Impact of zinc and arginine on antioxidant status of weanling piglets raised under commercial conditions

Nadia Bergeron, Frédéric Guay

Département des Sciences Animales, Université Laval, Québec, G1V 0A6, Canada

1. Introduction

When piglets are raised under conditions described as natural, the process of weaning begins at 10 weeks of age and ends at 4 months of age (Jensen and Recén, 1989; Jensen and Stangel, 1992). This normally involves a gradual reduction in milk intake and an increase in water and solid (cereal-based) feed intake. In contrast, weaning under commercial conditions begins at about 3 to 4 weeks of age, when the piglets are separated from the sow and moved to pens shared with unfamiliar cohorts. This abrupt change in diet, ambient sounds and social environment causes the animal to experience increased systemic inflammation and over-expression of pro-inflammatory cytokines in intestinal tissues (Pie et al., 2004). Haptoglobin, a protein marker of acute-phase inflammation, also increases in piglets weaned in this manner (Petersen et al., 2004; Sauerwein et al., 2005), as do other signs of systemic and intestinal oxidative stress associated with or caused by the increased inflammation (Sauerwein et al., 2005; Zhu et al., 2012; Yin et al., 2014). Oxidative stress is an unbalanced equilibrium between reactive oxygen species (ROS) production and the antioxidant system of the animal, resulting in an increase in oxidation products such as malondialdehyde, an indicator of oxidative stress (Jaeschke, 2011).

The effects of dietary zinc and L-arginine supplements on the weight gain, feed efficiency, antioxidant capacity and oxidative status of weanling piglets raised under commercial conditions were examined. A total of 288 piglets aged 21 d were fed for 15 d a diet supplemented or not with 2,500 mg/kg of zinc (provided as zinc oxide) and 1% L-arginine HCl. The 4 treatments were distributed in a randomized complete block design with 6 initial body weight categories (12 animals per pen). Access to feed and water was ad libitum. Data were analyzed as a 2 × 2 factorial experiment using the SAS MIXED procedure, with zinc and arginine as the main independent variables. Blood collection day (d 8 and 15, samples were collected from the same 2 piglets in each pen before the morning feeding) was included as a third factor.

The zinc supplement increased the average daily gain (ADG) from d 0 to 7, d 8 to 15 and d 0 to 15 (0.289 vs. 0.217 kg/d), average daily feed intake (ADFI) from d 8 to 15 and d 0 to 15 (0.953 vs. 0.957 kg/d) and the gain to feed (G:F) ratio from d 0 to 7 and d 0 to 15 (0.86 vs. 0.77) (P < 0.001). Both supplements significantly decreased the malondialdehyde concentration (zinc: 4.37 vs. 3.91 µmol/L, P = 0.005; arginine: 4.38 vs. 3.89 µmol/L, P = 0.002). Total antioxidant capacity and reduced glutathione (GSH) concentrations on d 8 were higher in response to the combined zinc and arginine interaction, P < 0.05). Piglets fed either Zn-supplemented diet had a lower haptoglobin serum concentration (509 vs. 1,417 mg/L; P < 0.001). In conclusion, the zinc supplement improved piglet growth performance (ADG and ADFI) and oxidative status (based on malondialdehyde concentration). The arginine supplement had a limited effect on growth performance and oxidative status under these conditions.

© 2019, Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Numerous experiments have shown that feeding pharmacological doses of zinc (2,000 to 3,000 mg/kg) to weaning piglets improves growth performance and reduces diarrhea (reviewed by Sales, 2013). Other studies have shown that L-arginine supplements (0.5% to 1% of the dry feed) improve growth and feed efficiency in weaned piglets (Tan et al., 2009; Wu et al., 2010; Yao et al., 2011). Arginine is known as an essential precursor for the synthesis of nitric oxide, a key mediator in several physiological functions. Increased production and concentration of nitric oxide is known to cause zinc release in endothelial cells and to increase metallothionein expression (Wiseman et al., 2006; Li et al., 2010). Metallothioneins are Zn-storing sulfo-proteins involved in zinc homeostasis and have significant antioxidant properties (Formigari et al., 2007). In 2 previous studies, we noted that zinc supplementation (2,500 mg/kg) reduced plasma malondialdehyde concentration in weaned piglets (Bergeron et al., 2014, 2017). We also observed that it improved antioxidant status under conditions of lipopolysaccharide-induced stress, especially when the diet included an arginine supplement (Bergeron et al., 2014). However, no zinc–arginine interaction was observed under conditions of chronic lipopolysaccharide-induced inflammation and oxidative stress (Bergeron et al., 2017).

It is known that deteriorating sanitary conditions in the breeding environment affect the antioxidant status of piglets, which can be measured in terms of decreased plasma glutathione (GSH) and increased plasma haptoglobin (Le Floch et al., 2006; Pastorelli et al., 2012a, b). At research stations, injections of lipopolysaccharide are used to activate the immune system and cause oxidative stress (Yi et al., 2016). However, the physiological conditions induced by such injections do not mimic entirely the alterations of immune system and antioxidant status that occur in conventional pig production facilities (Le Floch et al., 2006).

The objective of this study was to determine the impact of dietary arginine and zinc supplements on antioxidant and inflammatory status in weanling piglets raised in a conventional pig production facility. Our hypothesis is that this status should improve under the supplemented condition and lead to better growth performance.

2. Materials and methods

2.1. Animals and housing

The experiment was carried out with 288 piglets (Génétiporc Fertilis 25 × Génétiporc G Performer 6.0, St-Bernard, QC, Canada) obtained from 26 litters born in a conventional pig production facility (St-Anselme, Québec, Canada). The animals were weaned at the age of 21 d then moved to 1.62 m × 1.82 m pens such that each housed 12 piglets of similar body weight; weight categories were low (5.0 to 6.0 kg), medium (6.0 to 7.0 kg) and high (>7.0 kg) body weight. Two nursery rooms with 12 pens was used for this trial. In each nursery room, 3 blocks of 4 pens with piglets of similar body weight were included for a total of 6 blocks (2 blocks of low, 2 of medium and 2 of high weight). Each pen was equipped with a self-feeder (4-hole space feeder) and a watering nipple. The room temperature was initially 26 °C then decreased by 0.5 °C each day down to 22 °C, where it was maintained until the end of experiment. All animal procedures were conducted according to the guidelines set by the Canadian Council on Animal Care (2009), and the experimental protocol received approval from the Université Laval Animal Use and Care Committee. The health status of the pig farm was rated as stable but positive for exposure to porcine reproductive and respiratory syndrome (PRRS), Circovirus-2, and Mycoplasma hyopneumonia.

2.2. Experimental design and diets

The feeding experiment was begun on d 0 (distribution of the piglets to the pens). Formulated to meet or exceed NRC (2012) recommendations for piglets, the 4 dietary treatments (Table 1) were designated as follows: a control diet (ZN0ARG0), the control diet supplemented with 2,500 mg/kg zinc in the form of zinc oxide (ZN2500ARG0), the control diet supplemented with 1% of arginine in the form of L-arginine-HCl (ZN0ARG1) and the control diet with both supplements (ZN2500ARG1). The treatments were distributed throughout the room and to each of the 6 weight categories according to a randomized complete block design. Feed and water were provided ad libitum. The piglets and uneaten feed in each pen were weighed each day.

2.3. Blood samples

Two piglets are randomly selected from the 12 of each pen for blood sampling. Blood was collected by jugular venipuncture into 2 Vacutainer tubes (one containing EDTA and heparin for plasma and one empty for serum) before the morning feeding on d 0, 8 and 15. Feeders were emptied 4 h before sampling to uniformize piglet nutrient status. Samples were centrifuged at 2,000 × g at 4 °C for 15 min to obtain plasma and serum, which were frozen at −80 °C for analysis.

2.4. Biochemical analysis

Malondialdehyde generation was measured in plasma according to the method of Jain et al. (1989) as an index of systemic lipid peroxidation and oxidative status (Michel et al., 2008). Phosphate-buffered saline (800 mL, pH 7.4) and butylated hydroxytoluene solution (25 mL, 0.88%) were mixed thoroughly with 200 mL of plasma. Trichloroacetic acid (500 mL, 30%) was added, and the samples were placed on ice for 2 h. After 15 min of centrifugation at 2,000 × g, 1 mL of supernatant was mixed with 75 mL of 0.1 mol/L EDTA and 250 mL of 1% thiobarbituric acid in 0.05 mol/L NaOH. The samples were then placed in boiling water (100 °C) for 15 min, followed by cooling to room temperature. Absorbance was measured at 532 nm. Intra-assay and inter-assay coefficient of variation (CV) values were respectively 6.0% and 5.5%.

The total antioxidant capacity (TAC) of plasma was assayed according to the methods of Erel (2004) and Maurice et al. (2007), which measure the concentration of antioxidants including vitamin C, vitamin E, GSH, polyphenols and protein thiol groups (Erel, 2004). The intra-assay and inter-assay CV values were respectively 2.5% and 3.0%.

Plasma total oxidant status (TOS), which is related linearly to the molar concentration of strong oxidizers (hydrogen peroxide, cumene hydroperoxide, tert-butil hydroperoxide) was assayed according to the method of Erel (2005). The intra-assay and inter-assay CV values were respectively 2.0% and 3.5%.

Reduced and total GSH in plasma was determined using the fluorescent detection kit K006-F5 (Arbor Assays, Ann Arbor, USA) according to the manufacturer’s instructions. The intra-assay and inter-assay CV values were respectively 3.7% and 9.1% for reduced GSH and 3.6% and 10.0% for total GSH. The difference between total and reduced GSH was presumed to be oxidized GSH (GSSG).

Serum haptoglobin was determined using the Pig Haptoglobin ELISA kit KT-349 (Kamiya Biomedical Company, Seattle, USA) according to the manufacturer’s instructions. The intra-assay and inter-assay CV values were respectively 5.0% and 6.2%.

Plasma zinc concentration was determined using the Quantichrom Zinc Assay Kit DIZN-250 (BioAssay Systems, Hayward, USA).
2.5. Feed analysis

Feed samples were ground in a sample mill (Cyloctec 1093, Foss Tecator, Sweden). Feed energy content was measured using a bomb calorimeter (Parr Instruments Co., Moline, IL, USA). The mineral (P, Ca, and Zn) contents were determined using the Leco Nitrogen Determinator (model TruSpec v1.10, Leco, MI, USA). The mineral (P, Ca, and Zn) contents were determined according to the combustion method using AOAC methods (procedures 985.01, 2005 version) (AOAC, MI, USA). The same samples were then analyzed with a spectro- scope (Optima 4300DV ICP-OES, PerkinElmer, MA, USA). Lysine and arginine contents were determined by HPLC (Waters HPLC system, Waters Corporation, MA, USA) as described by Guay et al. (2006).

2.6. Statistical analysis

Results were analyzed as a 2 × 2 factorial experiment in a randomized complete block design (initial body weight category) with zinc and arginine supplementation as the main independent variables. For the analysis of the blood measurements, the sampling day was added as a third factor and the analysis was then a 2 × 2 × 2 factorial arrangement. The SAS MIXED procedure (SAS inst. Cary, NC) was used. Treatment means and interactions were calculated for malondialdehyde, TAC, TOS, zinc, GSH (reduced, oxidized and total), haptoglobin and growth performance (ADG, ADFI and gain to feed [G:F] ratio). For growth performance analysis, the model was: $Y_{ijkl} = \mu + R_{k} + B_{i} + F_{j} + BF_{ij} + e_{ijkl}$, where $Y_{ijkl}$ = dependent variable, $R_{k}$ = block factor, $B_{i}$ = zinc factor, $F_{j}$ = arginine factor, $BF_{ij}$ = residual error; and for blood measurements: $Y_{ijk} = \mu + R_{k} + B_{i} + F_{j} + G_{k} + BF_{ij} + BG_{ik} + FG_{jk} + BF_{ijk} + e_{ijk}$, where $Y_{ijk}$ = dependent variable, $B_{i}$ = zinc factor, $F_{j}$ = arginine factor, $G_{k}$ = day of blood sampling and $e_{ijk}$ = residual error. For each analysis, the pen was considered as the experimental unit. Differences were considered significant at $P<0.05$, while tendency refers to $0.05<P<0.10$. For parameters measured in plasma and serum, the baseline value (at d 0) was added as a covariable in all statistical models.

3. Results

3.1. Growth performance

As expected, zinc supplementation had a positive impact on the growth performance of piglets raised under commercial conditions (Table 2). It was associated with a higher ADG and G:F during the 15-d post-weaning period ($P<0.05$). These piglets also had a significantly higher ADFI ($P<0.001$) during the second week and for the 15 d overall.

Arginine supplementation tended to reduce ADG during the second week ($P=0.091$) and over the 15 d ($P=0.090$). For the 15-d period overall, this supplement was associated with an ADFI reduced by 5%. There was no significant effect of arginine on the G:F ratio.

### Table 1

| Item | Experimental diets |
|------|--------------------|
|      | ZN0ARG0 | ZN2500ARG0 | ZN0ARG1 | ZN2500ARG1 |
| Ground corn | 31.5 | 31.5 | 31.5 | 31.5 |
| Soybean meal | 22.5 | 22.5 | 22.5 | 22.5 |
| Whey powder | 20.0 | 20.0 | 20.0 | 20.0 |
| Hamlet protein 300 | 9.5 | 9.5 | 9.5 | 9.5 |
| Choice fat | 5.0 | 5.0 | 5.0 | 5.0 |
| Spray-dried animal plasma | 3.5 | 3.5 | 3.5 | 3.5 |
| Blood meal | 2.5 | 2.5 | 2.5 | 2.5 |
| Limestone | 1.1 | 1.1 | 1.1 | 1.1 |
| Di-calcium phosphate | 1.0 | 1.0 | 1.0 | 1.0 |
| Sodium chloride | 0.3 | 0.3 | 0.3 | 0.3 |
| Vitamin mix | 0.25 | 0.25 | 0.25 | 0.25 |
| Mineral mix | 0.25 | 0.25 | 0.25 | 0.25 |
| Lysine-HCl | 0.25 | 0.25 | 0.25 | 0.25 |
| DL-methionine | 0.15 | 0.15 | 0.15 | 0.15 |
| L-threonine | 0.10 | 0.10 | 0.10 | 0.10 |
| Corn starch | 0.40 | 0.40 | 0.40 | 0.40 |
| Zinc oxide | – | 0.40 | – | 0.40 |
| L-arginine-HCl | – | – | 1.00 | 1.00 |
| L-alanine | 1.70 | 1.70 | – | – |
| ME, MJ/kg | 18.67 | 18.72 | 18.68 | 18.69 |
| CP | 25.18 | 24.49 | 24.77 | 24.81 |
| Calcium | 0.78 | 0.78 | 0.73 | 0.77 |
| Phosphorus | 0.58 | 0.61 | 0.53 | 0.57 |
| Zinc | 1.34 | 2.224 | 1.23 | 2.342 |
| Total lysine | 1.69 | 1.65 | 1.67 | 1.67 |
| Total arginine | 1.52 | 1.49 | 2.30 | 2.31 |
| Calculated nutrient composition |
| Digestible lysine | 1.89 | 1.89 | 1.89 | 1.89 |
| Digestible arginine | 1.65 | 1.65 | 2.45 | 2.45 |

1 ZN0ARG0: control diet (n = 6); ZN2500ARG0: control diet + 2,500 mg of zinc (n = 6); ZN0ARG1: control diet + 1% arginine (n = 6); ZN2500ARG1: control diet + 2,500 mg of zinc + 1% arginine (n = 6).

2 Provided per kilogram of diet: vitamin A palmitate 5,000 IU; vitamin D3 1,000 IU; vitamin E acetate 22.5 IU; menadione sodium bisulphite 3.75 mg; thiamin HCl 1.0 mg; riboflavin 4.5 mg; niacin 20.0 mg; calcium pantothenate 25.0 mg; pyridoxine HCl 0.25 mg; choline bitartrate 375 mg; vitamin B12 0.25 mg.

3 Provided per kilogram of diet: Zn (as carbonate) 100 mg; Fe (as ferric citrate) 100 mg; Cu (as cupric carbonate) 25 mg; I (as potassium iodate) 0.28 mg; Mn (as manganous carbonate) 46 mg; Se (as sodium selenite) 0.30 mg.

4 Values for nutritional composition were calculated according to Sauvant et al. (2004).
The combined supplement (treatment ZN2500ARG1) increased the total GSH concentration measured in plasma on d 8 but not on d 15 (Table 4, day × zinc × arginine, P = 0.017), while no effect could be attributed to either supplement alone. The reduced GSH concentration was not affected by either supplement, although the average for all 4 treatments increased from 2.22 μmol/L on d 8 to 3.37 μmol/L on d 15 (P < 0.001). The GSSH:total GSH ratio was affected by arginine as measured on d 15 (P < 0.05) but not on d 8. The apparent differences between the arginine, zinc and combined treatments on d 15 are not significant. However, the ratio averaged over the 4 treatments decreased from 0.201 on d 8 to 0.130 on d 15 (P < 0.001).

Finally, the zinc supplement had the effect of lowering the serum concentration of haptoglobin (P < 0.001), while the arginine supplement only tended to do so on d 8 (P = 0.081). In spite of what the values suggest, there was no interaction between zinc and arginine.

### 4. Discussion

#### 4.1. Growth performance

The objective of this study was to determine the impact of zinc and arginine dietary supplements on the growth performance and antioxidant and oxidative status of weanling piglets raised in a commercial pig production facility. The farm where the experiment was conducted was positive for known pathogens including Circovirus-2, PRRSV and Mycoplasma hyopneumoniae, which could affect the immune and antioxidant responses (Krakowka et al., 2001).

In the present study, the zinc supplement had a positive impact on ADFI and ADG and increased the G:F ratio. Supplementation of the weanling piglet diet with 2,500 to 3,000 mg/kg of zinc in the form of zinc oxide is known to improve growth performance and reduce

### Table 2

Average daily gain (ADG), average daily feed intake (ADFI) and gain to feed (G:F) ratio of weanling piglets fed a diet with or without zinc and/or arginine supplementation under commercial conditions.

| Item\(^1\) | ADG, g/d | ADFI, g/d | G:F ratio |
|-----------|----------|-----------|-----------|
|           | d 0 to 7 | d 8 to 15 | d 0 to 15 | d 0 to 7 | d 8 to 15 | d 0 to 15 |
| ZN0ARG0   | 0.159    | 0.290     | 0.231     | 0.193    | 0.378     | 0.294     | 0.821    | 0.768     | 0.783     |
| ZN0ARG1   | 0.130    | 0.267     | 0.209     | 0.181    | 0.344     | 0.269     | 0.761    | 0.778     | 0.771     |
| ZN2500ARG0| 0.190    | 0.379     | 0.294     | 0.193    | 0.462     | 0.340     | 0.986    | 0.822     | 0.865     |
| ZN2500ARG1| 0.198    | 0.357     | 0.285     | 0.200    | 0.446     | 0.335     | 0.995    | 0.801     | 0.854     |
| SEM       | 0.015    | 0.026     | 0.019     | 0.019    | 0.022     | 0.022     | 0.046    | 0.018     | 0.015     |
| P-value   |          |           |           |          |           |           |          |           |           |

\(NS = \) not significant (\(P \geq 0.10\).)

\(1\) ZN0ARG0: control diet (n = 6); ZN0ARG1: control diet + 1% arginine (n = 6); ZN2500ARG0: control diet + 2,500 mg of zinc (n = 6); ZN2500ARG1: control diet + 2,500 mg of zinc + 1% arginine (n = 6).

### Table 3

Plasma malondialdehyde, total antioxidant capacity (TAC), total oxidant capacity (TOS), TAC:TOS ratio and zinc concentration on d 8 and 15 in piglets fed diets with or without zinc and/or arginine supplementation.

| Item\(^1\) | Malondialdehyde, μmol/L | TAC, μmol/L | TOS, μmol/L | TAC:TOS ratio | Zinc, μmol/L |
|-----------|--------------------------|-------------|-------------|----------------|--------------|
|           | d 8 | d 15 | d 8 | d 15 | d 8 | d 15 | d 8 | d 15 | d 8 | d 15 | d 8 | d 15 | d 8 | d 15 |
| ZN0ARG0   | 4.59 | 4.66 | 123.5 | 198.4 | 20.3 | 7.3 | 13.0 | 44.8 | 2.34 | 3.49 |
| ZN0ARG1   | 4.09 | 4.14 | 70.5  | 158.3 | 12.2 | 18.9 | 19.3 | 18.6 | 2.26 | 3.25 |
| ZN2500ARG0| 4.16 | 4.16 | 149.6 | 152.5 | 37.4 | 7.1 | 12.1 | 30.8 | 5.42 | 7.89 |
| ZN2500ARG1| 4.15 | 3.21 | 132.8 | 186.2 | 25.4 | 10.4 | 34.4 | 46.8 | 6.97 | 8.62 |
| SEM       | 0.33 | 0.21 | 29.1  | 9.7   |       |     | 8.9  | 0.58 |       |       |
| P-value   |       |       |Arginine | 0.002 |          | NS  | NS  | NS  |       | NS  |
|          |       |       |Zinc | 0.005 |          | NS  | NS  | NS  |       | <0.001 |
|          |       |       |Day |       |          | NS  | NS  | NS  |       | <0.001 |
|          |       |       |Zinc × Arginine | 0.003 |          | NS  | NS  | NS  |       | 0.091 |
|          |       |       |Day × Zinc |       |          | NS  | NS  | NS  |       | 0.099 |
|          |       |       |Day × Arginine |       |          | NS  | NS  | NS  |       | NS  |
|          |       |       |Day × Zinc × Arginine |       |          | NS  | NS  | NS  |       | NS  |

\(NS = \) not significant (\(P \geq 0.10\).)

\(1\) ZN0ARG0: control diet (n = 6); ZN0ARG1: control diet + 1% arginine (n = 6); ZN2500ARG0: control diet + 2,500 mg of zinc (n = 6); ZN2500ARG1: control diet + 2,500 mg of zinc + 1% arginine (n = 6).
the incidence of diarrhea (reviewed by Sales, 2013). However, in previous studies conducted under good sanitary conditions, such supplementation has been found not to have any effect on growth performance, even in conjunction with lipopolysaccharide challenge of the immune system (Bergeron et al., 2014, 2017). Other growth promoters (antibiotics and spray-dried animal plasma) are known to be more effective at improving piglet growth and health under poor sanitary conditions (inadequate pen maintenance, high microbiological loads) in commercial settings (Williams et al., 1997).

In contrast, the arginine supplement tended to reduce ADG and ADFI. Studies have shown that such supplements (0.5% to 1% for totals of 1.64% to 2.14% arginine) improve growth and feed efficiency and enhance small intestinal growth in weanling piglets (Wu et al., 2009, 2010; Yao et al., 2011). However, Chen et al. (2012) found no effect of 0.5% to 1.2% added arginine (0.8% to 1.6%) on malondialdehyde concentration in piglets 8 d after weaning, and Zhan et al. (2008) found no effect of 0.5% to 1% added arginine (n = 6); ZN2500ARG0: control diet + 2,500 mg of zinc (n = 6); ZN2500ARG1: control diet + 2,500 mg of zinc + 1% arginine (n = 6).

Table 4

| Item | GSH, μmol/L | Total GSH, μmol/L | GSSG, μmol/L | GSSG: Total GSH ratio | Haptoglobin, mg/L |
|------|-------------|------------------|-------------|----------------------|------------------|
|      | d 8 d 15    | d 8 d 15         | d 8 d 15    | d 8 d 15             |                  |
| ZN0ARG0 | 2.77 3.60 | 4.19<sup>abc</sup> 4.37 | 1.04<sup>b</sup> 0.46 | 0.234 0.087<sup>c</sup> | 1,715 1,359 |
| ZN0ARG1 | 2.01 2.87 | 3.42<sup>ab</sup> 4.56 | 0.67<sup>b</sup> 0.84 | 0.202 0.195<sup>b</sup> | 1,303 1,292 |
| ZN2500ARG0 | 1.66 3.50 | 2.57<sup>b</sup> 4.88 | 0.41<sup>b</sup> 0.72 | 0.141 0.134<sup>c</sup> | 768 572 |
| ZN2500ARG1 | 2.96 3.49 | 5.42<sup>c</sup> 4.36 | 1.20<sup>b</sup> 0.40 | 0.224 0.103<sup>b</sup> | 376 320 |
| SEM | 0.55 | 0.55 | 0.24 | 0.052 | 0.103<sup>b</sup> |

GSH = glutathione; GSSG = oxidized glutathione; NS = not significant (P > 0.10).

<sup>ab</sup>, <sup>c</sup> Within a column, means without a common superscript differ significantly (P < 0.05).

<sup>1</sup> ZN0ARG0: control diet (n = 6); ZN0ARG1: control diet + 1% arginine (n = 6); ZN2500ARG0: control diet + 2,500 mg of zinc (n = 6); ZN2500ARG1: control diet + 2,500 mg of zinc + 1% arginine (n = 6).

4.2. Antioxidant and oxidative status

Weaning is well known to decrease antioxidant status and increase oxidative stress in piglets as measured in plasma and the intestinal mucosa in next days after weaning. Following this oxidative stress, the oxidative status of piglets improves during the next weeks (Zhu et al., 2012; Yin et al., 2014; Buchet et al., 2017). We found also increases in TAC, TAC:TOS ratio and total GSH and decreased GSSG:total GSH ratio from d 8 to 15, suggesting a lowering of oxidative stress in second week after weaning. The plasma zinc concentration increased during this post-weaning period, suggesting an improvement in zinc status and corroborating at least one study (Carlson et al., 2007). In another study, weaning itself was associated with a reduction in plasma zinc (Davin et al., 2013).

Although neither zinc nor arginine had any impact on GSH overall (reduced, total GSH or GSSG) or TAC values, both supplements reduced malondialdehyde concentration. Zinc supplementation of the piglet diet has been found to decrease plasma malondialdehyde measured 8 or 15 d after weaning (Bergeron et al., 2014, 2017) and to increase blood total superoxide dismutase activity measured 14 and 28 d after weaning (Zhu et al., 2017), although this latter effect was not observed in a similar previous study (Bergeron et al., 2014). Zinc is known as an essential co-factor for the function of CuZn-superoxide-dismutase (SOD), which catalyzes the dismutation of superoxide anion O2•− to H2O2 and is stimulated during periods of oxidative stress (Fukai and Ushio-Kukai, 2011). Increased zinc intake also elevates levels of metallothionein 1 (MT1) in tissues and plasma in weaning piglets (Martínez et al., 2004, 2005; Bergeron et al., 2014, 2017). The zinc-containing sulpho-protein MT1 is involved in zinc homeostasis and has a significant antioxidant function (Formigari et al., 2007).

Previous studies have shown no effect of increased dietary arginine (0.8% to 1.6%) on malondialdehyde concentration in piglets 8 d after weaning (Zheng et al., 2013; Bergeron et al., 2014, 2017). A similar arginine supplement has been found to have a positive effect on antioxidant status measured as ferric reduction by plasma in lipopolysaccharide-challenged piglets (Bergeron et al., 2014) and on the total antioxidant capacity value measured in the plasma and liver of weanling piglets injected with diquat to induce oxidative stress (Zheng et al., 2013). Arginine could provide protection against reactive oxygen species by direct chemical interaction with O2•−, thereby improving antioxidant status (Lass et al., 2002).

Although the present results show overall that zinc and arginine supplements did not modify GSH metabolism or TAC value, either supplement alone (but not the combination) did decrease total GSH and GSSG on d 8. Combined zinc and arginine supplementation in conjunction with LPS injection 5 d after weaning has been found to increase GSH concentrations in plasma (Bergeron et al., 2017). Zinc...
injections have been shown to increase the concentration of GSH in the liver of rats (Izard et al., 1995). Increased hepatic concentrations of GSSG in piglets fed diets supplemented with 0.5% or 1% arginine have been observed after injection of lipopolysaccharide (Li et al., 2012). However, there is no obvious explanation of how arginine or zinc increase the release or synthesis of GSH. It is possible that by stimulating nitric oxide synthesis (Poeze et al., 2011), an arginine supplement acts on GSH metabolism through the release of zinc from the protein MT1 (Wiseman et al., 2006; Li et al., 2010). In the present study, zinc supplementation increased plasma zinc concentration as expected and observed previously (Walk et al., 2015), but the effect was enhanced by the arginine supplement, suggesting either a release of zinc from tissues or better intestinal absorption of Zn.

In addition to improving oxidative status in weanling piglets, the dietary zinc supplement appeared to act on inflammation by reducing serum haptoglobin. This may prevent the inflammation induced by weaning from reaching the acute phase (Petersen et al., 2004; Sauwerman et al., 2005). Reduced haptoglobin concentrations have been observed in a study of piglets fed a zinc-supplemented diet and challenged with lipopolysaccharide 12 d after weaning (Bergeron et al., 2017). It has been observed also that interleukin 10 (IL10), a known anti-inflammatory factor (Kubo and Motomura, 2012), is overexpressed (based on mRNA) in the mucosa of the ileum of weanling piglets fed a Zn-supplemented diet (Bergeron et al., 2014).

5. Conclusion

In this study, we demonstrated that increasing the dietary intake of zinc had a positive effect on the growth performance of weaning piglets through a mechanism that remains to be determined but likely involves improvement of the systemic antioxidant capacity (estimated as malondialdehyde concentration) and control of inflammation (haptoglobin concentration). Also in this study, increasing the dietary intake of arginine had no positive effect on growth performance despite a positive impact on malondialdehyde status. Zinc and arginine supplements may act synergistically on GSH metabolism, but the mechanism involved remains to be determined.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can appropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgements

Financial support for this work was provided by a grant from the Natural Sciences and Engineering Research Council of Canada awarded to F. Guay and by a studentship from La fédération des producteurs de porcs du Québec (FPPO) awarded to N. Bergeron.

References

Association of Official Analytical Chemists. Official methods of analysis. 17th ed. Arlington, VA: AOAC; 2005.

Bergeron N, Robert C, Guay F. Antioxidant status and inflammatory response in weaning piglets fed diets supplemented with arginine and zinc. Can J Anim Sci 2014;94(1):87–97.

Bergeron N, Robert C, Guay F. Feed supplementation with arginine and zinc on antioxidant status and inflammatory response in challenged weaning piglets. Anim Nutr 2017;3(3):236–46.

Buchet A, Belloc C, Leblanc-Maridor M, Merlet E. Effects of age and weaning conditions on blood indicators of oxidative status in pigs. PLoS One 2017;12(5): e0174963.

Care CCOA. The care and use of farm animals in research, teaching and testing. Ottawa, ON: CCAC; 2009. p. 12–5.

Carlson D, Beattie JH, Poulsen HD. Assessment of zinc and copper status in weaned piglets in relation to dietary zinc and copper supply. J Anim Physiol Anim Nutr 2007;91(1):19–28.

Chen Y, Chen D, Tian G, He J, Lao X, Mao Q, et al. Dietary arginine supplementation alleviates immune challenge induced by Salmonella enterica serovar Choleraesuis bacterin potentially through the Toll-like receptor 4-myeloid differentiation factor 88 signalling pathway in weaned piglets. Br J Nutr 2012;108(6):1069–76.

Davin R, Mananizania EG, Klaing KC, Pérez JF. Effect of weaning and in-feed high dose zinc oxide on zinc levels in different body compartments of piglets. J Anim Physiol Anim Nutr 2013;97(1):6–12.

Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37(4):77–85.

Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38(12):1103–11.

Formigari A, Iratro F, Santon A. Zinc, antioxidant systems and metallothionein in metal mediated-apoptosis: biochemical and cytotoxic aspects. Comp Biochem Physiol, C 2007;146(4):443–50.

Fukai T, Ushio-Kuikar M. Superoxide dismutases: role in redox signaling, vascular function, and disease. Antioxidants Redox Signal 2011;15(5):1583–606.

Guay F, Derayean SM, Trotier N. Biochemical and morphological development of the digestive tract of rat. Cells are partially impaired in intestinal mucosa from growing pigs fed reduced-protein diets supplemented with crystalline amino acids. J Anim Sci 2006;84(7):1749–60.

Izard MB, Liu J, Klaassen CD. Effect of several metallothionein inducers on oxidative stress defense mechanisms in rats. Toxicology 1995;104(1):25–33.

Jaeschke H. Reactive oxygen and mechanisms of inflammatory liver injury: present concepts. J Gastroenterol Hepatol 2011;26(Suppl 1):173–9.

Jain SK, McVeir R, Duett J, Herbst JJ. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes 1989;38(12):1539–43.

Jensen P, Recen B. When to wean – observations from free-ranging domestic pigs. Appl Anim Behav Sci 1989;23(1–2):49–60.

Jensen P, Stangel G. Behaviour of piglets during weaning in a seminatural enclosure. Appl Anim Behav Sci 1992;33(2–3):227–38.

Karakovka S, Ellis JA, McNeilly F, Kingsler S, Rings DM, Allan G. Activation of the immunosuppressive system is the pivotal event in the production of wasting disease in pigs infected with porcine circovirus-2 (PCV-2). Vet Pathol 2001;38(1):31–42.

Kubo M, Motomura Y. Transcriptional regulation of the anti-inflammatory cytokine IL-10 in acquired immune cells. Front Immunol 2012;3:275.

Lais A, Suesenbacher A, Wölkert C, Mayer B, Brunner F. Functional and analytical evidence for scavenging of oxygen radicals by L-arginine. Mol Pharmacol 2002;61(5):1081–8.

Le Floch N, Jondreville C, Matte JJ, Seve B. Importance of sanitary environment for growth performance and plasma zinc in nutrition. Ann N Y Acad Sci 2006;1080:23–34.

Li Q, Liu Y, Che Z, Zhu H, Meng G, Hou Y, et al. Dietary L-arginine supplementation effects on tissue mineral concentrations, metallothionein, and apparent mineral digestibility in newly weaned pigs. J Nutr 2011;141(5):1069–76.

Ottewell JP, Blazey F, Laffite J, Seve B, Oswald IP. Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. J Nutr 2004;134(3):641–7.
Poeze M, Bruins MJ, Kessels F, Luiking YC, Lamers WH, Deutz NEP. Effects of L-arginine pretreatment on nitric oxide metabolism and hepatosplanchnic perfusion during porcine endotoxemia. Am J Clin Nutr 2011;93(6):1237–47.

Sales J. Effects of pharmacological concentrations of dietary zinc oxide on growth of post-weaning pigs: a metaanalysis. Biol Trace Elem Res 2013;152(3):343–9.

Sauerwein H, Schmitz S, Hess S. The acute phase protein haptoglobin and its relation to oxidative status in piglets undergoing weaning-induced stress. Redox Rep 2005;10(6):295–302.

Sauvant D, Perez JM, Tran G. T Tables de composition et de valeur nutritive des matières premières destinées aux animaux d’élevage: Porcs, volailles, bovins, ovins, caprins, lapins, chevaux, poissons. 2004. p. 304. INRA Editions Versailles, 2e édition revue et corrigée, ISBN 2-7380-1158-6.

Tan B, Li XG, Kong X, Huang R, Ruan Z, Yao K, et al. Dietary L-arginine supplementation enhances the immune status in early-weaned piglets. Amino Acids 2009;37(2). 323–311.

Walk CL, Wilcock P, Magowan E. Evaluation of the effects of pharmacological zinc oxide and phosphorus source on weaned piglet growth performance, plasma minerals and mineral digestibility. Animal 2015;9(7):1145–52.

Williams NH, Stahly TS, Zimmerman DR. Effect of chronic immune system activation on nitrogen retention, partial efficiency of lysine utilization, and lysine needs of pigs. J Anim Sci 1997;75:2472–81.

Wiseman DA, Wells SM, Willham J, Hubbard M, Welker JE, Black SM. Endothelial response to stress from exogenous Zn2+ resembles that of NO-mediated nitrosative stress, and is protected by MT-1 overexpression. Am J Physiol Cell Physiol 2006;291(3):555–68.

Wu G, Bazer FW, Davis TA, Kim SW, Li P, Rhoads JM, et al. Arginine metabolism and nutrition in growth, health and disease. Amino Acids 2009;37(1):153–68.

Wu X, Ruan Z, Gao Y, Yin Y, Zhou X, Wang L, et al. Dietary supplementation with L-arginine or N-carbamylglutamate enhances intestinal growth and heat shock protein-70 expression in weaning pigs fed a corn- and soybean meal-based diet. Amino Acids 2010;39(3):831–9.

Yao K, Guan S, Li T, Huang R, Wu G, Ruan Z, et al. Dietary L-arginine supplementation enhances intestinal development and expression of vascular endothelial growth factor in weaning piglets. Br J Nutr 2011;105(5):703–9.

Yi D, Hou Y, Wang L, Zhao D, Ding B, Wu T, et al. Gene expression profiles in the intestine of lipopolysaccharide-challenged piglets. Front Biosci (Landmark Ed) 2016;21:487–501.

Yin J, Wu MM, Xiao H, Ren WK, Duan JL, Yang G, et al. Development of an antioxidant system after early weaning in piglets. J Anim Sci 2014;92(2):612–9.

Zhan Z, Ou D, Piao X, Kim SW, Liu Y, Wang J. Dietary arginine supplementation affects microvascular development in the small intestine of early-weaned pigs. J Nutr 2008;138(7):1304–9.

Zheng P, Yu B, He J, Tian G, Luo Y, Mao X, et al. Protective effects of dietary arginine supplementation against oxidative stress in weaned piglets. Br J Nutr 2013;109(12):2253–60.

Zhu C, Lv H, Chen Z, Wang L, Wu X, Chen Z, et al. Dietary zinc oxide modulates antioxidant capacity, small intestine development, and jejunal gene expression in weaned piglets. Biol Trace Elem Res 2017;175(2):331–8.

Zhu LH, Zhao KL, Chen XL, Xu JX. Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs. J Anim Sci 2012;90(8):2581–9.