peroxidation driven
314 by reactive oxygen and nitrogen species (Campbell *et al.*, 2013). At weaning, piglets
315 can be subjected to an accumulation of ROS that could exceed the antioxidant
316 capacities of the animal, leading to a high susceptibility to oxidative stress (Zhu *et al.,*
317 2012).

As a strong effect of the LPS challenge on immune response can be highlighted in this trial, the general lack of significant changes in oxidative biomarkers, with the exception of a trend for decreased serum content of TAOC, can be attributed to other numerous factors. Moreover, independently from the applied dosage to mimic a chronic inflammation in the present trial, it must be noted that the effects of an immune challenge did not always lead to univocal results on oxidative stress biomarkers, depending on the quantification of different antioxidant or pro-oxidant components of oxidative stress (van de Crommenacker *et al.*, 2010). In the present trial oxidative markers such as plasma SOD activity, ROS and 8-oxo-dGuo were not influenced by melon pulp administration, while TAOC and HT_{50}RBC were improved in MPC groups. If the obtained results on ROS can be attributed to their reported very short half-life and high instability, the SOD activity could have been influenced by several factors and can be interpreted in different ways, as the activity of major antioxidant enzymes (**AOX**) is quite variable depending on the experimental design adopted, the challenge performed or the animal species. Increased activity of AOX (SOD, CAT, GPx) can be related both to the reaction of the organism to oxidative stress, or to the stimulation of antioxidant defences when supplying antioxidant compounds in the diet. To discriminate between these two opposite interpretations, additional biomarkers of oxidative stress, such as oxidized proteins (e.g. protein carbonyls) or oxidized lipids (e.g. TBARS, MDA, isoprostanes, lipid peroxides) could have been measured. In fact a study by Royer *et al.* (2016) found that a decreased concentration of oxidative stress biomarkers and an increased activity of AOX confirmed the efficacy of melon pulp concentrate supplementation in pigs. Although dietary SOD mechanism of action is not fully understood at the present moment, Carillon *et al.* (2013a) suggested that melon pulp concentrate administration could induce increased antioxidant defence, as

343 outlined by TAOC and RBC blood resistance to haemolysis in the present trial, through
344 the activation of mRNA transcription or the regulation or induction of complex cellular
345 pathways, involving different transcription factors. These authors hypothesized that the
346 induction of antioxidant enzymes could be regulated at the transcriptional level through
347 the nuclear-factor-E2-related factor (Nrf2)/antioxidant response as demonstrated in
348 some other studies with different antioxidant supplements in humans and *in vitro*
349 (Muchová *et al.*, 2001; Zhang *et al.*, 2012).

350 In the present study, decreased FI and impaired growth performance during
351 LPS challenge period were observed according with Gessner *et al.* (2017). Lower
352 performance under the challenged corresponded to a higher concentration of
353 inflammation markers and an increased rectal temperature of the piglets at two hours
354 after each LPS injection as a sign of the presence of a systemic inflammation (Ceciliani
355 *et al.*, 2012). When an infection occurs, metabolic shifts are characterized by the
356 redistribution of nutrients away from the growth processes toward the immune system
357 function, with a subsequent decrease in feed efficiency for growth (Gessner *et al.*,
358 2017). Moreover the suboptimal concentration of tryptophan supplied in the present
359 trial (approximately 78% of the requirements on total basis for piglets ranging from 7
360 to 25 kg BW) (NRC, 2012) could have represent an amplification factor for the
361 effectiveness of the applied challenge, since tryptophan metabolic demand is strongly
362 increased by the synthesis of acute phase proteins during immune stimulation, with a
363 consequent lower availability for growth (de Ridder *et al.*, 2012).

364 Besides the inflammation effect of the applied challenge, the administration of
365 melon pulp concentrate led to increased performance, which instead was not observed
366 by Lallès *et al.* (2011). It must be outlined that the total duration of administration in the
367 work of Lallès *et al.* (2011) was 5 or 12 days, after an initial two-days period of fasting

to induce greater stress protein concentrations. Animals were then slaughtered on days 7 and 14 after weaning. The lack of improved performance in the work of Lallès *et al.* (2011) can be probably due to the short duration of melon pulp concentrate supplementation to translate any positive effect in the gastrointestinal tract or on immune response into improved growth performance.

In conclusion, the present study demonstrates that oral supplementation with melon pulp concentrate rich in SOD during the post-weaning period can enhances the total antioxidant capacity, half-haemolysis time of red blood cells and growth rate of post-weaning piglets when oxidative stress and/or inflammation are increased.

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Declaration of interest

The authors declare no conflicts of interest

Ethics statement

The protocol for care, handling, and sampling of animals defined in the present study was reviewed and approved by the Università degli Studi di Milano Animal Care and Use Committee (Protocol No 82/14).

Software and data repository resources

Data or models from the present work are not deposited in an official repository

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498 **Tables**

499 **Table 1** *Ingredients (% w/w) and chemical composition (g/100g dry matter) of the basal diet*

500 *fed to the experimental piglets*

501

Ingredients	Feeds
Wheat meal	29.47
Barley meal	23.12
Wheat flaked	14.00
Soybean meal, 48.0% CP	17.00
Sweet whey powder	6.00
Soybean oil	3.00
Corn gluten meal	2.50
Dextrose monohydrate	1.50
Dicalcium phospate	1.30
L-lysine, 78% Lys	0.57
Calcium carbonate	0.50
Sodium chloride	0.30
Vitamin-mineral premix[1]	0.25
L-Thr	0.23
DL-Met	0.18
Flavour	0.05
Sweetener[2]	0.01
Zinc oxide	0.01
Cu sulphate	0.01
Chemical composition[3] (g/100g DM)	
DM	88.70
CP	18.01

Ether extract	4.52
Ash	5.08
NDF	14.59
Ca	0.75
Total P	0.57
Digestible energy (Mcal/kg DM)	3.44
Net energy, (Mcal/kg DM)	2.45
Lys	1.25
Met+Cyst	0.80
Thr	0.85
Trp	0.21

Lys = lysine; Thr = threonine; Met = methionine; Cyst = cysteine; Trp = tryptophan

[1]The vitamin-mineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A, 10,500 IU; vitamin D3, 2500 IU; vitamin E, 15 mg; vitamin B1, 1.5 mg; vitamin B2,3.8 mg; vitamin B12, 0.025 mg; vitamin B6, 1.6 mg; calcium pantotenate, 12 mg; nicotinic acid, 15 mg; biotin, 0.15 mg; folic acid, 0.5 mg; vitamin K3, 3 mg; Fe, 100 mg; Cu, 6 mg; Co, 0.75 mg; Zn, 150 mg; Mn, 65 mg; I, 0.75 mg; Se, 0.3 mg; ethoxyquin, 150 mg.

[2]The sweetener (Optisweet®) was provided by Nutriad (Dendermonde, Belgium)

[3]Feeds were analysed for DM, CP, ether extract, ash, NDF, Ca and total P according to AOAC (2005). All other values were calculated from NRC (2012).

Table 2 *Mean concentration of oxidative biomarkers[1,2] of post-weaning piglets supplemented with melon pulp concentrate in the diet and subjected to a chronic LPS challenge[3,4].*

	LPS challenge				SEM[5]	*P*-values		
	-		+					
	Dietary treatments[2]							
	CTR	MPC	CTR	MPC		Diet	Challenge	Diet X Challenge
N. of piglets[6]	12	12	12	12				
SOD (IU/mL)	54.50	54.50	54.28	55.54	3.69	0.66	0.77	0.65
TAOC (mM Trolox equivalent)	4.97	7.94	4.11	6.49	1.71	<0.01	0.08	0.66
ROS (Abs)	0.16	0.15	0.15	0.15	0.03	0.73	0.62	0.74
8-oxo-dGuo (ng/mL)	0.89	0.84	0.87	0.91	0.06	0.99	0.40	0.26
HT_{50}WB (min)	106.05	112.23	113.38	114.17	4.60	0.20	0.09	0.32
HT_{50}RBC (min)	65.31	71.19	67.51	70.23	2.40	<0.01	0.66	0.26
HT_{50}PC (min)	40.74	41.03	45.87	43.94	3.89	0.72	0.08	0.62

LPS = lipopolysaccharyde; SEM = standard error of the means; SOD = superoxide dismutase; TAOC = total antioxidant activity, ROS = reactive oxygen species; 8-oxo-dGuo = 8-oxo-7, 8-dihydro-2'-deoxyguanosine, HT_{50}WB = half haemolysis time of whole blood, HT_{50}RBC = half haemolysis time of red blood cells; HT_{50}PC = plasma contribution to half haemolysis.

516

517 [1]SOD and TAOC were performed on plasma samples; ROS and 8-oxo-dGuo were performed in serum; HT_{50}WB and HT_{50}RBC were performed on whole
518 blood. HT_{50}PC was obtained as the difference between HT_{50}WB-HT_{50}RBC.

519 [2]Total antioxidant activity was performed by KRL (Kit Radicaux Libres) biological test (Rossi *et al.*, 2013). Results are expressed as the time (min) required to
520 reach 50% of maximal haemolysis (HT_{50}), which refers to the whole blood, red blood cells and plasma resistance to free-radical attack.

521 [3]The challenge was performed from day 19 to 25 of the trial with increasing dosages of lipopolysaccharide (LPS from *E. coli* serotype 055:B5). Subsequent
522 intramuscular injections of LPS were performed on days 19, 21, 23 and 25. Initial concentration of LPS was 60 µg/kg of BW and the dosage was increased by
523 12% at each subsequent injection to reduce endotoxin tolerance and mimic a chronic inflammation in piglets. The applied concentrations of LPS from the second
524 to the fourth injection day were 67.2, 75.26, and 84.30 µg/kg of BW. Individual body weight was determined prior each LPS injection to calculate individual total
525 LPS amount to be injected.

526 [4]Dietary treatments: CTR-= piglets fed the basal diet and not subjected to the LPS challenge; CTR+= piglets fed the basal diet and subjected to the LPS
527 challenge; MPC-=piglets fed the basal diet added with 30g/ton of melon pulp concentrate (Melofeed, Lallemand SAS, Blagnac, France) and not subjected to
528 LPS challenge; MPC+= piglets fed the basal diet added with 30g/ton of melon pulp concentrate (Melofeed, Lallemand SAS, Blagnac, France) and subjected to
529 LPS challenge.

530 [5]SEM = pooled SEM. Means are presented as least square means.

531 [6]Piglets were reared in individual pens (0.47 m^2) with *ad libitum* access to feed and water.

532 **Table 3** *Body weight (BW), average daily gain (ADG), feed intake (FI) and gain:feed (G:F) of post-weaning piglets supplemented with melon*

533 *pulp concentrate in the diet and subjected LPS challenge*[1]

	LPS challenge				SEM[4]	*P*-values		
	-		+					
	Dietary treatments[2]							
	CTR	MPC	CTR	MPC		Diet	Challenge	Diet X Challenge
N. of piglets[3]	12	12	12	12				
BW (kg)								
Day 0	7.79	7.79	7.78	7.79	0.71	0.70	0.47	0.73
Day 29	17.94	18.48	16.98	17.37				
ADG (g/d)								
Days 0 to19	298	297	343	328	51.98	0.79	0.13	0.78
Days 19 to 29	464	517	255	327	60.69	0.05	<0.01	0.75
Days 0 to 29	350	368	317	331	56.34	0.50	0.14	0.91
FI[4] (kg/period)								
Days 0 to 19	8.56	8.55	9.50	9.31	1.24	0.87	0.18	0.89
Days 19 to 29	7.31	8.22	4.99	6.15	0.67	<0.01	<0.01	0.73

Days 0 to 29	15.74	16.63	14.65	15.56	1.75	0.31	0.22	0.10
G:F								
Days 0 to19	0.64	0.65	0.69	0.66	0.04	0.71	0.12	0.32
Days 19 to 29	0.62	0.63	0.51	0.53	0.05	0.64	<0.01	0.72
Days 0 to 29	0.64	0.64	0.63	0.61	0.03	0.69	0.18	0.41

534 LPS = lipopolysaccharyde; SEM = standard error of the means

535 [1]The challenge was performed from d 19 to 25 of the trial with increasing dosages of lipopolysaccharide (LPS from *E. coli* serotype 055:B5). Subsequent
536 intramuscular injections of LPS were performed on days 19, 21, 23 and 25. Initial concentration of LPS was 60 µg/kg of BW and the dosage was increased by
537 12% at each subsequent injection to reduce endotoxin tolerance and mimic a chronic inflammation in piglets. The applied concentrations of LPS from the second
538 to the fourth injection day were 67.2, 75.26, and 84.30 µg/kg of BW. Individual body weight was determined prior each LPS injection to calculate individual total
539 LPS amount to be injected.

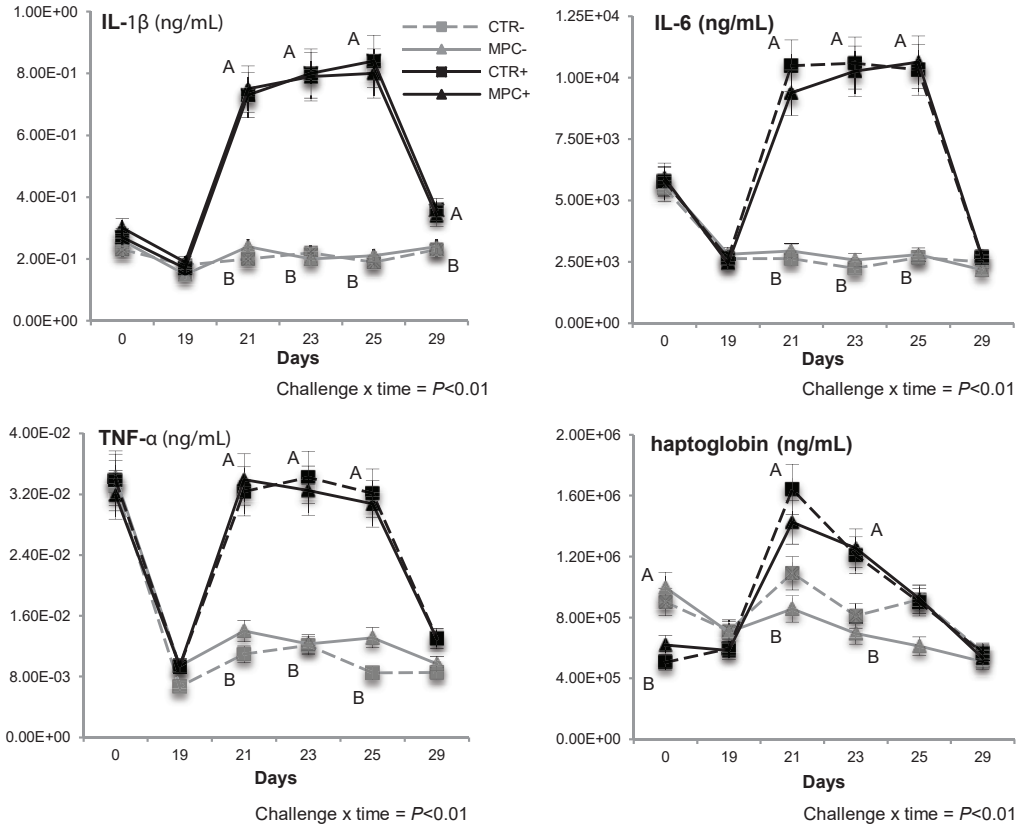
540 [2]Dietary treatments: CTR-= piglets fed the basal diet and not subjected to the LPS challenge; CTR+= piglets fed the basal diet and subjected to the LPS
541 challenge; MPC-=piglets fed the basal diet added with 30g/ton of melon pulp concentrate (Melofeed, Lallemand SAS, Blagnac, France) and not subjected to
542 LPS challenge; MPC+= piglets fed the basal diet added with 30g/ton of melon pulp concentrate (Lallemand SAS, Blagnac, France) and subjected to LPS
543 challenge.

544 [3]Piglets were reared in individual pens (0.47 m^2) with *ad libitum* access to feed and water.

[4]SEM = pooled SEM. Means are presented as least square means.

List of figure captions

Figure 1 Challenge effect on Interleukin 1β (IL-1β), Interleukin 6 (IL-6), Tumour Necrosis Factor α (TNF-α) and haptoglobin serum concentration in post-weaning piglets supplemented with melon pulp concentrate[1] in the diet and subjected to chronic LPS challenge[2,3]



[1]Dietary treatments: CTR-= piglets fed the basal diet and not subjected to the LPS challenge; CTR+= piglets fed the basal diet and subjected to the LPS challenge; MPC-=piglets fed the basal diet added with 30g/ton of melon pulp concentrate (Melofeed, Lallemand SAS, Blagnac, France) and not subjected to LPS challenge; MPC+= piglets fed the basal diet added with 30g/ton of melon pulp concentrate (Melofeed, Lallemand SAS, Blagnac, France) and subjected to LPS challenge.

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[3A,B]Different letters refer to significant differences between challenged and not challenge piglets for $P<0.01$.