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Original Research Article

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

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ABSTRACT

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 (0.338 vs. 0.279 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) increased from d 8 to 15 (0.953 vs. 1.391 μmol/L, 2.22 vs. 3.37 μmol/L, $P < 0.05$) regardless of dietary treatment. Total and oxidized GSH concentrations on d 8 were higher in response to the combined supplements (zinc × 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.

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 (Pié 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).

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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 znic 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 znic 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 Floc'h 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 Floc'h 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 hyopleumonia*.

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 znic 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 μL, pH 7.4) and butylated hydroxytoluene solution (25 μL, 0.88%) were mixed thoroughly with 200 μL of plasma. Trichloroacetic acid (500 μL, 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 μL of 0.1 mol/L EDTA and 250 μL 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-butyl 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 Quanti-Chrom Zinc Assay Kit DIZN-250 (BioAssay Systems, Hayward, USA).

Table 1
Composition of experimental diets (%, as fed basis).

Item	Experimental diets[1]			
	ZN0ARG0	ZN2500ARG0	ZN0ARG1	ZN2500ARG1
Ingredients				
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[2]	0.25	0.25	0.25	0.25
Mineral mix[3]	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	–	1.1	0.70
Zinc oxide	–	0.40	–	0.40
L-arginine·HCl	–	–	1.00	1.00
L-alanine	1.70	1.70	–	–
Analysed nutrient composition				
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	134	2,224	123	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[4]				
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 D_3 1,000 IU; vitamin E acetate 22.5 IU; menadione sodium bisulfite 3.75 mg; thiamin HCl 1.0 mg; riboflavin 4.5 mg; niacin 20.0 mg; calcium pantothenate 25.0 mg; pyridoxine HCl 1.5 mg; biotin 0.2 mg; choline bitartrate 375 mg; vitamin B_{12} 25.0 μg.
[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).

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). Nitrogen content was determined according to the combustion method using the Leco Nitrogen Determinator (model TruSpec v1.10, Leco, MI, USA). The mineral (P, Ca, and Zn) contents were determined using AOAC methods (procedures 985.01, 2005 version) (AOAC, 2005). The same samples were then analyzed with a spectroscope (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. Inc. 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: $Y_{hij} = \mu + R_h + B_i + F_j + BF_{ij} + e_{hij}$, where Y_{hij} = dependent variable, R_h = block factor, B_i = zinc factor, F_j = arginine factor, e_{hij} = residual error; and for blood measurements: $Y_{ijk} = \mu + R_h + B_i + F_j + G_k + BF_{ij} + BG_{ik} + FG_{jk} + BFG_{ijk} + e_{ijk}$, 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 ($P = 0.100$). 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 2
Average daily gain (ADG), average daily feed intake (ADFI) and gain to feed (G:F) ratio of weaning 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	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									
Zinc	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001	0.019	<0.001
Arginine	NS	0.091	NS	NS	NS	NS	NS	NS	NS
Zinc × Arginine	NS	NS	NS	NS	NS	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 ($n = 6$).

3.2. Antioxidant and oxidative status

The average concentration of plasma malondialdehyde was reduced from 4.37 to 3.91 μmol/L by zinc ($P = 0.005$) and from 4.38 to 3.89 μmol/L by arginine ($P = 0.002$), as measured on d 8 and 15 (Table 3). In contrast, neither supplement had any effect on plasma TAC or TOS during the same period. However, TAC averaged for all 4 treatments increased from 119.1 μmol/L on d 8 to 173.9 μmol/L on d 15 ($P = 0.003$), and TAC:TOS ratio increased likewise, from 19.7 to 35.3 μmol/L ($P = 0.044$). The zinc supplement tended to increase the TAC:TOS ratio, but only in the presence of the arginine supplement (zinc × arginine, $P = 0.091$). As expected, the average concentration of plasma znic measured on d 8 and 15 was higher in piglets fed Zn-supplemented diets ($P < 0.001$). However, the increase was more pronounced when the piglets also received the arginine supplement (zinc × arginine, $P = 0.042$) and tended to be greater on d 15 than on d 8 (day × zinc, $P = 0.099$). The average daily increase in plasma znic was 0.29 μmol/L per d in piglets fed the Zn-supplemented diet vs. 0.15 μmol/L per d in those fed the control diet.

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. Supplementing the weanling piglet diet with 2,500 to 3,000 mg/kg of znic in the form of zinc oxide is known to improve growth performance and reduce

Table 3
Plasma malondyaldehyde, 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
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		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		0.003		NS		0.044		<0.001	
Zinc × Arginine	NS		NS		NS		0.091		0.042	
Day × Zinc	NS		NS		NS		NS		0.099	
Day × Arginine	NS		NS		NS		NS		NS	
Day × Zinc × Arginine	NS		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 ($n = 6$).

Table 4
Plasma reduced GSH, total GSH, GSSG and serum haptoglobin concentrations on d 8 and 15 post-weaning in piglets fed a diet with or without zinc and/or arginine supplementation.

Item[1]	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	d 8	d 15
ZN0ARG0	2.27	3.60	4.19^{abc}	4.37	1.04^{b}	0.46	0.234	0.087^{a}	1,715	1,359
ZN0ARG1	2.01	2.87	3.42^{ab}	4.56	0.67^{a}	0.84	0.202	0.195^{b}	1,303	1,292
ZN2500ARG0	1.66	3.50	2.57^{a}	4.88	0.41^{a}	0.72	0.141	0.134^{ab}	768	572
ZN2500ARG1	2.96	3.49	5.42^{c}	4.36	1.20^{b}	0.40	0.224	0.103^{ab}	376	320
SEM	0.55		0.55		0.24		0.052		244	
P-value										
Arginine	NS		NS		NS		NS		0.081	
Zinc	NS		NS		NS		NS		<0.001	
Day	<0.001		NS		0.068		<0.001		NS	
Zinc × Arginine	NS		NS		NS		NS		NS	
Day × Zinc	NS		NS		NS		NS		NS	
Day × Arginine	NS		NS		NS		NS		NS	
Day × Zinc × Arginine	NS		0.017		<0.001		0.002		NS	

GSH = glutathione; GSSG = oxidized glutathione; NS = not significant ($P \geq 0.10$).
[a, b, c] Within a column, means without a common superscript differ significantly ($P < 0.05$).
[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) and Zhan et al. (2008) found no effect of 0.5% to 1.2% added arginine (for totals of 1.52% to 2.14%) on ADG and ADFI. Zhan et al. (2008) suggest that 1.2% added arginine could lead to an amino acid imbalance by lowering the lysine to arginine ratio from 2.30 to 0.93. These authors observed a significant reduction in the plasma lysine concentration under this condition. In our experiment, the supplement raised the total arginine concentration to 2.45% and lowered the lysine:arginine ratio to 0.77. This lower ratio might explain the reduced growth performance. Since lysine and arginine share certain chemical properties, excess dietary arginine could increase the requirement for lysine, as has been observed in chicks (O'Dell and Savage, 1966). This lysine deficiency may explain that despite its positive effect on oxidative status (see below), supplementation with arginine did not have a positive effect on growth performance.

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 znic concentration increased during this post-weaning period, suggesting an improvement in znic status and corroborating at least one study (Carlson et al., 2007). In another study, weaning itself was associated with a reduction in plasma znic (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 $\dot{O}_2^-$ to H_2O_2 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 weanling piglets (Martinez et al., 2004, 2005; Bergeron et al., 2014, 2017). The Zn-containing sulpho-protein MT1 is involved in znic 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 $\dot{O}_2^-$, 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 (Iszard et al., 1995). Increased hepatic concentrations of GSSG in piglets fed diets supplemented with 0.5% or 1% arginine have been noted 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 znic from the protein MT1 (Wiseman et al., 2006; Li et al., 2010). In the present study, zinc supplementation increased plasma znic concentration as expected and observed previously (Walk et al., 2015), but the effect was enhanced by the arginine supplement, suggesting either a release of znic 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; Sauerwein 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 weanling 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 inappropriately 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.

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