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Original Research Article

Feed supplementation with arginine and zinc on antioxidant status and inflammatory response in challenged weanling piglets

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ABSTRACT

Although supplementing the diet with zinc oxide and arginine is known to improve growth in weanling piglets, the mechanism of action is not well understood. We measured the antioxidant status and inflammatory response in 48 weanling castrated male piglets fed diets supplemented with or without zinc oxide (2,500 mg Zn oxide per kg) and arginine (1%) starting at the age of 20 days. The animals were injected with lipopolysaccharide (100 μg/kg) on day 5. Half of them received another injection on day 12. Blood samples were taken just before and 6, 24 and 48 h after injection and the mucosa lining the ileum was recovered following euthanizing on days 7 and 14. Zinc supplementation increased reduced and total glutathione (GSH) (reduced and total) during days 5 to 7 and arginine decreased oxidized GSH measured on days 5 and 12 and the ratio of total antioxidant capacity to total oxidative status during days 12 to 14. Zinc decreased plasma malondialdehyde measured on days 5 and 12 and serum haptoglobin measured on day 12 and increased both metallothionein-1 expression and total antioxidant capacity measured in the ileal mucosa on day 14. Tumour necrosis factor α concentration decreased from days 5 to 12 (all effects were significant at $P < 0.05$). This study shows that the zinc supplement reduced lipid oxidation and lipopolysaccharide-induced inflammation during the post-weaning period, while the arginine supplementation had only a limited effect.

1. Introduction

In weanling piglets, the abrupt change in diet from milk to cereal-based solid feed leads to specific systemic and intestinal perturbations, including over-expression of pro-inflammatory cytokines (Pié et al., 2004) and increased levels of haptoglobin, an acute-phase protein in blood (Petersen et al., 2004; Sauerwein et al., 2005). An increase in oxidative stress is also noted (Sauerwein et al., 2005; Zhu et al., 2012; Yin et al., 2014). Oxidative stress results from production of reactive oxygen species (ROS) exceeding the capacity of the antioxidant system. Its indicators include increases in oxidation products such as malondialdehyde and oxidized glutathione (GSH) (Jaeschke, 2011).

Numerous experiments have shown that feeding pharmacological doses of inorganic zinc (2,000 to 3,000 mg Zn oxide per kg of feed) to weanling piglets improves growth performance, reduces diarrhoea, has a positive impact on the immune response and reduces intestinal malformations (Hu et al., 2012a, 2012b, 2013; Sales, 2013). In our recent study of piglets, we found that zinc supplementation at 2,500 mg/kg after weaning decreased lipid oxidation measured as plasma malondialdehyde concentration (Bergeron et al., 2014). The supplementation improves the total antioxidant capacity measured in the mucosae of the jejunum and ileum 3 h after an acute inflammatory challenge with lipopolysaccharide (Bergeron et al., 2014). It remains to be determined whether or not similar effects are observed for a lipopolysaccharide-induced chronic inflammatory condition. Being major components of the outer membrane of Gram-negative bacteria, lipopolysaccharide injected into the bloodstream can cause a wide variety of pathological effects, including tissue injury, release of various pro-inflammatory cytokines and increased production of ROS, nitric

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oxide (NO) and lipid peroxides (Wyns et al., 2015). The intensity of these effects depends on the dose and time after injection (Wyns et al., 2015).

Studies have also shown that arginine supplements (0.5% to 1% of the diet) improve growth and feed efficiency in weaned piglets (Wu et al., 2010; Yao et al., 2011). We have observed that this supplement improves systemic antioxidant status 3 h after acute challenge with lipopolysaccharide (Bergeron et al., 2014). Arginine is an essential precursor for the synthesis of NO, a key mediator in several physiological functions. Increased production and concentration of NO is known to cause Zn^{2+} release in endothelial cells and to increase metallothionein-1 expression (Wiseman et al., 2006; Li et al., 2010). Metallothionein-1 (MT-1) is a zinc-storing sulpho-protein involved in zinc homoeostasis and has significant antioxidant properties (Formigari et al., 2007). Arginine supplementation could therefore activate zinc release from MT-1 via NO production.

The goal of this study was to determine the impact of zinc and arginine supplementation on antioxidant and inflammatory status in weaning piglets using a model of chronic inflammation and oxidative stress obtained by injecting coliform lipopolysaccharide. Our hypothesis is that this supplementation should improve antioxidant status and reduce inflammation.

2. Materials and methods

2.1. Animals and housing

Forty-eight 20-day-old (±1 day) male-castrated and weaned piglets (Yorkshire × Landrace) were moved from the farrowing room of a commercial farm (La Coopérative Fédérée, QC, Canada) to an experimental farm (Centre de recherche en sciences animales de Deschambault, Québec, Canada) and distributed in pairs to pens (1 m × 2 m) each equipped with a feeder and watering nipple and maintained at 32 °C. The ambient temperature was decreased gradually to 26 °C over the next 7 days, at which point one pig per pen was euthanized (48 h after injection of lipopolysaccharide as described below). The photoperiod was 12 h light and 12 h dark for the duration of the 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.

2.2. Experimental design and diets

Four diets (Table 1) were formulated to meet or exceed recommendations suggested by the NRC (2012) for piglets. The diets were designated as follows: ZN0ARG0 or control, ZN2500ARG0 (containing 2,500 mg Zn/kg), ZN0ARG1 (containing 1% arginine) and ZN2500ARG1 (containing 2,500 mg Zn/kg and 1% arginine). Animals were distributed according to their initial body weight among the 4 treatments in a randomized complete block design (initially 12 per treatment) and were paired according to weight (difference minimized). Feed was provided daily in 3 equal portions throughout the 14-day period for a total of 60 g per piglet per day on day 0 and increased daily by 45% of the intake on the previous day. During the lipopolysaccharide challenge periods, feeding was maintained at the same level as before the injection and refusal was noted daily. In spite of this limiting of feed, refusals were observed each day in all pens, indicating that feed intake was not a limiting factor. The piglets had *ad libitum* access to water during the experiment. They were weighed on days 0, 7 and 14.

2.3. Challenge with lipopolysaccharide

On day 5, lipopolysaccharide (*Escherichia coli* LPS, K-235 Sigma Aldrich, St-Louis, MO, USA) was administered to all piglets by intramuscular injection (100 μg per kg of body weight). Blood samples were taken just before the injection and then 6, 24 and 48 h after from one piglet per pen. One piglet was sacrificed on day 7. On day 12, the remaining piglet was injected again with the same dose and blood samples were collected according to the same sampling schedule. All samples were placed on ice and then centrifuged at 2,000 × *g* for 15 min at 4 °C. Plasma was stored at −80 °C for further analysis.

2.4. Tissue collection

Shortly after obtaining the 48 h post-injection blood sample on day 7 or 14, animals were sedated with an intramuscular injection of azaperone (Stresnil, Vetoquinol Canada Inc. QC, Canada) at 2 mg/kg. The sedated animals were euthanized by CO_2 inhalation. The entire intestine was removed and freed from the mesentery. The segment ending 50 cm cranial from the caecum was considered the ileum (Yen, 2001). The middle 20 cm this segment was used for biochemical analyses and determination of mRNA expression levels of *MT-1*, tumour necrosis factor-α (*TNF-α*) and inductive nitric oxide synthase (*iNOS*). The segment was rinsed with ice-cold saline solution (0.9% NaCl), opened lengthwise and blotted dry. The mucosa was scraped from the underlying tissue using a glass slide, snap-frozen immediately in liquid nitrogen and then stored at −80 °C until analysis.

2.5. Biochemical analysis

Malondialdehyde generation in samples of plasma and ileum mucosa was measured according to the method of Jain et al. (1989) as an index of lipid peroxidation and oxidative status (Michel et al., 2008). Although the spectrometric determination may have given higher values than an HPLC reference method, the concentrations measured in the present study were close to values published in other studies of weanling piglets. Intra-assay and inter-assay coefficients of variability (CV) values were 6.0% and 5.5%, respectively. Samples of ileum tissue (0.5 g) were homogenized directly (Ultra-Turrax T18, IKA-Labortechnick, Stenfer, Germany) with 5 mL of ice-cold PBS pH 7.4 and then centrifuged at 2,000 × *g* for 15 min. The supernatant (100 μL) was used for the assay as described previously for plasma. The intra-assay and inter-assay CV values were 7.0% and 6.0%, respectively.

Plasma TNF-α concentrations were determined using an ELISA kit (# KSC3012/KSC3011, Invitrogen Corporation, Carlsbad, USA). The intra-assay and inter-assay CV values were 6.0% and 7.2%, respectively.

Total antioxidant capacities (TAC) of plasma and intestinal mucosa (supernatant obtained as described above) were assayed (80 μL sample volume) according to the method of Erel (2004) and Maurice et al. (2007). Total antioxidant capacities are a measurement of the concentration of antioxidants including vitamin C, vitamin E, reduced GSH, polyphenol compounds and protein thiol groups (Erel, 2004). The intra-assay and inter-assay CV values were 2.5% and 3.0%, respectively. Serum total oxidant status (TOS) was assayed according to the method of Erel (2005). Total oxidant status is a linear function of the molar concentration of oxidant substances (hydrogen peroxide, cumene hydroperoxide, tert-butyl hydroperoxide). The intra-assay and inter-assay CV values were 2.0% and 3.5%, respectively.

Reduced and total GSH in plasma were determined using fluorescent detection kit K006-F5 (Arbor Assays, Ann Arbor, USA)

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