ALINY KÉTILIM NOVAIS

CHARACTERIZATION OF MITOCHONDRIAL FUNCTION, OXIDATIVE STRESS, INFLAMMATION AND APOPTOSIS IN LOW AND NORMAL BIRTHWEIGHT PIGLET THROUGHOUT THE PERI-WEANING PERIOD

Londrina
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Tese apresentada ao Programa de Pós-Graduação em Ciência Animal da Universidade Estadual de Londrina como requisito parcial para a obtenção do título de Doutor.

Orientador: Prof. Dr. Caio Abércio da Silva

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Londrina, 20 de fevereiro de 2020

DEDICATÓRIA

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RESUMO

O período pós-desmame é caracterizado por uma alta incidência de deficiência energética celular, distúrbios intestinais e infecções bacterianas que podem levar a doenças graves. Evidências indicam que a função mitocondrial e o estresse oxidativo estão intimamente relacionados no controle da eficiência energética celular, resposta imune e bactérias patogênicas. Portanto, estratégias eficazes que visam melhorar a saúde metabólica dos leitões e aumentar vigor durante o período pós-desmame precisam ser desenvolvidas. No entanto, mesmo que tal conceito seja altamente promissor, poucas informações estão disponíveis atualmente em relação à intensidade e duração desse estresse em leitões com normal e baixo peso ao nascimento. Portanto, objetivou-se caracterizar marcadores do metabolismo energético e estresse oxidativo ao longo do período peri-desmame. Um total de 30 (trinta) porcas multíparas foram inseminadas e as leitegadas foram padronizadas em 10 leitões. Os leitões foram distribuídos para um dos dois grupos experimentais: leitões de peso normal ao nascimento (NPN, 1,73 ± 0,01 kg, n = 60) e leitões de baixo peso ao nascimento (BPN, 1,01 ± 0,01 kg, n = 60). Em seguida, 10 leitões de cada grupo foram selecionados aos 14, 21, 23, 25, 29 e 35 dias de idade para a coleta de amostras de plasma e órgãos (fígado, jejuno e rins). Observou-se aumento significativo ($P < 0,05$) do dano oxidativo ao DNA (8-OHdG) e das proteínas plasmáticas (grupo carbonila). Uma diminuição ($P < 0,05$) no nível de energia celular na forma de adenosina trifosfato (ATP) foi observada no fígado após o desmame. Essa diminuição na produção de energia mitocondrial está relacionada à atividade mitocondrial maior ($P<0,05$) da enzima antioxidante glutationa peroxidase (GPx) e superóxido dismutase (SOD) no fígado, mucosa intestinal e rins de leitões após o desmame. Além disso, os níveis de dano oxidativo ao DNA (8-OHdG) mensurados no plasma e protéina hépatica foram maiores ($P < 0,05$) nos leitões BPN. Na sequência foram analisados ensaio de RT2 Profiler™ PCR de genes relacionadas à função mitocondrial, estresse oxidativo, processos de inflamação e apoptose em leitões NPN (23d, 35d vs. 14d). A expressão dos genes selecionados foi avaliada por RT- PCR quantitativo. Essas análises revelaram que a expressão de RNAm das moléculas inflamatórias (*IL-8* e *CCL19*) aumentou após o desmame na mucosa intestinal, enquanto a expressão de genes que codificam subunidades da cadeia respiratória mitocondrial (*NDUFA2* e *NDUFA5*) foi reduzida no fígado de ambos os grupos ($P < 0,05$). Os principais moduladores da apoptose mitocondrial (*BCL2A1* e *BNIP3*) e do sistema de defesa antioxidante (*TXNRD2*, *GPx3*, *HMOX1*) foram altamente expressos em leitões BPN comparados a NPN ($P < 0,05$). Além disso, os níveis sistêmicos de moléculas inflamatórias (*TNF-α* e *IL1-β*) aumentaram logo após o desmame e foram maiores nos leitões NPN ($P < 0,05$). Esses resultados indicaram que o desmame causa diminuição da energia mitocondrial no fígado, intestino e rim, em associação com condições de estresse oxidativo. Além disso, esses resultados sugerem que defesas antioxidantes, resposta inflamatória e apoptose estão comprometidas em leitões BPN.

Palavras-chave: Antioxidantes. Apoptose. Desmame. Estresse oxidativo. Mitocôndria.

NOVAIS, Aliny Kétilim. **Characterization of mitochondrial function, oxidative stress, inflammation and apoptosis in low and normal birthweight piglet throughout the peri-weaning period**. 140 pages. Thesis (Doctor´s Degree in Animal Science) – Universidade Estadual de Londrina, Londrina, 2020.

ABSTRACT

The period following weaning is characterised by a high incidence of energetic deficiencies, intestinal disturbances and bacterial infections that led to serious diseases. Evidence indicates that mitochondrial function and oxidative stress are closely related to controlling cellular energetic efficiency, immune response and bacterial pathogenesis. Thus, effective strategies aiming to improve metabolic health of piglets and increased their robustness during the post-weaning period need to be developed. However,little information is currently available regarding the intensity and duration of this stress in both normal and low weight piglets. The objective was to characterize markers of energy metabolism and oxidative status throughout the peri-weaning period. Thirty (30) multiparous sows were inseminated, and litters were standardized to 10 piglets. Piglets were designed to one of two experimental groups: normal birth weight piglets (NBW, 1.73 ± 0.01 kg, n = 60) and low birth weight piglets (LBW, 1.01 ± 0. 01 kg, n = 60). Then, 10 piglets from each group were selected at 14, 21, 23, 25, 29 and 35 days of age to collect plasma and organ (liver, intestinal mucosa and kidney) samples. Analysis revealed a significant increase ($P < 0.05$) in plasma levels of both oxidative damage to DNA (8-OHdG) and proteins (protein carbonyls) measured in plasma increased after weaning. A decrease ($P < 0.05$) in cellular energy level in the form of adenosine triphosphate (ATP) content, known to be produced in mitochondria, was observed in the liver after weaning. This decrease in mitochondrial energy production was found to be linked to significantly higher ($P < 0.05$) mitochondrial activity major antioxidant glutathione peroxidase (GPx) and superoxide dismutase (SOD) in liver, intestinal mucosa and kidney of piglets after weaning. Additionally, the levels of oxidative damage to DNA (8-OHdG) measured in plasma and of liver protein carbonyls were found to be higher ($P < 0.05$) in LBW piglets. RT2 Profiler ™ PCR arrays were assessed for genes related to mitochondrial function, oxidative stress, inflammation and apoptosis in NBW piglets (d 23 and 35 vs. d 14). The expression of selected genes was assessed by quantitative PCR. These analyzes revealed that the mRNA expression of inflammatory molecules (*IL-8* and *CCL19*) increased after weaning in the intestinal mucosa, while the expression of genes encoding subunits of the mitochondrial respiratory chain (*NDUFA2* and *NDUFA5*) was reduced in the liver of both groups ($P <0.05$). The main modulators of mitochondrial apoptosis (*BCL2A1* and *BNIP3*) and the antioxidant defense system (*TXNRD2*, *GPx3*, *HMOX1*) were highly expressed in LBW piglets compared to NBW ($P <0.05$). In addition, the systemic levels of inflammatory molecules (*TNF-α* and *IL1-β*) increased in the days after weaning and are higher in NBW piglets ($P <0.05$). These results indicated that weaning causes decrease in mitochondrial energy in liver, intestine and kidney after associated with oxidative stress conditions. Further, these findings suggest that antioxidant defenses, inflammatory response and apoptosis, are compromised in LBW piglets.

Keywords: Antioxidants. Apoptosis. Oxidative stress. Mitochondria. Weaning.

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LISTA DE ABREVIATURAS E SIGLAS

ADP	Adenosine Diphosphate
AR	Antibiotic Resistant
ATP	Adenosine Triphosphate
CoQ10	Ubiquinol
Cu/ZnSOD	Copper Zinc Superoxide Dismutase
DNA	Deoxyribonucleic acid
ECSOD	Extracellular superoxide Dismutase
FAD	Reduced Flavin Adenine Dinucleotide
$FADH_2$	Oxidized Flavin Adenine Dinucleotide
GPx	Glutathione Peroxidase
GSH	Glutathione Reductase
H_2O_2	Hydrogen Peroxide
IUGR	Intrauterine Growth Retardation
LBW	Low Birth Weights
MnSOD	Manganese Superoxide Dismutase
NAD	Reduced Nicotinamide Adenine Dinucleotide
NADH	Oxidized Nicotinamide Adenine Dinucleotide
NBW	Normal Birth Weights
NO	Nitric Oxide
O_2	Oxygen
O^{2-}	Superoxide anions
OH	Hydroxyl Radical
RNA	Ribonucleic acid

ROO	Peroxide Radical
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
TCA	Tricarboxylic Acid

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1. INTRODUCTION

In the past 20 years, increased litter sizes have led to increased productivity. Genetic selection is one of the drivers of this improvement, along with better nutrition, overall management and improved health status, thus allowing animals to better express their genetic potential (ROTHSCHIL; RUVINSKY, 2011). However, the increased prolificity has significant consequences for the offspring, including decreased birth weight and increased heterogeneity of piglet weight (QUINIOU et al., 2002).

Weaning is a critical period in swine production in fact, weaning is probably the most stressful event for piglets (WEARY et al., 2007), in which they are subjected to nutritional, environmental, and social stressors that will impact feed intake and, consequently, development of the digestive system, growth, and feed efficiency (CAMPBELL et al., 2013). During the post-weaning period, feeding is key to the success of the subsequent stages of the animal's life. After pigs are weaned, they must adapt to a solid diet that is based on plant protein and concentrate. Whenever the digestive system is disturbed, pathological conditions result (digestive issues, diarrhea), slowed growth, uneven litter sizes, and occasionally, higher mortality (RHOUMA et al., 2017). These conditions lead to an increased use of antibiotics (prophylactic and therapeutic use) (BUROW et al., 2014). Also, the introduction of regulations to curb the use of antibiotics after weaning for pigs, such as those in Europe, could cause these digestive and metabolic disturbances to worsen (RHOUMA et al., 2017).

Weaning in piglets could increase energy demand and lead to the production of reactive oxygen species (ROS), leading to oxidative stress (BUCHET et al., 2017). In addition, oxidative metabolism and the production of ROS are related to

impaired immunity and could also be involved in increased inflammatory responses. Piglets under oxidative be stress can be rendering more susceptible to disease (LUO et al., 2016). An improved antioxidative potential could be part of a nutritional strategy to promote animal health status and decrease or even eliminate the use of antibiotics. Therefore, this review will describe oxidative stress and its interactions with the energy metabolism and inflammatory response and the effect that this can have on piglets during the post-weaning period.

2. **LITERATURE REVIEW**

2.1 WEANING

2.1.1 WEANING STRESS

Weaning is probably the most stressful event for piglets. Early weaning leads to significant nutritional, environmental, social, and psychological stress (MOESER et al., 2007). These disorders can be due to the abrupt separation from the sow, the introduction of a new diet, transportation, management by humans, a new social hierarchy to be established between piglets from different litters, different physical surroundings, and exposure to pathogens.

Generally, piglets are weaned between 18 and 30 days, depending on production methods (COLSON et al., 2006, VAN DER MEULEN et al., 2010). During this period, these stressors often lead to temporary anorexia, significant changes to intestinal anatomy and physiology, and decreased growth and feed efficiency (PLUSKE et al., 1997, LALLÈS et al., 2004, CAMPBELl et al., 2013, XIONG et al., 2019). In fact, after weaning (at 21 days), the weight of the small intestine (tissue and mucosa) is significantly reduced (LALLÈS et al., 2004), as is the height of the villi (PLUSKE et al., 1997).

These structural changes are followed by an increased gut permeability (BOUDRY et al., 2004, MOESER et al., 2007). Moeser et al. (2007) showed a significant drop in transepithelial resistance (TER) in the jejunum and colon of piglets weaned at 19 days and fed for 24 hours with a basal post-weaning diet in comparison to nursing piglets. In addition, the role of the barrier, which impedes the transepithelial migration of pathogens and antigens as well as the digestion and absorption of nutrients and water, must be preserved to ensure the efficiency of the digestive tract. The integrity of the epithelial and mucosal barriers is, therefore, an important factor in ensuring these functions (MOESER, 2010).

During weaning, research shows that the genes that regulate pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are highly expressed after exposure to these different types of stresses. Also, this inflammatory response appears to be associated with anatomical and functional gut disorders (PIÉ et al., 2004).

Furthermore, David et al., (2002) showed that heat shock proteins are expressed in large amounts after weaning in different sections of piglets' gut. These proteins are involved in protecting and maintaining cellular integrity after stress. Research showed that there is an increase in the concentration of heat shock proteins (HSP27 and HSP90) in the gut (stomach, jejunum and proximal colon) (LALLÈS; DAVID, 2011). They noted a decrease in the weight of the small intestine, atrophy of the crypt and villi, and decreased alkaline phosphatase activity in the duodenal mucosa, showing the effect the nutritional stress has on the digestive physiology.

These after weaning changes to the digestive tract can lead to pathological conditions such as diarrhea, slow growth, more heterogeneous litters of

piglets, and in some cases, increased mortality (PLUSKE et al, 1997; LALLÈS et al., 2004, 2007; CAMPBELL et al., 2013). In reality, issues relating to the post-weaning digestive functions are the main causes of mortality and lost efficiency in swine production (MOESER, 2010). These pathological conditions, whose etiology is not fully understood, and which include a microbial, immune, and nutritional component, lead to increased antibiotic use (in either prophylaxis or treatment). The enactment of regulations imposing the reduced use or removal of certain antibiotics after weaning could increase the severity or incidence of digestive and metabolic disorders (RHOUMA et al., 2017).

These anatomical, physiological and dysfunctional changes to the gut after weaning are particularly important for piglets with low birth weight. In fact, as sows become more prolific, the variation in the birth weights of the piglets in each litter increases. This intrauterine growth retardation (IUGR) can significantly impact the metabolism, development, weight gain, and growth of pigs (LE COZLER et al., 2004; LIU et al., 2013; QUINIOU et al., 2002; WU et al., 2006). Michiels et al., (2013) noted that pigs at 45 to 55 days of age that were weaned at 27 days and affected by IUGR showed slower gut development compared to those born with average birth weight. Also, post-natal mortality in pigs affected by IUGR is higher and carcass yield is lower when compared with piglets born with higher birthweights (LE COZLER et al., 2004; QUINIOU et al., 2002).

2.1.2 Highly-Prolific Sows and Low Birth-Weight Piglets

The high-prolificity of sows and the increased survival rate lead to a

higher incidence of IUGR, which can impact the pre- and post-natal development of the litter. The intense selection of lineages for prolificity and the common use of maternal heterosis through the crossing of two breeds has influenced the size of the litter at birth. This increased fertility of sows and mortality of piglets has been observed in many countries, including Switzerland, France, Great Britain, and Poland (BEE, 2007; BOULOT et al., 2008; ORZECHOWSKA; MUCHA, 2010).

The pre-natal survival rate is related to the amount of intrauterine space. Fetuses developing in a limited intra-uterine space have a higher chance of facing perinatal disease and post-natal mortality compared with piglets developing under normal conditions (CHEN; DZIUK, 1993; RYAN; VANDENBERGH, 2002). The fetus requires at least 36 cm of uterine length at day 50 of gestation to develop normally (WU et al., 1999), a condition that is not always found as fertility increases.

According to research, the production efficiency of modern genotype females is determined by an increase in the number of born and weaned piglets/litter/year over the reproductive life (QUINTON et al., 2006; ROCADEMBOSCH et al., 2016). Brüssow et al., (2011) indicate that heritability for reproductive traits has low heritability and small repeatability. Even animals with high genetic value can have their genotype impacted by antagonist effects of the external and internal environments that continuously occur (ONTERU, 2011).

Research shows that when the size of the litter increases, the variation in birth weight within the litter increases (QUINIOU et al., 2002; BOULOT et al., 2008); heterogenicity increased as a consequence (MILLIGAN et al., 2002; BEE, 2007; BEAULIEU et al., 2010). In a study involving 965 litters, Quiniou et al., (2002) found that in approximately 45% increase in litter size reduced the average weight at birth by over 20% and that there was a 16% increase in the number of piglets born

with less than 1 kg. According to Quiniou et al., (2002) and Boulot et al., (2008), the mortality rate in the group of piglets weighing less than 1 kg is 3- to 5- fold higher than in the group of piglets with weight > 1 kg. The low body weight and the high weight variability at birth are negatively correlated with piglet survival (HERPIN et al., 2002) and can impact productive traits such as growth, weight gain, and carcass fat. Therefore, preference in breeding is given to gilts born from sows females with high uterine capacity as it increases the chances of improving the size of the litter (VALLET et al., 2002).

2.1.3 Weaning and the Use of Antibiotics

Weaning is known to cause stress to piglets, and the post-weaning period is known for a high incidence of gut disorders, bacterial infections, and energy deficiency that can lead to serious disease (PIÈ et al., 2004; LALLÈS et al., 2007; WIJTTEN et al., 2011). Unfortunately, this issue was intensified by the selection of highly-prolific sows with a view to increasing the size of the litter and its profitability, which has led to a higher variability in birthweights and an increase in the number of piglets with low birth weights (LBW) (DAMGAARD et al., 2003). These low-birthweight piglets are more likely to suffer from an inadequate pre- and post-natal transfer of nutrients which make them more susceptible to infectious gut diseases and energetic deficiencies (DE VOS et al., 2014).

The health status of post-weaned piglets has been associated with the intense use of antibiotics as a prophylactic treatment in swine production in an attempt to limit the incidence of disease (AARESTRUP, 2005). However, the current removal of many antibiotics for growth-promoters and disease prevention and other restrictions adopted by several countries has been associated with an increase in

health issues in recently-weaned pigs (JENSEN; HAYES, 2014). As a result, effective strategies aimed at improving the metabolic health of the piglets and their defence mechanisms against bacterial infections are required to limit the use of antibiotics in swine production and ensure profitability (DIANA et al., 2019). Also, there is evidence that the mitochondria of recently-weaned piglets require optimal conditions in terms of antioxidant protection and the availability of metabolic substrate to ensure proper energy status, intestinal health and disease resistance (CAO et al., 2018). However, even if such a concept is highly promising, no focused nutritional strategy is actually available to producers to provide for such outcomes.

Antibiotics have been widely used in the past few decades to promote growth and as prophylactic treatment to prevent swine disease (BARTON, 2014). There are three important components of antibiotic-resistance (AR): the antibiotics, the AR bacteria and the AR genes. However, several countries, including Canada, are increasingly concerned with the development of antibiotic-resistant (AR) bacterial strains and the development of genes resistance that could impact human health (SHEIKH et al., 2012; DURSO; COOK, 2014). There is broad consensus that AR bacteria and the genes associated with this resistance can be physically transferred to humans. In fact, the World Health Organization (WHO) recognized that the use of antibiotics in animals will likely impact the incidence of human antibiotic resistance (CAPITA; ALONSO-CALLEJA, 2013). Also, a significant correlation between the use of antibiotics and the spread of resistant enterotoxic *E.coli* isolated in the bovine species was recently established in a number of countries (CHANTZIARAS et al., 2014).

It is known that AR bacteria antibiotics can be transmitted from animals to humans through food, direct contact, and environmental dissemination of

fecal residues (MARSHALL; LEVY, 2011). Food-borne disease are responsible for the highest number of AR bacteria transmitted from farms to consumers, particularly when adequate food safety standards are not consistently applied throughout the food chain (BYWATER; CASEWELL, 2000; VAN DEN BOGAARD; STOBBERINGH, 2000). This is very hard to quantify in fact, given the delay between food exposure and infection, it is somewhat difficult to find evidence that links human infections to resistant bacteria sourced from foods prepared from animals treated with antibiotics. Several published studies establish a direct correlation between antibiotic-resistant bacteria in food to human disease (TEALE, 2002; VARMA et al., 2006, DURSO; COOK, 2014).

Consequently, several countries have taken or are beginning to take measures to control and reduce the use of antibiotics in swine production (BARTON, 2014). The non-use of antibiotics and other restrictions have not yet been linked to a reduction in the total use of antimicrobials or to a drop in bacterial resistance. In fact, the elimination of antibiotics in swine prophylaxis has been linked to an increase in the number of health problems in animals that require the use of antibiotic therapy, particularly among the recently weaned (JENSEN; HAYES, 2014).

Therefore, effective strategies aimed at improving the metabolic health of piglets and boosting their defence mechanisms against bacterial infections during the post-weaning period need to be developed to limit the use of antibiotics in swine production (AARESTRUP et al., 2008; STANTON, 2013).

2.2 ENERGY DEFICIENCY AND OXIDATIVE STRESS

2.2.1 Mitochondria and Energy Production

The mitochondria are the interface between energy supply and requirements of individual body organs. In response to energy requirements, various substrates such as carbohydrates, proteins, and fatty acids are metabolized through several pathways, including glycolysis, β-oxidation, tricarboxylic acid (TCA) or Krebs Cycle, and the transmission of electrons through the respiratory chain and ultimately leading to energy synthesis in the form of ATP via oxidative phosphorylation (OXPHOS) (GREEN; TZAGOLOFF, 1966).

The mitochondria are efficient source of cellular ATP. The process involves the oxidative phosphorylation (OXPHOS) steps of the mitochondrial respiratory chain which is made up of five complexes located in the internal mitochondrial membrane. The reduced form of the nicotinamide adenine dinucleotide (NADH) produced by the TCA cycle is initially oxidized by complex I. Given that the NADH electrons are transferred to the first electron receptor, where it is either oxidized into coenzyme Q10 ubiquinol (CoQ10) or the energy is converted by proton pumping of the mitochondrial matrix to the intermembrane space. Coenzyme Q10 may also accept complex II electrons donated by the reduced form of the flavin-adenine dinucleotide ($FADH_2$), another product of the TCA cycle. Then, the CoQ10 transfers electrons to the complex III. The electrons are then transferred to the second mobile element of the respiratory chain, the cytochrome c, which reduces the molecular structure of the oxygen to make water. This final dissipation of the redox energy is associated also with a final matrix proton ejection. The transfer of protons to the intermembrane space, which generates an electrochemical gradient that eventually leads to the phosphorylation of the ADP into ATP through the complex V or proton ATPase when re-introducing the matrix. The ATP is then transferred to the outside of the mitochondria through the adenine nucleotide translocase, and the

energy remains available for cellular processes (Figure 1). The mitochondria that produce energy and fail to generate a mitochondrial membrane potential become a target for destruction through mitophagy. A significant correlation between the levels of expression of mitochondrial proteins involved in energy metabolism and productivity was recently observed (GRUBBS et al., 2013). As a result, whenever the metabolic demands are high, the demand on the mitochondrial respiratory chain to respond to these energy requirements is also high to provide large amounts of ATP. The physiological status of an animal determines the nutritional demand and the energy efficiency of the mitochondrial function. Countless factors, such as high metabolic activity and hypoxia, can significantly impact the use of the substrate and the activities of the main mitochondrial enzymes.

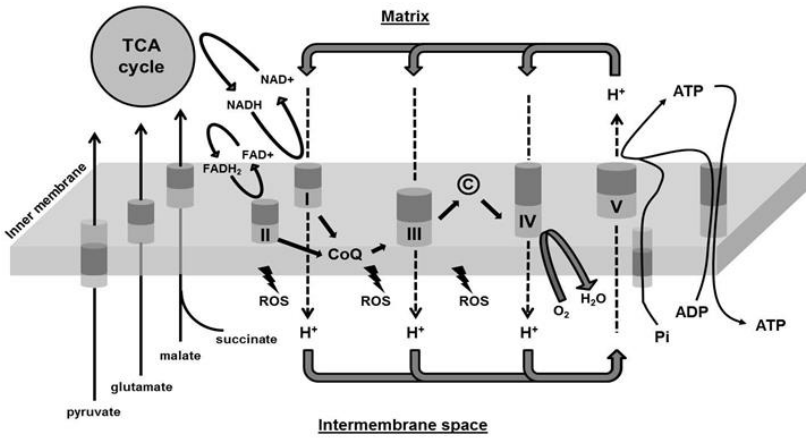

Figure 1. Energy production system of mitochondrial electron transportation chain and oxidative phosphorylation (Lapointe, 2014). The TCA cycle, tricarboxylic acid cycle; mitochondrial I to V complex; CoQ, coenzyme Q or ubiquinone; (c) cytochrome c; H^+, protons; Pi, phosphate; ADP, diphosphate adenosine; ATP, adenosine triphosphate; ROs, reactive oxygen species; O_2, oxygen; H_2O, water; NAD, oxidized or reduced nicotinamide adenine dinucleotide (NADH); FAD, oxidized or reduced flavin-adenine dinucleotide ($FADH_2$).

2.2.2 Products of Reactive Oxygen Species (ROS) and Oxidative Damage

During aerobic metabolism, cells produce energy in the form of ATP

through a mitochondrial process of oxidative phosphorylation. During this process, oxygen is reduced to water (H_2O) in a reaction catalyzed by the enzyme cytochrome c oxidase in the mitochondrial respiratory chain, which generally requires four electrons and four protons ($O_2 + 4H^+ + 4é = 2H_2O$) (HALLIWELL; GUTTERIDGE, 2015). However, the process of cellular respiration and energy metabolism produces reactive oxygen species (ROS). Reactive oxygen species are oxidizing agents that can be more reactive than O_2 (KALYANARAMAN, 2013; HALLIWELL; GUTTERIDGE, 2015). In fact, some of the components of the mitochondrial transportation electrons can generate superoxide anions (O_2•-), a highly-reactive molecule. This free radical is unstable and can oxidize molecules and lead to the production of other ROS. Several types of compounds derived from oxygen (ROS) and nitrogen (RNE) may result from nitric oxide (NO), hydroxyl radical (OH), peroxide radical (ROO), and hydrogen peroxide (H_2O_2). At the cellular level, reactive oxygen species are involved in maintaining homeostasis and also in important biological functions, such as immunity and reproduction (DELAMIRANDE; GAGNON, 1993; HURST, 2012; DEVASAGAYAM et al., 2004).

In addition, some of these ROS can leave the mitochondria, react with other cellular components or even with other cells (BOVERIS et al., 1972). This is particularly true in the case of the hydrogen peroxide, a more stable ROS, capable of crossing membranes. Finally, the excessive production of ROS can damage mitochondrial and cytoplasm macromolecules, such as proteins, fats, and DNA. Once oxidized, these damaged macromolecules can cause a loss of biological functions and DNA mutations (DEVASAGAYAM et al., 2004; LYKKESFELDT; SVENDSEN, 2007). This imbalance between oxidizing and antioxidant compounds lead to oxidation of macromolecules in the cell and is designated as oxidative stress (Figure

2). The cells have defence mechanisms against ROS and for the removal of damaged macromolecules (SIES et al., 2017). In fact, an antioxidant network allows for a first control of ROS.

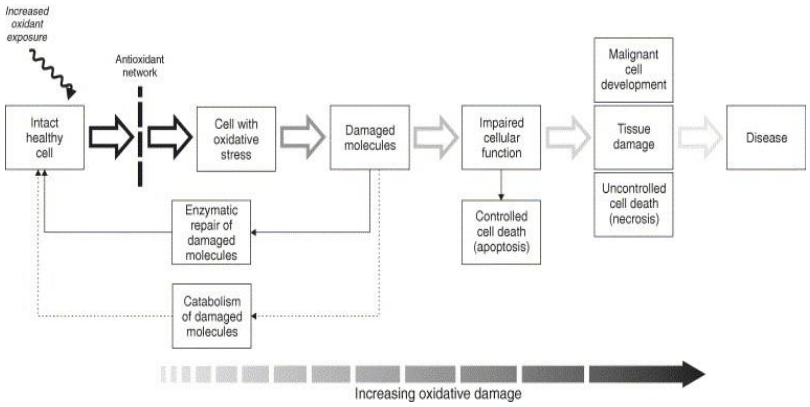

Figure 2. Representation of cellular defence against oxidative stress. Adapted by (Lykkesfeldt; Svendsen, 2007).

The reactive oxygen species, including free radicals, are generated mainly by aerobic respiration and metabolism by mitochondria. During the stage of mitochondrial electron transport, the reduction of oxygen is the main source of the superoxide (O_2•-), when mitochondrial superoxide oxygen dismutase (SOD) convert hydrogen peroxide (H_2O_2) and O_2. This reaction is important because the H_2O_2 is relatively stable and permeable to the membrane, and able to diffuse from the mitochondria to the cytoplasm (VEAL et al., 2007). Reactive oxygen species can, therefore, cause serious damage to mitochondrial and cytoplasmic macromolecules such as lipids, nucleic acids, and proteins. Poly-unsaturated fatty acids are susceptible to ROS oxidation because once fat peroxidation is initiated, a chain reaction follows (NIKI, 2009). The DNA bases are also susceptible to the ROS attack, and it is believed that the oxidation of the DNA bases lead to mutations and deletions

in both nuclear and mitochondrial genomes (FRAGA et al., 1990). Almost all aminoacidic residues found in proteins can be oxidized by ROS, and these changes can lead to loss of function (UGARTE et al., 2010).

Exposure to ROS seems unavoidable for cells in aerobic environments, and ROS toxicity is controlled by a complex network of enzymatic and non-enzymatic antioxidants, including superoxide dismutase (SODs), glutathione peroxidases (GPxs), thioredoxin reductase (TRxs), peroxiredoxins (PRxs), catalase, and glutathione reductase (GSH) (YU, 1994; FLOHE, 2010). Therefore, oxidative stress can be defined as any imbalance between ROS production and detoxification.

During periods of high energy demand, ROS production by mitochondria exceeds, to a great extent, the antioxidant potential, leading to conditions of oxidative stress and leading to oxidative damage in the main cellular structures (BALABAN et al., 2005). It is known that reproductive performance, disease resistance, immune response, and longevity can be negatively impacted by mitochondrial oxidative stress in mammals (AGARWAL et al., 2006). The links between oxidative stress and adverse physiological development represent important issues in animal science, particularly in animals under metabolic stress (Figure 3).

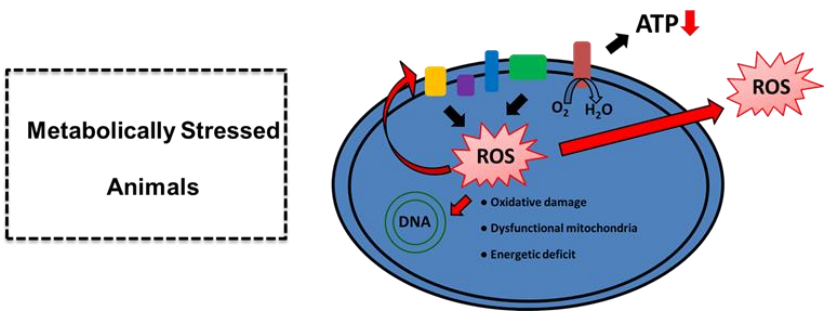

Figure 3. Representation of the mitochondrial function and production associated with ROS in animals under metabolic stress. Whenever the metabolic demand is high, as is the case with the swine species, the demand on the mitochondrial respiratory chain to respond to all energy requirements is also high in order to provide large amounts of ATP. Mitochondrial energy production is associated with the production of ROS, and highly prolific sows and

weaned pigs have to deal with substantial amounts of ROS, which makes them susceptible to oxidative damage, including impairment of reproductive function, and more vulnerable to disease and to decreased longevity.

2.2.3 Biological Implications of Mitochondrial Oxidative Stress

Oxidative metabolism and the production of ROS are also related to the immune system and to the inflammatory response. In fact, reactive species are involved in intracellular signaling and the activation of cells in the immune system (GEISZT; LETO, 2004), synthesis of pro-inflammatory cytokines (WEST et al., 2011), cellular adhesion (CHIARUGI et al., 2003) and the oxidative process of phagocytosis (HURST, 2012). In addition, oxidative stress and inflammation appear to be involved in disorders of the intestinal barrier (JOHN et al., 2011). Oxidative stress is an important factor in the development of some syndromes of poor absorption and gastrointestinal inflammation (REZAIE et al., 2007, JOHN et al., 2011, KIM et al., 2012).

Superoxide ion production occurring in the cellular respiratory chain increases with cellular activity whenever there is an increase in energy demand or a large supply of oxygen (BOVERIS; CHANCE, 1973). This is important because after weaning, pigs undergo a transient anorexia, and as energy demand increases, ROS production also increases, leading to increased oxidative stress. This increase in oxidative status could damage cell structures, further stressing the pigs. In fact, Yin et al., (2014) noticed that piglets weaned at 14 days of age had a significant increase in the levels of some cellular oxidation markers, such as malonaldehyde, a marker of lipid oxidation, 8-hydroxydeoxyguanosine, a marker of DNA oxidation and of protein carbonylation, a marker of protein oxidation.

Growing evidence suggests that mitochondrial function and oxidative

stress are closely related to the innate immune response (WEST et al., 2011), intestinal inflammation (CIRCU; AW, 2012; LÓPEZ-ARMADA et al., 2013), reproduction (AGARWAL et al., 2006), and bacterial pathogenesis (ARNOULT et al., 2009).

The toxicity of the mitochondrial ROS is only one aspect of its action in living cells as ROS from mitochondria or other parts of the cell can also influence the signaling of several pathways. In fact, under physiological and stress conditions, the transitory production of ROS within limits appears to be key to maintaining cellular homeostasis.

The reactive oxygen species of mitochondria are also the main defence mechanism of the host against infections and harmful agents, and the mitochondria ROS are also known as the main mediators of inflammation. It has been shown that pro-inflammatory factors hinder the mitochondrial activity and increase mitochondrial dysfunction as they can modulate inflammatory responses through redox-sensitive inflammatory pathways and direct activation of the inflammasome (LÓPEZ-ARMADA et al., 2013). Therefore, mitochondria could be a part of these two pathways, leading to an overstimulation of the inflammatory response. A drop in mitochondrial function is key to the inflammatory phenotype found in degenerative, acute disease, and aging (HAAS, 2019). Mitochondria are now considered central to regulation of the immune cells. Several studies have shown that in immune cells, the mitochondria participate in the signaling through the production of ROS, availability of metabolites, and protein interactions (WEST et al., 2011, WEINBERG et al., 2015). The mitochondrial signals appear to be necessary for immune cells to fulfill their specific role in the immune response in innate and adaptive environments of several challenges. The mitochondrial signaling determines

the macrophage polarization and function, regulates the activation of the T cells, and controls the CD8+ memory T cells (LEAVY, 2013).

Also, the mitochondria were identified as the target of a growing number of bacterial proteins that are transferred to the cell by virulent bacteria such as enterotoxic *E. coli* (ETEC) and *Salmonella (*S. enterica) (RUDEL et al., 2010; JIANG TONG et al., 2012; HICKS; GALÁN, 2013). These pathogens have developed multiple strategies to subvert the mechanism of cellular death in the host, including mitochondria-mediated mechanisms, to (i) avoid the premature death of infected cells to facilitate intracellular replication; (ii) kill infected cells at a late stage of the microbial life-cycle to favour dissemination of the pathogen; or (iii) destroy non-infected immune cells to subvert the antimicrobial mechanism. These bacteria are problematic for pigs in the post-weaning period and lead to severe loss of mitochondrial energy and to rupture of the epithelial barrier. This leads to significant losses per year due to mortality, cost of medication, and reduced growth (SARGEANT et al., 2011; MIARELLI et al., 2016).

2.3 ANTIOXIDANTS

2.3.1 Non-enzymatic antioxidants

Antioxidants can be defined as any substance that, when present in baseline concentrations, in the presence of a substrate that can be oxidized, retards or inhibits the oxidation of this substrate (HALLIWELL; GUTTERIDGE, 2007). The antioxidant defence system is composed of enzymatic and non-enzymatic compounds and can be found inside the body's cells or in the blood stream. Examples are ubiquinone, α-lipoic acid, N-acetylcysteine, vitamins A, C, and E, and

glutathione (GSH).

Ubiquinone or CoQ10 is a bioactive lipid known mostly for its function as transporter of electrons of complexes I and II to complex III in the mitochondrial respiratory chain. In its reduced form (ubiquinol), the CoQ10 can act as an antioxidant, protecting mitochondrial lipids, proteins, and DNA from oxidative damage. It is also known for its anti-apoptotic as well as anti-inflammatory properties, and is widely used as a nutritional supplement (BENTINGER et al., 2010). The CoQ10 is actively incorporated in the mitochondrial membrane where it can reverse the mitochondrial dysfunction, alleviate oxidative stress conditions and improve cellular function in several conditions (BHAGAVAN; CHOPRA, 2006; LAPOINTE et al., 2012).

The α-lipoic acid is a coenzyme found naturally in mitochondria and is involved in energy metabolism. The reduced form of the α-lipoic acid, dihydrolipoic acid, is a powerful mitochondrial antioxidant. It is involved in the reduction of vitamins C and E, recycles CoQ10, increases the intracellular levels of both glutathione and ascorbic acid, chelated iron and copper, and activates the main enzymatic antioxidants (PACKER et al., 1995). Supplementation studies of α-lipoic acid to animals found that it led to a decrease in the level of damage, reduced production of oxidizing compounds, and to the improvement of mitochondrial function (HAGEN et al., 1999). N-acetylcysteine (NAC), a by-product of the N-acetyl of the amino acid cysteine, is also known for its antioxidant properties. It has been reported to increase glutathione and its related enzymatic antioxidants (SCHIFF et al., 2011). Vitamin A and carotenoids are also known as antioxidants. Dietary beta-carotene is efficiently incorporated by the mitochondria and participates in protecting the mitochondrial DNA and cytochrome c against oxidative stress associated with aging (LIU; AMES,

2005). It has also been recently described in vitro that retinol (generally the dietary form of vitamin A) is a regulator of mitochondrial function (ACIN-PEREZ et al., 2010).

Vitamin C (ascorbic acid) is a water-soluble vitamin that has been used in mitochondrial disorders because of its antioxidant properties. In fact, ascorbate can be oxidized (dehyroascorbate) and reduced back to ascorbate by antioxidant enzymes and GSH. In addition to its antioxidant properties, vitamin C is a co-factor for the hydroxylation of collagen and is involved in the biosynthesis of endogenous carnitine (LIU; AMES, 2005).

Vitamin E has antioxidant properties, is liposoluble and includes four tocopherols and four tocotrienols with α-tocopherol having the highest biologic activity. Tocopherols have important antioxidant properties. They accumulate in the cellular membrane and are particularly abundant in mitochondrial membranes. The main role of vitamin E is to eliminate mitochondrial ROS and to inhibit lipid peroxidation, thereby maintaining the integrity of the membrane (MARRIAGE et al., 2003). Evidence suggests that it is possible to strengthen the mitochondria through dietary supplementation with vitamin E (LAURIDSEN; JENSEN, 2012).

Glutathione (GSH) is a tripeptide (L-glutamyl-L-cystenylglycine) with multiple cell functions. It includes a thiol group in the form of cysteine residue and has antioxidant properties; it is able to interact with ROS and RNS and to participate as a co-factor of several enzymes (DIAZ VIVANCO et al., 2010).

Initially, GSH was described as a powerful antioxidant; however, it was later associated with other cell functions. It is currently accepted that the GSH acts not only as a reduction agent and as an important antioxidant inside the cells but also as the mediator of several other physiological reactions, including xenobiotic metabolism, thiol-disulfide exchange, cellular reactions, and cellular signaling

(regulation of the cellular cycle, proliferation, and apoptosis). Despite the fact that it is synthesized in the cytoplasm only, GSH is distributed into the intracellular organelles, including the endoplasmic reticulum, nucleus, and mitochondria. In the nucleus, GSH maintains the critical protein sulfhydryl required for the repair and expression of DNA (VALKO et al., 2007).

GSH is found mainly in its reduced form, except in the endoplasmic reticulum, where it is primarily found as oxidized glutathione (GSSG), which is the main source favouring the formation of disulfide bonds (HWANG et al., 1992). However, in the mitochondria, GSH is found mainly in its reduced form and represents a smaller fraction of the total GSH (10-15%) (MÅRTENSSON et al., 1990). Glutathione (GSH) undergoes reversible oxidation or reduction reactions key to the cellular redox balance to ensure the activity of certain antioxidant enzymes (MARÌ et al., 2009).

2.3.2 Enzymatic antioxidants

Enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase allow for a first level of cellular defence. Thereafter, whenever the macromolecules are damaged by reactive oxygen species, the cells are able to use certain enzymes that are capable of detecting and correcting damage to DNA molecules or to metabolize and eliminate the non-functional ones, e.g., damaged proteins and lipids (DEMPLE; HARRISON, 1994; PARZYCH; KLIONSKY, 2014; SIES et al., 2017). However, when the damage to the cell is significant, apoptosis is initiated to eliminate these damaged cells (HADDAD, 2004). Finally, cellular death can also damage tissues and even lead to certain pathologies (LYKKESFELDT; SVENDSEN, 2007).

2.3.2.1 Glutathione peroxidases (GPx)

The glutathione peroxidases (GPxs) belong to a family of genetically related enzymes. An example in mammals is GPx1 through GPx4, which are selenoproteins that have a selenocysteine (Sec) in the catalytic centre (MARGIS et al., 2008). Selenium is a mineral that participates in the synthesis of selenoproteins with strong antioxidant properties, such as glutathione peroxidases (GPxs) and thioredoxin reductase (TRxs) (DURSUN et al., 2011).

For decades, GPxs were known for catalyzing the reduction of H_2O_2 or organic hydroperoxides into water or corresponding alcohols, respectively, using glutathione (GSH) as the reducing agent (URSINI et al., 1995). The presence of selenocysteine with the catalytic portion ensures a quick reaction with the hydroperoxide and a quick reduction by the GSH. This characterization was widely accepted given that only the first four GPxs had been identified. Therefore, they appeared to have an antioxidant role in different compartments of the cell GPx1 in the cytoplasm and mitochondria, GPx2 in the intestinal epithelium, GPx3 in the plasma (all three in the aqueous phase), and GPx4 in the cell membrane, working against oxidative stress. However, GPx5 was also found; it has a cysteine rather than a selenocysteine at the active centre and has been characterized as an epididymis secreted protein (GHYSELINCK; DUFAURE, 1990). There is also GPx6, a selenoprotein found in humans but not found in rats or mice (KRYUKOV et al., 2003), which expressed in the olfactory epithelium (DEAR; CAMPBELL, 1991). GPx7 and GPx8 contain cysteine but little GPx activity (BRIGELIUS-FLOHÉ and FLOHÉ, 2011).

Glutathione peroxidase 1 (GPx1) was the first selenoprotein identified (ROTRUCK et al.,1973, FLOHÉ et al., 1973), found mainly in the cytoplasm and showing lower mitochondrial matrix expression; it is therefore a primary defence

against mitochondrial membrane damage due to oxidation (MARÌ et al., 2010). It is a homotetramer and reacts with H_2O_2 and with soluble hydroperoxides of low molecular mass, such as t-butyl hydroperoxide, cumene hydroperoxide, hydroperoxide fatty acids (FLOHÉ, 1989), and even lysophosphate hydroperoxides (MARINHO et al., 1997), but does not react with more complex fatty hydroperoxides, a role that is performed by GPx4.

Knockout GPx1 studies in mice showed that the mice developed normally, indicating that a lack of antioxidant defence can be compensated for by other proteins or that oxidative stress conditions does not cause major damage (HO et al., 1997; HAAN et al., 1998). In contrast, acute oxidative stress killed GPx1 -/- mice regardless of selenium supplementation; in contrast, wild-type mice supplemented with selenium survived (CHENG et al.,1998). These results suggest that GPx1 cannot be replaced by any other protein with the goal of protecting against generalized oxidative stress, and that it performs a primary antioxidant role in vivo. Within the hierarchy of the SecGPxs (selenocysteine) family, GPx2 is the highest ranked and is followed by GPx4, GPx3 and GPx1 (WINGLER et al., 1999).

In the hierarchy of selenoproteins, GPx1 is the lowest-ranked one, suggesting that selenoproteins are not evenly supplied with selenium, particularly when selenium is not a limiting factor (CHAMBERS et al., 1986).

Glutathione peroxidase 2 (GPx2) is a homotetramer and strictly related to GPx1. It is expressed mainly in the gastrointestinal system, including the epithelium of the esophagus and, in humans, also in the liver. Therefore, it has been named GI-GPx or GPx-GI and is believed to work as a barrier against the absorption of hydroperoxides of food origin (CHU et al.,1993). GPx2 concentrations are highest at the base of the crypt, lowering towards the tops of the crypts of the colon or in the

villi in the small intestine, respectively (FLORIAN et al., 2001). The rapid regeneration of the intestinal surface is due to the cellular proliferation at the base of the intestinal crypt regulated by the Wnt pathway, which also promotes the expression of the glutathione peroxidase 2, suggesting that it plays an important role in the intestinal turnover (KIPP et al., 2012).

Prevention of the absorption of hydroperoxide is GPx2's main role, given that the crypt base is not the main location for absorption. Studies in Knockout mice (GPx2) show no clear phenotype (ESWORTHY et al., 2000), but an increase in allergic inflammation of the airways when challenged with ovalbumin has been observed (DITTRICH et al., 2010). A histochemical characterization showed a high number of apoptotic cells at the base of the colonic crypt, particularly when mice were fed a selenium-deficient diet (FLORIAN et al., 2010).

Glutathione peroxidase 3 (GPx3) is similar to GPx1, a tetramer, and contains two of the four arginines responsible for binding of GSH. It was described initially as being synthesized in the proximal convoluted tubule of the kidney (AVISSAR et al., 1994) and secreted into the plasma (WHITIN et al., 2002), but it has been found also in the lungs, heart, and in the intestine (KINGSLEY et al., 1998). Along with selenoprotein P, GPx3 is responsible for over 97% of all plasma selenium in mice (OLSON et al., 2010). The high amounts of GPx3 in the plasma could be suggestive of selenium transportation or homeostasis. However, no indication has been found to support this suggestion (OLSON et al., 2010). GPx3 is also found in other extracellular body fluids, such as in the eyes, in the thyroid colloid (KÖHRLE, 2005, SCHOMBURG; KÖHRLE, 2008), fatty tissue (MAEDA et al., 1997), and amniotic fluid (FLOHÉ, 1997). It has been recently determined that GPx3 connects to basal membranes of the tubular cells of the kidney cortex and also to the basal

membranes of the epithelial cells along the intestine, epididymis, bronchia, and type II pneumocytes (BURK et al., 2011).

Studies in GPx3 Knockout mice showed viable animals; however, no clear phenotype was determined (OLSON et al., 2010), while in the treatment with ADP (adenosine diphosphate) a prothrombotic state was evidenced along with vessel dysfunction leading to arterial thrombosis (JIN et al., 2011).

Glutathione peroxidase 4 (GPx4) is a monomer and loses the dimer and tetramer interface, as do all amino acids involved in bonding GSH with GPx1 (AUMANN et al., 1997). In spite of the loss of binding sites, GPx4 still reacts with GSH (URSINI et al., 1997) and not with the thioredoxin (TAKEBE et al., 2002). GPx4 was initially characterized as a lipid peroxidation-inhibiting protein (URSINI et al., 1982) due to its capacity to reduce not only H_2O_2 and small hydroperoxides in general but also complexes such as hydroperoxides, phospholipids, and cholesterol, even when inserted in biomembranes or lipoproteins (THOMAS et al., 1990). There are three different GPx4 isoforms: cytosolic (cGPx4), mitochondrial (mGPx4) and nuclear in the sperm (nSGPx4). Although a systemic failure of the entire GPx4 gene is lethal, knockout mice were fully viable and developed normally (SCHNEIDER et al., 2009).

2.3.2.2 Superoxide dismutase (SOD)

Superoxide dismutase enzymes specialize in the elimination of superoxide anionic radicals resulting from extracellular stimuli, including radiation and oxidative injury, added to radicals produced mainly inside the mitochondrial matrix as by-products of oxygen metabolism through the electron transportation chain (MCCORD; FRIDOVICH, 1969).

Three distinct isoforms of SOD have been identified and characterized in mammals: copper/zinc superoxide dismutase (Cu/ZnSOD, coded by gene SOD1), manganese superoxide dismutase (MnSOD, coded by gene SOD2), and extracellular superoxide dismutase (ECSOD, coded by gene SOD3). These forms of SOD have similar roles, but the characteristics of their protein structures, the location of the chromosome, metal cofactor requirements, gene distribution, and cell compartmentalization are different. Genetic comparisons indicate that in terms of amino acid homology, the genes SOD1 and SOD3 share similarities, while the SOD2 does not share any similarities (PARGE et al., 1992).

The regulation of SOD genes performs a fundamental role in the balance of the concentration of reactive oxygen species. The compartmentalization and control of SODs in terms of expression and activity contribute to the SOD level and, as a consequence localized ROS level (FORMAN, 2007; LIU et al., 2008).

The first superoxide dismutase to be characterized was Cu/ZnSOD (SOD1) and it can be found in the cytoplasm, nucleus, microsomes as well as in the mitochondrial intermembrane space (OKADO-MATSUMOTO; FRIDOVICH, 2001).

In SOD1 knockout mice, physiological compromise has occurred, including lower fertility in females, vascular degeneration of liver tumours leading to death (TURNER; TALBOT, 2008).

When comparing the three SOD isoforms, MnSOD (SOD2) proved vital for the survival of aerobic organisms (CARLIOZ; TOUATI, 1986). The role of the MnSOD as a cytoprotective isoenzyme was confirmed by the short life of the MnSOD knockout mice, which died shortly after birth with dilated cardiomyopathy and neurodegeneration (LI et al., 1995; LEBOVITZ et al., 1996). The recently synthesized polypeptide for MnSOD requires transportation through two mitochondrial

membranes into the mitochondrial matrix, where the enzyme is converted into its active form (ROSENBLUM et al., 1996).

ECSOD (SOD3) containing copper and zinc was initially found in extracellular fluids, including plasma, lymph, and synovial fluid. It is a hydrophobic glycoprotein normally found as a homotetramer with molecular weight of approximately 135,000 Da (MARKLUND et al.,1982).

2.4 MITOCHONDRIA AND NUTRITION

Mitochondrial energy production is strongly associated with the production of reactive oxygen species (ROS) and, in this case, highly-prolific sows and post-weaning piglets deal with significant amounts of ROS. These conditions lead to susceptible to the oxidative damage and are likely lead to reproductive issues, increasing their vulnerability to several diseases and decreasing their longevity (JANSEN; BURTON, 2004; PIECZENIK; NEUSTADT, 2007). As a result, mitochondria require optimal conditions in terms of antioxidant protection and availability of metabolic substrates to ensure reproductive success, health, and long-term productivity.

However, the goal of optimizing mitochondrial function can be perceived as complex and ambitious, and this organelle is known for being sensitive to the amount and variety of nutrients provided by diet. Evidence suggests that providing naturally-sourced specific nutrients to mitochondria, currently referred to as mitochondrial nutrients or mitonutrients, could efficiently prevent and improve several conditions associated with mitochondrial dysfunction. Recent studies show that directed nutritional interventions can be beneficial in the prevention and improvement of mitochondrial function, particularly by stimulating the production of mitochondrial

energy, improving mitochondrial metabolism (biogenesis and degradation) as well as decreasing the oxidative stress. These nutrients can exercise their function directly by being actively involved in mitochondrial biochemical pathways or, indirectly, by increasing the expression of genes that codify proteins involved in the mitochondrial biology (BALTZER et al., 2010). In other words, the mitonutrients could be incorporated and become immediately active in the mitochondria or have nutrigenomic effects over the long run. Several nutrients with antioxidant properties, such as lipoic acid, coenzyme Q10 (CoQ10), vitamin E, selenium, and vitamin B2 have shown positive results in mitochondrial function (WESSELINK et al., 2018).

The α-lipoic acid is a powerful mitochondrial antioxidant, and a coenzyme naturally found in mitochondria, involved in energy metabolism. It is known that a diet supplemented with α-lipoic acid has anti-inflammatory properties inside the cells (KORIYAMA et al., 2013).

Coenzyme Q10 is a bioactive lipid that acts as a carrier of electrons in the mitochondrial respiratory chain. However, it also has antioxidant, anti-apoptotic, and anti-inflammatory properties, and can be found in mitochondrial membranes when fed as a nutritional supplement (BENTINGER et al., 2010; LAPOINTE et al., 2012). Vitamin E is a liposoluble antioxidant found in mitochondrial membranes (LAURIDSEN; JENSEN 2012).

Selenium is a mineral that participates in the synthesis of selenoproteins with strong antioxidant properties, such as glutathione peroxidase (GPxs) and thioredoxin (TRxs). Selenium cellular levels is tightly related to protects against mitochondrial dysfunction (DURSUN et al., 2011).

Complex B vitamins are particularly important in supporting mitochondrial function, acting directly as co-factors of mitochondrial enzymes or as

657 precursors of important co-factors. It is known that the vitamin B complex is key to
658 mitochondrial aerobic respiration and energy production. In addition, mitochondrial
659 functions and integrity are compromised in the absence of this vitamin complex.
660 Riboflavin (vitamins B2) is a hydrosoluble vitamin and the main component of flavin-
661 adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which work as redox
662 co-factors in the mitochondrial respiratory chain (DEPEINT et al., 2006).

663 Other mitochondrial nutrients, such as carnitine and L-carnitine, are
664 able to act as energy stimulants. L-carnitine and its acetyl derivative (acetyl-L-
665 carnitine) transport long-chain fatty acids to the mitochondria for β-oxidation and
666 production. Likewise, it has been demonstrated that a combination of α-lipoic acid
667 and L-carnitine significantly improves mitochondrial function and stimulates
668 mitochondrial biogenesis (TARNOPOLSKY, 2008).

669 In addition, it has been demonstrated that some nutrient
670 combinations can have unique roles and be more efficient than individual nutrients.
671 Several studies indicate that the antioxidant CoQ10 and/or its energy potential could
672 be more efficient when provided along with α-tocopherol, α-lipoic acid, or creatine
673 (MARRIAGE et al., 2003). Creatine is actively incorporated into the mitochondria to
674 act as an energy propelling compound, increasing creatine/phosphocreatine stocks
675 and, consequently, preventing the depletion of ATP.

676 This PhD research aimed to identify and characterize for the first time
677 the cellular and mitochondrial biomarkers of energy metabolism and oxidative stress
678 in low and normal birthweigths piglets troughtout the peri-weaning period.

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REFERENCES

AARESTRUP, F.M. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. **Basic and Clinical Pharmacology and Toxicology**, v.96, n. 4, p. 271-281, 2005.

AARESTRUP, F. M.; OLIVER DURAN, C.; BURCH, D. G. Antimicrobial resistance in swine production. **Animal Health Research Reviews**, Wallingford, v. 9, n. 2, p. 135-148, 2008.

ACIN-PEREZ, R.; HOYOS, B.; ZHAO, F.; VINOGRADOV, V.; FISCHMAN, D. A.; HARRIS, R. A.; LEITGES, M.; WONGSIRIROJ, N.; BLANER, W. S.; MANFREDI, G.; HAMMERLING, U. Control of oxidative phosphorylation by vitamin a illuminates a fundamental role in mitochondrial energy homoeostasis. **Faseb Journal**, Bethesda, v. 24, n. 2, p. 627-636, 2010.

AGARWAL, A.; GUPTA, S.; SEKHON, L.; SHAH, R. Redox considerations in female reproductive function and assisted reproduction: from molecular mechanisms to health implications. **Antioxidants & Redox signaling**, Larchmont, v. 10, n. 8, p. 1375-1403, 2008.

AGARWAL, A.; GUPTA, S.; SIKKA, S. The role of free radicals and antioxidants in reproduction. **Current Opinion in Obstetrics & Gynecology**, London, v. 18, n. 3, p. 325-332, 2006.

ARNOULT, D.; CARNEIRO, L.; TATTOLI, I.; GIRARDIN, S. E. The role of mitochondria in cellular defense against microbial infection. **Seminars in Immunology**, London, v. 21, n. 4, p. 223-232, 2009.

AUMANN, K. D.; BEDORF, N.; BRIGELIUS-FLOHÉ, R.; SCHOMBURG, D.; FLOHÉ, L. Glutathione peroxidase revisited simulation of the catalytic cycle by computer-assisted molecular modelling. **Biomedical and Environmental Sciences**, Beijing, v. 10, n. 2, p. 136-155, 1997.

AVISSAR, N.; ORNT, D. B.; YAGIL, Y.; HOROWITZ, S.; WATKINS, R. H.; KERL, E. A.; TAKAHASHI, K.; PALMER, I. S.; COHEN, H. J. Human kidney proximal tubules are the main source of plasma glutathione peroxidase. **American Journal of Physiology**, Bethesda, v. 266, p. c367–c375, 1994.

BALABAN, R. S.; NEMOTO, S.; FINKEL, T. Mitochondria, oxidants, and aging. **Cell**, Cambridge, v. 120, n. 4, p. 483-495, 2005.

BALTZER, C.; TIEFENBÖCK, S. K.; FREI, C. Mitochondria in response to nutrients and nutrient-sensitive pathways. **Mitochondrion**, Amsterdan, v. 10, n. 6, p. 589-597, 2010.

BARTON, M. D. Impact of antibiotic use in the swine industry. **Current Opinion in Microbiology**, London, v. 19, p. 9-15, 2014.

BEAULIEU, A. D.; AALHUS, J. L.; WILLIAMS, N. H.; PATIENCE, J. F. Impact of piglet birth weight, birth order, and litter size on subsequent growth performance,

carcass quality, muscle composition and eating quality of pork. **Journal of Animal Science**, Champaign, v. 88, n. 8, p. 2767-2778, 2010.

BEE, G. Birth weight of litters as source of variation in postnatal growth, and carcass and meat quality. *In*: UNIVERSITY OF ALBERTA. **Advances in pork production**. Edmonton: University of Alberta, 2007. v. 18, p. 191-195.

BENTINGER, M.; TEKLE, M.; DALLNER, G. Coenzyme q--biosynthesis and functions. **Biochemical and Biophysical Research Communications**, New York, v. 396, n. 1, p. 74-79, 2010.

BHAGAVAN, H. N.; CHOPRA, R. K. Coenzyme q10: absorption, tissue uptake, metabolism and pharmacokinetics. **Free Radical Research**, Yverdon, v. 40, n. 5, p. 445-453, 2006.

BOUDRY, G.; PÉRON, V.; LE HUËROU-LURON, I.; LALLÈS, J. P.; SÈVE, B. Weaning induces both transient and long-lasting modifications of absorptive, secretory, and barrier properties of piglet intestine. **The Journal of Nutrition**, Rockville, v. 134, n. 9, p. 2256-2262, 2004.

BOULOT, S.; QUESNEL, H.; QUINIOU, N. Management of high prolificacy in french herds: can we alleviate side effects on piglet survival? **Advances in Pork Production**, Canadá, v. 19, p. 213-220, 2008.

BOVERIS, A.; CHANCE, B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. **Biochemistry Journal**, London, v. 134, n. 3, p. 707-716, 1973.

BOVERIS, A.; OSHINO, N.; CHANCE, B. The cellular production of hydrogen peroxide. **Biochemistry Journal**, London, v. 128, n. 3, p. 617-630, 1972.

BRIGELIUS-FLOHÉ, R. Tissue-specific functions of individual glutathione peroxidases. **Free Radical Biology and Medicine**, New York, v. 27, n. 9, p. 951-965, 1999.

BRIGELIUS-FLOHÉ, R.; FLOHÉ, L. Basic principles and emerging concepts in the redox control of transcription factors. **Antioxidants & Redox Signaling**, Larchmont, v. 15, n. 15, p. 2335-2381, 2011.

BRÜSSOW, K. P.; WÄHNER, M.; JAŚKOWSKI, J. M. Biological limit of fecundity in sows – do they exist? **Electronic Journal of Polish Agricultural Universities**, Wroclaw, Polônia, v. 14, n. 3, p. 3, 2011.

BUCHET, A.; BELLOC, C.; LEBLANC-MARIDOR, M.; MERLOT, E. Effects of age and weaning conditions on blood indicators of oxidative status in pigs. **Plos One**, Califórnia, v. 12, n. 5, p. 178487, 2017.

BURK, R. F.; OLSON, G. E.; WINFREY, V. P.; HILL, K. E.; YIN, D. Glutathione peroxidase-3 produced by the kidney binds to a population of basement membranes in the gastrointestinal tract and in other tissues. **American Journal of Physiology Gastrointestinal and Liver Physiology**, Bethesda, v. 301, n. 1, p. G32-G38, 2011.

761 BUROW, E.; SIMONEIT, C.; TENHAGEN, B.-A.; KÄSBOHRER, A. Oral
762 antimicrobials increase antimicrobial resistance in porcine E. coli – A systematic
763 review. **Preventive Veterinary Medicine**, Amsterdam, v. 113, n. 4, p. 364-375, 2014.

764 BYWATER, R. J.; CASEWELL, M. W. An assessment of the impact of antibiotic
765 resistance in different bacterial species and of the contribution of animal sources to
766 resistance in human infections. **Journal of Antimicrobial Chemotherapy**, London,
767 v. 46, n. 6, p. 1052, 2000.

768 CAMPBELL, M. J.; CRENSHAW, J. D.; POLO, J. The biological stress of early
769 weaned piglets. **Journal of Animal Science Biotechnology**, London, v. 4, n. 1, p.
770 19, 2013.

771 CAPITA, R.; ALONSO-CALLEJA, C. Antibiotic-resistant bacteria: a challenge for the
772 food industry. **Critical Reviews in Food Science and Nutrition**, Boca Raton, v. 53,
773 n. 1, p. 11-48, 2013.

774 CHAMBERS, I.; FRAMPTON, J.; GOLDFARB, P.; AFFARA, N.; MCBAIN, W.;
775 HARRISON, P. R. The structure of the mouse glutathione peroxidase gene: the
776 selenocysteine in the active sites encoded by the ‘termination’ codon, TGA. **Embo**
777 **Journal**, London, v. 5, n. 6, p. 1221-1227, 1986.

778 CHANTZIARAS, I.; BOYEN, F.; CALLENS, B.; DEWULF, J. Correlation between
779 veterinary antimicrobial use and antimicrobial resistance in food-producing animals: a
780 report on seven countries. **The Journal of Antimicrobial Chemotherapy**, London,
781 v. 69, n. 3, p. 827-834, 2014.

782 CHENG, W. H.; HO, Y. S.; VALENTINE, B. A.; ROSS, D. A.; COMBS JUNIOR, G. F.;
783 LEI, X. G. Cellular glutathione peroxidase is the mediator of body selenium to protect
784 against paraquat lethality in transgenic mice. **The Journal of Nutrition**, Rockville, v.
785 128, n. 7, p. 1070-1076, 1998.

786 CHENZ, Y.; DZIUKP, J. Influence of initial length of uterus per embryo and gestation
787 stage on prenatal survival, development, and sex ratio in the pig. **Journal of Animal**
788 **Science**, Champaign, v. 71, n. 7, p. 1895-1901, 1993.

789 CHIARUGI, P.; G. PANI, E.; GIANNONI, L.; TADDEI, R.; COLAVITTI, G.; RAUGEI,
790 M.; SYMONS, S.; BORRELLO, T.; GALEOTTI, E. T.; RAMPONI, G. Reactive oxygen
791 species as essential mediators of cell adhesion: the oxidative inhibition of a fak
792 tyrosine phosphatase is required for cell adhesion. **Journal of Cellular Biology**,
793 New York, v. 161, n. 5, p. 933-44, 2003.

794 CHU, F. F.; DOROSHOW, J. H.; ESWORTHY, R. S. Expression, characterization,
795 and tissue distribution of a new cellular selenium-dependent glutathione peroxidase,
796 gshpx-gi. **Journal of Biological Chemistry**, Baltimore, v. 268, n. 4, p. 2571-2576,
797 1993.

798 CIRCU, M. L.; AW, T. Y. Intestinal redox biology and oxidative stress. **Seminars In**
799 **Cell & Developmental Biology**, London, v. 23, n. 7, p. 729-737, 2012.

COLSON, V.; ORGEUR, P.; FOURY, A.; MORMEDE, P.Consequences of weaning piglets at 21 and 28 days on growth, behaviour and hormonal responses. **Applied Animal Behaviour Science**, v.98, p.70-88, 2006.

DAMGAARD, L. H.; RYDHMER, P.; LOVENDAHL, P.; GRANDINSON, K. Genetic parameters for within-litter variation in piglet birth weight and change in within litter variation during suckling. **Journal of Animal Science**, Champaign, v. 81, n. 3, p. 604-610, 2003.

DAVID, J. C.; GRONGNET, J. F.; LALLES, J. P. Weaning affects the expression of heat shock proteins in different regions of the gastrointestinal tract of piglets. **The Journal of Nutrition**, Rockville, v. 132, n. 9, p. 2551-2561, 2002.

DE HAAN, J. B.; BLADIER, C.; GRIFFITHS, P.; KELNER, M.; O'SHEA, R. D.; CHEUNG, N. S.; BRONSON, R. T.; SILVESTRO, M. J.; WILD, S.; ZHENG, S. S.; BEART, P. M.; HERTZOG, P. J.; KOLA, I. Mice with a homozygous null mutation for the most abundant glutathione peroxidase, gpx1, show increased susceptibility to the oxidative stress- inducing agents paraquat and hydrogen peroxide. **Journal of Biological Chemistry**, Baltimore, v. 273, n. 35, p. 22528-22536, 1998.

DE VOS, M.; CHE, L.; HUYGELEN, V.; WILLEMEN, S.; MICHIELS, J.; VAN CRUCHTEN, S.; VAN GINNEKEN, C. Nutritional interventions to prevent and rear low-birthweight piglets. **Journal of Animal Physiology and Animal Nutrition**, Berlin, v. 98, n. 4, p. 609-619, 2014.

DEAR, T. N.; CAMPBELL, K.; RABBITTS, T. H. Molecular cloning of putative odorant-binding and odorant-metabolizing proteins. **Biochemistry**, Washington, v. 30, n. 43, p. 10376-10382, 1991.

DELAMIRANDE, E.; GAGNON, C. Human sperm hyperactivation and capacitation as parts of an oxidative process. **Free Radical Biology and Medicine**, New York, v. 14, n. 2, p. 157-166, 1993.

DEMPLE, B.; HARRISON, L. Repair of oxidative damage to dna: enzymology and biology. **Annual Review of Biochemistry**, Palo Alto, v. 63, p. 915-948, 1994.

DEPEINT, F., W. R. BRUCE, N. SHANGARI, R. MEHTA AND P. J. O'BRIEN. Mitochondrial function and toxicity: role of the b vitamin family on mitochondrial energy metabolism. **Chemico-Biological Interactions**, Amsterdam, v. 163, n. 1-2, p. 94-112, 2006.

DEVASAGAYAM, T. P.; TILAK, J. C.; BOLOOR, K. K.; SANE, K. S.; GHASKADBI ET, S. S.; LELE, R. D. Free radicals and antioxidants in human health : current status and future prospects. **The Journal of Association of Physicians of India**, Bombay, v. 52, p. 794-804, 2004.

DIANA A, BOYLE LA, LEONARD FC, CARROLL C, SHEEHAN E, MURPHY D, MANZANILLA EG. Removing prophylactic antibiotics from pig feed: how does it affect their performance and health? **BioMediCentral Veterinary Research.**;v.15n.67, 2019

DIAZ VIVANCOS, P.; WOLFF, T.; MARKOVIC, J.; PALLARDÓ, F. V.; FOYER, C. H. A nuclear glutathione cycle within the cell cycle. **Biochemical Journal**, London, v. 431, n. 2, p. 169-178, 2010.

DITTRICH, A. M.; MEYER, H. A.; KROKOWSKI, M.; QUARCOO, D.; AHRENS, B.; KUBE, S. M.; WITZENRATH, M.; ESWORTHY, R.S.; CHU, F. F.; HAMELMANN, E. Glutathione peroxidase-2 protects from allergen-induced airway inflammation in mice. **European Respiratory Journal**, Copenhagen, v. 35, n. 5, p. 1148-1154, 2010.

DURSO, L. M.; COOK, K. L. Impacts of antibiotic use in agriculture: what are the benefits and risks? **Current Opinion Microbiology**, London, v. 19, p. 37-44, 2014.

DURSUN, N.; TASKIN, E.; YERER AYCAN, M. B.; SAHIN, L. Selenium-mediated cardioprotection against adriamycin-induced mitochondrial damage. **Drug and Chemical Toxicology**, New York, v. 34, n. 2, p. 199-207, 2011.

ESWORTHY, R. S.; MANN, J. R.; SAM, M.; CHU, F. F. Low glutathione peroxidase activity in gpx1 knockout mice protects jejunum crypts from gamma-irradiation damage. **American Journal of Physiology of Gastrointestinal and Liver Physiology**, Bethesda, v. 279, n. 2, p.G426-G436, 2000.

FLOHE, L. Changing paradigms in thiology from antioxidant defense toward redox regulation. **Methods in Enzymology**, New York, v. 473, p. 1-39, 2010.

FLOHÉ, L. The selenoprotein glutathione peroxidase. *In*: DOLPHIN, D.; AVRAMOVIC, O.; POULSON, R. (ed.). **Glutathione**. New York: John Wiley & Sons, 1989. p. 644-731.

FLOHÉ, L.; GÜNZLER, W. A.; SCHOCK, H. H. Glutathione peroxidase: a selenoenzyme. **Febs Letters**, Amsterdam, v. 32, n. 1, p. 132-134,1973.

FLORIAN, S.; KREHL, S.; LÖWINGER, M.; KIPP, A.; BANNING, A.; ESWORTHY, S.; CHU, F. F.; BRIGELIUS-FLOHÉ, R. Loss of GPx2 increases apoptosis, mitosis, and gpx1 expression in the intestine of mice. **Free Radicals Biology Medicine**, New York v. 49, n. 11, p. 1694-1702, 2010.

FLORIAN, S.; WINGLER, K.; SCHMEHL, K.; JACOBASCH, G.; KREUZER, O. J.; MEYERHOF, W.; BRIGELIUS-FLOHÉ, R. Cellular and subcellular localization of gastrointestinal glutathione peroxidase in normal and malignant human intestinal tissue. **Free Radical Research**, Yverdon, v. 35, n. 6, p. 655-663, 2001.

FORMAN, H. J. Use and abuse of exogenous h2o2 in studies of signal transduction. **Free Radical Biology and Medicine**, New York, v. 42, n. 7, p. 926-932, 2007.

FRAGA, C. G.; SHIGENAGA, M. K.; PARK, J. W.; DEGAN, P.; AMES, B. N. .Oxidative damage to dna during aging: 8-hydroxy-2'-deoxyguanosine in rat organ dna and urine. **Proceedings of National Academic of Science of United States of America**, Washington, v. 87, n. 12, p. 4533-4537, 1990.

FUJII, J.; IUCHI, Y.; OKADA, F. Fundamental roles of reactive oxygen species and protective mechanisms in the female reproductive system. **Reproductive Biology and Endocrinology**, London, v. 3, p. 43, 2005.

GEISZT, M.; LETO, T. L. The NOx family of NAD(P)H oxidases: host defense and beyond. **Journal of Biological Chemistry**, Baltimore, v. 279, n. 50, p. 51715-51718, 2004.

GHYSELINCK, N. B.; DUFAURE, J. P. A mouse cDNA sequence for epididymal androgen regulated proteins related to glutathione peroxidase. **Nucleic Acids Research**, London, v. 18, n. 23, p. 7144, 1990.

GREEN, D. E.; TZAGOLOFF, A. The mitochondrial electron transfer chain. **Archives of Biochemistry and Biophysics**, New York, v. 116, n. 1, p. 293-304, 1966.

GRUBBS, J. K.; FRITCHEN, A. N.; HUFF-LONERGAN, E.; GABLER, N. K.; LONERGAN, S. M. Selection for residual feed intake alters the mitochondria protein profile in pigs. **Journal of Proteomics**, Amsterdam, v. 80, p. 334-345, 2013.

HAAS, R. H. Mitochondrial dysfunction in aging and diseases of aging. **Biology**, v. 8, n.2 p.48, 2019.

HADDAD, J. J. Redox and oxidant-mediated regulation of apoptosis signaling pathways: immuno-pharmaco-redox conception of oxidative siege versus cell death commitment. **International Immunopharmacology**, Amsterdam, v. 4, n. 4, p. 475-493, 2004.

HAGEN, T. M.; INGERSOLL, R. T.; LYKKESFELDT, J.; LIU, J.; WEHR, C. M.; VINARSKY, V.; BARTHOLOMEW, J. C.; AMES, A. B. (R)-alpha-lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, andincreased metabolic rate. **Faseb Journal**, Bethesda, v. 13, n. 2, p. 411-418, 1999.

HALLIWELL, B.; GUTTERIDGE, J. M. C. **Free radicals in biology and medicine**. 4. ed. Oxford: Oxford University Press, 2007.

HALLIWELL, B.; GUTTERIDGE, J. M. C. **Free radicals in biology and medicine**. 5. ed. New York: Oxford University Press, 2015.

HERPIN, P.; DAMON, M.; LEDIVIDICH, J. Development of thermoregulation and neonatal survival in pigs. **Livestock Production Science**, Amsterdam, v. 78, n. 1, p. 25-45, 2002.

HICKS, S. W.; GALÁN, J. E. Exploitation of eukaryotic subcellular targeting mechanisms by bacterial effectors. **Nature Reviews Microbiology**, London, v. 11, n. 5, p. 316-326, 2013.

HO, Y. S.; MAGNENAT, J. L.; BRONSON, R. T.; CAO, J.; GARGANO, M.; SUGAWARA, M.; FUNK, C. D. Mice deficient in cellular glutathione peroxidase develop normally and show no increased sensitivity to hyperoxia. **The Journal of Biological Chemistry**, Baltimore, v. 272, n. 26, p. 16644-16651, 1997.

HURST, J. K. What really happens in the neutrophil phagosome? **Free Radical Biology and Medicine**, New York, v. 53, n. 3, p. 508-520, 2012.

HWANG, C.; SINSKEY, A. J.; LODISH, H. F. Oxidized redox state of glutathione in the endoplasmic reticulum. **Science**, New York, v. 257, n. 5076, p. 1496-1502, 1992.

JANSEN, R. P.; BURTON, G. J. Mitochondrial dysfunction in reproduction. **Mitochondrion**, Amsterdam, v. 4, n. 5-6, p. 577-600, 2004.

JENSEN, H. H.; HAYES, D. J. Impact of denmark's ban on antimicrobials for growth promotion. **Current Opinion In Microbiology**, London, v. 19, p. 30-36, 2014.

JIANG, J.-H.; TONG, J.; GABRIEL, K. Hijacking mitochondria: bacterial toxins that modulate mitochondrial function. **International Union of Biochemistry and Molecular Biology**, London, v. 64, n. 5, p. 397-401, 2012.

JIN, R. C.; MAHONEY, C. E.; COLEMAN ANDERSON, L.; OTTAVIANO, F.; CROCE, K.; LEOPOLD, J. A.; ZHANG, Y. Y.; TANG, S. S.; HANDY, D. E.; LOSCALZO, J. Glutathione peroxidase-3 deficiency promotes platelet-dependent thrombosis in vivo, **Circulation**, Hagerstown, v. 123, n. 18, p. 1963-1973, 2011.

JOHN, L. J.; FROM, M.; SCHULZKE, J. D. Epithelial barriers in intestinal inflammation. **Antioxidant & Redox signaling**, Larchmont, v. 15, n. 5, p. 1255-1270, 2011.

KALYANARAMAN, B. Teaching the basics of redox biology to medical and graduate students: oxidants, antioxidants and disease mechanisms. **Redox Biology**, Amsterdam, v. 1, n. 1, p. 244-257, 2013.

KIM, M.; CHRISTLEY, S.; JOHN, C. A.; DONALD, L.; GARY, A. Immature oxidative stress management as a unifying principle in the pathogenesis of necrotizing enterocolitis: insights from an agent-based model. **Surgical Infections**, Larchmont, v. 13, n. 1, p. 18-32, 2012.

KIPP, A. P.; MÜLLER, M. F.; GÖKEN, E. M.; DEUBEL, S.; BRIGELIUS-FLOHÉ, R. The selenoproteins gpx2, trxr2 and trxr3 are regulated by WNT signalling in the intestinal epithelium. **Biochemistry & Biophysical Acta**, Amsterdam, v. 1820, n. 10, p. 1588-96, 2012.

KÖHRLE, J. Selenium and the control of thyroid hormone metabolism. **Thyroid**, New York, v. 15, n. 8, p. 841-853, 2005.

KORIYAMA, Y.; NAKAYAMA, Y.; MATSUGO, S.; SUGITANI, K.; OGAI, K.; TAKADERA, T.; KATO, S. Anti-inflammatory effects of lipoic acid through inhibition of gsk-3β in lipopolysaccharide-induced bv-2 microglial cells. **Neuroscience Research**, New York, v. 77, n. 1-2, p. 87-96, 2013.

KRYUKOV, G. V.; CASTELLANO, S.; NOVOSELOV, S. V.; LOBANOV, A. V.; ZEHTAB, O.; GUIGÓ, R.; GLADYSHEV, V. N. Characterization of mammalian selenoproteomes. **Science**, New York, v. 300, n. 5624, p. 1439-1443, 2003.

LALLES, J. P.; BOSI, P.; SMIDT, H.; STOKES, C. R. Nutritional management of gut health in pigs around weaning. **The Proceedings of the Nutrition Society**, London, v. 66, n. 2, p. 260-268, 2007.

LALLÈS, J. P.; BOUDRY, G.; FAVIER, C.; LE FLOC'H, N.; LURON, I.; MONTAGNE, L.; OSWALD, I. P.; PIÉ, S.; PIEL, C.; SÈVE, B. Gut function and dysfunction in young pigs: physiology. **Animal Research**, Cambridge, v. 53, n. 4, p. 301-316, 2004.

LALLÈS, J. P.; DAVID, J. C. Fasting and refeeding modulate the expression of stress proteins along the gastrointestinal tract of weaned pigs. **Journal of Animal Physiology and Animal Nutrition**, Berlin, v. 95, n. 4, p. 478-488, 2011.

LAPOINTE, J.; WANG, Y.; BIGRAS, E.; HEKIMI, S. The submitochondrial distribution of ubiquinone affects respiration in long-lived mclk1+/- mice. **Journal of Cell Biology**, New York, v. 199, n. 2, p. 215-224, 2012.

LAURIDSEN, C.; JENSEN, S. K. α-tocopherol incorporation in mitochondria and microsomes upon supranutritional vitamin e supplementation. **Genes & Nutrition**, New Orleans, v. 7, n. 4, p. 475-482, 2012.

LE COLZLER, Y.; PICHODO, X.; ROY, H.; GUYOMARC'H, C.; PELLOIS, H.; QUINIOU, N.; LOUVEAU, I.; LEBRET, B.; LEFAUCHEUR, L.; GONDRET, F. Influence du poids individuel et de la taille de la portée à la naissance sur la survie du porcelet, ses performances de croissance et d'abattage et la qualité de la viande. **Journées Recherche Porcine en France**, Paris, v. 36, p. 443-450, 2004.

LEAVY, O. T cells: mitochondria and t cell activation. **Nature Reviews. Immunology**, London, v. 13, n. 4, p. 224, 2013.

LEBOVITZ, R. M.; ZHANG, H.; VOGEL, H.; CARTWRIGHT JUNIOR, J.; DIONNE, L.; LU, N.; HUANG, S.; MATZUK, M. M. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase deficient mice. **Proceedings of National Academy of Sciences of United States of America**, Washington, v. 93, n. 18, p. 9782-9787, 1996.

LI, Y.; HUANG, T. T.; CARLSON, E. J.; MELOV, S.; URSELL, P. C.; OLSON, J. L.; NOBLE, L. J.; YOSHIMURA, M. P.; BERGER, C.; CHAN, P. H.; WALLACE, D. C.; EPSTEIN, C. J. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. **Nature Genetics**, New York, v. 11, n. 4, p. 376-381, 1995.

LIM, J.; LUDERER, U. Oxidative damage increases and antioxidant gene expression decreases with aging in the mouse ovary. **Biology of Reproduction**, Champaign, v. 84, n. 4, p. 775-782, 2011.

LIU, B.; CHEN, Y.; CLAIR, D. K. Ros and p53: a versatile partnership. **Free Radical Biology and Medicine**, New York, v. 44, n. 8, p. 1529-1535, 2008.

LIU, C.; LIN, G.; WANG, X. Q.; WANG, T. J.; WU, G. Y.; LI, D. F.; WANG, J. Intrauterine growth restriction alters the hepatic proteome in fetal pigs. **The Journal of Nutritional Biochemistry**, New York, v. 24, n. 6, p. 954-959, 2013.

LIU, J.; AMES, B. N. Reducing mitochondrial decay with mitochondrial nutrients to delay and treat cognitive dysfunction, Alzheimer's disease, and Parkinson's disease. **Nutritional Neuroscience**, Abingdon, v. 8, n. 2, p. 67-89, 2005.

LÓPEZ-ARMADA, M. J.; RIVEIRO-NAVEIRA, R. R.; VAAMONDE-GARCÍA, C.; VALCÁRCEL-ARES, M. N. Mitochondrial dysfunction and the inflammatory response. **Mitochondrion**, Amsterdam, v. 13, n. 2, p. 106-118, 2013.

LUO, Z.; ZHU, W.; GUO,Q.; LUO,W.; ZHANG,J.; XU,W.; XU, J. Weaning Induced Hepatic Oxidative Stress, Apoptosis, and Aminotransferases through MAPK Signaling Pathways in Piglets. **Oxidative Medicine and Cellular Longevity**, New York, v. 2016, p. 4768541, 2016.

LYKKESFELDT, J.; SVENDSEN, O. Oxidants and antioxidants in disease: oxidative stress in farms animals. **Veterinary Journal**, London, v. 173, n. 3, p. 502-511, 2007.

MAEDA, K.; OKUBO, K.; SHIMOMURA, I.; MIZUNO, K.; MATSUZAWA, Y.; MATSUBARA, K. Analysis of an expression profile of genes in the human adipose tissue. **Gene**, Amsterdam, v. 190, n. 2, p. 227-235, 1997.

MARGIS, R.; DUNAND, C.; TEIXEIRA, F. K.; MARGIS-PINHEIRO, M. Glutathione peroxidase family: an evolutionary overview. **Febs Journal**, Oxford, v. 275, n. 15, p. 3959-3970, 2008.

MARI, M.; COLELL, A.; MORALES, A.; VON MONTFORT, C.; GARCIA-RUIZ, C.; FERNANDEZ-CHECA, J. C. Redox control of liver function in health and disease. **Antioxidants & Redox Signaling**, Larchmont, v. 12, n. 11, p. 1295-1331, 2009.

MARINHO, H. S.; ANTUNES, F.; PINTO, R. E. Role of glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase in the reduction of lysophospholipid hydroperoxides. **Free Radical Biology & Medicine**, New York, v. 22, n. 5, p. 871-883, 1997.

MARKLUND, S. L.; HOLME, E.; HELLNER, L. Superoxide dismutase in extracellular fluids. **Clinica Chimica Acta**, Amsterdam, v. 126, n. 1, p. 41-51, 1982.

MARRIAGE, B.; CLANDININ, M. T.; GLERUM, D. M. Nutritional cofactor treatment in mitochondrial disorders. **Journal of the American Dietetic Association**, Chicago, v. 103, n. 8, p. 1029-1038, 2003.

MARSHALL, B. M.; LEVY, S. B. Food Animals and Antimicrobials: Impacts on Human Health. **Clinical Microbiology Review**, v.24, p.718–733, 2011.

MÅRTENSSON, J.; LAI, J. C.; MEISTER, A. High-affinity transport of glutathione is part of a multicomponent system essential for mitochondrial function. **Proceedings of the National Academic of Sciences of the United States of America**, Washington, v. 87, n. 18, p. 7185-7189, 1990.

MCCORD, J. M.; FRIDOVICH, I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). **The Journal of biological Chemistry**, Baltimore, v. 244, n. 22, p. 6049-6055, 1969.

MIARELLI, M.; DRUMO, R.; SIGNORELLI, F.; MARCHITELLI, C.; PAVONE, S.; PESCIAROLI, M.; RUGGIERI, J.; CHIRULLO, B.; AMMENDOLA, S.; BATTISTONI, A.; ALBORALI, G. L.; MANUALI, E.; PASQUALI, P. Salmonella typhimurium infection

primes a nutriprive mechanism in piglets. **Veterinary Microbiology**, Amsterdam, v. 186, p. 117-125, 2016.

MICHIELS, J.; DE VOS, M.; MISSOTTEN, J.; OVYN, A.; DE SMET, S.; VAN GINNEKEN, C. Maturation of digestive function is retarded and plasma antioxidant capacity lowered in fully weaned low birth weight piglets. **British Journal of Nutrition**, Cambridge, v. 109, n. 1, p. 65-75, 2013.

MILLIGAN, B. N.; FRASER, D.; KRAMER, D. L. Within-litter birth weight variation in the domestic pig and its relation to pre-weaning survival, weight gain, and variation in weaning weights. **Livestock Production Science**, Amsterdam, v. 76, n. 1-2, p. 181-191, 2002.

MOESER, A. J. Weaning: what's going on at the gut level? In: ALLEN D. LEMAN SWINE CONFERENCE, 2010, Saint Paul. **Anais** […]. Saint Paul: University of Minnesota, 2010. p. 124-128. Disponível em: https://conservancy.umn.edu/bitstream/handle/11299/131177/Moeser.pdf?sequence=1. Acesso em: 5 mar. 2019.

MOESER, A. J.; KLOCK, C. V.; RYAN, K. A.; WOOTEN, J. G.; LITTLE, D.; COOK, V. L.; BLIKSLAGER, A. T. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. **American Journal Physiology Gastrointestinal and Liver Physiology**, Bethesda, v. 292, n. 1, p. G173-G181, 2007.

NAVARRO, A.; TORREJON, R.; BÁNDEZ, M. J.; LOPEZ-CEPERO, J. M.; BOVERIS, A. Mitochondrial function and mitochondria-induced apoptosis in an overstimulated rat ovarian cycle. **American Journal of Physiology. Endocrinology and Metabolism**, Bethesda, v. 289, n. 6, p. e1101-e1109, 2005.

NIKI, E. Lipid peroxidation: physiological levels and dual biological effects. **Free Radical Biology & Medicine**, New York, v. 47, n. 5, p. 469-484, 2009.

OKADO-MATSUMOTO, A.; FRIDOVICH, I. Subcellular distribution of superoxide dismutases (SOD) in rat liver: Cu, Zn-sod in mitochondria. **Journal of Biological Chemistry**, Baltimore, v. 276, n. 42, p. 38388-38393, 2001.

OLSON, G. E.; WHITIN, J. C.; HILL, K. E.; WINFREY, V. P.; MOTLEY, A. K.; AUSTIN, L. M.; DEAL, J.; COHEN, H. J.; BURK, R. F. Extracellular glutathione peroxidase (gpx3) binds specifically to basement membranes of mouse renal cortex tubule cells. **American Journal of Physiology. Renal Physiology**, Bethesda, v. 298, n. 5, p. f1244–f1253, 2010.

ONTERUS, K.; FAN, B.; NIKKILA, M. T.; GARRIK, D. J.; STALDEK, J.; ROTHSCHILD, M. F. Whole-genome association analyses for lifetime reproductive traits in the pig. **Journal of Animal Science**, Champaign, v. 89, n. 4, p. 988-995, 2011.

ORZECHOWSKA, B.; MUCHA, A. Results of reproductive performance of breeding sows. **Report on Pig Breeding in Poland**, Kraków, v. 29, p. 3-18, 2010.

PACKER, L.; WITT, E. H.; TRITSCHLER, H. J. Alpha-lipoic acid as a biological antioxidant. **Free Radical Biology & Medicine**, New York, v. 19, n. 2, p. 227-250, 1995.

PARGE, H. E.; HALLEWELL, R. A.; TAINER, J. A. Atomic structures of wild-type and thermostable mutant recombinant human cu,zn superoxide dismutase. **Proceedings of the national academic science of United States of America**, Washington, v. 89, n. 13, p. 6109-6113, 1992.

PARZYCH, K. R.; KLIONSKY, D. J. An overview of autophagy: morphology, mechanism, and regulation. **Antioxidants & Redox Signaling**, Larchmont, v. 20, n. 3, p. 460-473, 2014.

PIE, S.; LALLES, J. P.; BLAZY, F.; LAFFITTE, J.; SEVE, B.; OSWALD, I. P. Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. **The Journal of Nutrition**, Rcockville, v. 134, n. 3, p. 641-647, 2004.

PIECZENIK, S. R.; NEUSTADT, J. Mitochondrial dysfunction and molecular pathways of disease. **Experimental and Molecular Pathology**, New York, v. 83, n. 1, p. 84-92, 2007.

PLUSKE, J. R.; HAMPSON, J. D.; WILLIAMS, I. H. Factors influencing the structure and function of the small intestine in the weaned pig: a review. **Livestock Production Science**, Amsterdam, v. 57, n. 1-3, p. 215-236, 1997.

QUINIOU, N.; DAGORN, J.; GAUDRE, D. Variation of piglets birth weight and consequences on subsequent performance. **Livestock Production Science**, Amsterdam, v. 78, p. 63-70, 2002.

QUINTON, V. M.; WILTON, J. W.; ROBINSON, J. A.; MATUR, P. K. Economic weights for sow productivity traits in nucleus pig populations. **Livestock Science**, Suwon, v. 99, p. 69-70, 2006.

RAMALHO-SANTOS, J.; VARUM, S.; AMARAL, S.; MOTA, P. C.; SOUSA, A. P.; AMARAL, A. Mitochondrial functionality in reproduction: from gonads and gametes to embryos and embryonic stem cells. **Human Reproduction Update**, Oxford, v. 15, n. 5, p. 553-572, 2009.

REZAIE, A.; PARKER, R. D.; ABDOLLAHI, M. Oxidative stress and pathogenesis of inflammatory bowel disease: an epiphenomenon or the cause? **Digestive Diseases and Sciences**, New York, v. 52, n. 9, p. 2015-2021, 2007.

RHOUMA, M.; FAIRBROTHER, J. M.; BEAUDRY, F.; LETELLIER, A. Post weaning diarrhea in pigs: risk factors and non-colistin-based control strategies. **Acta Veterinaria Scandinavica**, Copenhagen, v. 59, p. 31, 2017.

ROCADEMBOSCH, J.; AMADOR, J.; BERNAUS, J.; FONT, J.; FRAILE, L. J. Production parameters and pig production cost: temporal evolution 2010-2014. **Porcine Health Management**, London, v. 2, p. 11, 2016.

ROSENBLUM, J. S.; GILULA, N. B.; LERNER, R. A. On signal sequence polymorphisms and diseases of distribution. **Proceedings of National Academic of Science of United States of America**, Washington, v. 93, n. 9, p. 4471-4473, 1996.

ROTHSCHILD, M.; RUVINSKY, A. **The genetics of the pig**. 2. ed. London: CAB, 2011.

ROTRUCK, J. T.; POPE, A. L.; GANTHER, H. E.; SWANSON, A. B.; HAFEMAN, D. G.; HOEKSTRA, W. G. Selenium: biochemical role as a component of glutathione peroxidase. **Science**, New York, v. 179, n. 4073, p. 588-590, 1973.

ROVER JÚNIOR, L.; HÖEHR, N. F.; VELLASCO, A. P.; KUBOTA, L. T. Sistema antioxidante envolvendo o ciclo metabólico da glutationa associado a métodos eletroanalíticos na avaliação do estresse oxidativo. **Química Nova**, São Paulo, v. 24, n. 1, p. 112-119, 2001.

RUDEL, T.; KEPP, O.; KOZJAK-PAVLOVIC, V. Interactions between bacterial pathogens and mitochondrial cell death pathways. **Nature Reviews Microbiology**, London, v. 8, n. 10, p. 693-705, 2010.

RYAN, B. C.; VANDENBERGH, J. G. Intrauterine position effects. **Neuroscience & Biobehavioral Reviews**, Oxford, v. 26, p. 665-678, 2002.

SARGEANT, H. R.; MILLER, H. M.; SHAW, M. A. Inflammatory response of porcine epithelial ipec j2 cells to enterotoxigenic e. Coli infection is modulated by zinc supplementation. **Molecular Immunology**, Oxford, v. 48, n. 15-16, p. 2113-2121, 2011.

SCHIFF, M.; BENIT, P.; COULIBALY, A.; LOUBLIER, S.; EL-KHOURY, R.; RUSTIN, P. Mitochondrial response to controlled nutrition in health and disease. **Nutrition Reviews**, Washington, v. 69, n. 2, p. 65-75, 2011.

SCHNEIDER, M.; FORSTER, H.; BOERSMA, A.; SEILER, A.; WEHNES, H.; SINOWATZ, F.; NEUMULLER, C.; DEUTSCH, M. J.; WALCH, A.; HRABE DE ANGELIS, M.; WURST, W.; URSINI, F.; ROVERI, A.; MALESZEWSKI, M.; MAIORINO, M.; CONRAD, M. Mitochondrial glutathione peroxidase 4 disruption causes male infertility. **Faseb Journal**, Bethesda, v. 23, n. 9, p. 3233-3242, 2009.

SCHOMBURG, L.; KÖHRLE, J. On the importance of selenium and iodine metabolism for thyroid hormone biosynthesis and human health. **Molecular Nutrition & Food Research**, Weinheim, v. 52, n. 11, p. 1235-1246, 2008.

SHEIKH, A. A.; CHECKLEY, S.; AVERY, B.; CHALMERS, G.; BOHAYCHUK, V.; BOERLIN, P.; REID-SMITH, R.; ASLAM, M. Antimicrobial resistance and resistance genes in escherichia coli isolated from retail meat purchased in Alberta, Canada. **Foodborne Pathogens and Disease**, Larchmont, v. 9, n. 7, p. 625-631, 2012.

SIES, H.; BERNDT, C.; JONES, P. D. Oxidative stress. **Annual Review of Biochemistry**, Palo Alto, v. 86, p. 715-748, 2017.

STANTON, T. B. A call for antibiotic alternatives research. **Trends in Microbiology**, Cambridge, v. 21, n. 3, p. 111-113, 2013.

TAKEBE, G.; YARIMIZU, J.; SAITO, Y.; HAYASHI, T.; NAKAMURA, H.; YODOI, J.; NAGASAWA, S.; TAKAHASHI, K. A comparative study on the hydroperoxide and thiol specificity of the glutathione peroxidase family and selenoprotein P. **Journal Biological Chemistry**, Baltimore, v. 277, n. 43, p. 41254–41258, 2002.

TARNOPOLSKY, M. A. The mitochondrial cocktail: rationale for combined nutraceutical therapy in mitochondrial cytopathies. **Advanced Drug Delivery Reviews**, Amsterdam, v. 60, n. 13-14, p. 1561-1567, 2008.

TEALE, C. J. Antimicrobial resistance and the food chain. **Journal of Applied Microbiology**, Oxford, v. 92, p. S85-S89, 2002.

THOMAS, J. P.; GEIGER, P. G.; MAIORINO, M.; URSINI, F.; GIROTTI, A. W. Enzymatic reduction of phospholipid and cholesterol hydroperoxides in artificial bilayers and lipoproteins. **Biochimica & Biophysica (Acta)**, Amsterdam, v. 1045, n. 3, p. 252-260, 1990.

TURNER, B. J.; TALBOT, K. Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. **Progress in Neurobiology**, Oxford, v. 85, n. 1, p. 94-134, 2008.

UGARTE, N.; PETROPOULOS, I.; FRIGUET, B. Oxidized mitochondrial protein degradation and repair in aging and oxidative stress. **Antioxidants & Redox Signaling**, Larchmont, v. 13, n. 4, p. 539-549, 2010.

URSINI, F.; MAIORINO, M.; BRIGELIUS-FLOHE, R.; AUMANN, K. D.; ROVERI, A.; SCHOMBURG, D.; FLOHE, L. Diversity of glutathione peroxidases. **Methods in Enzymology**, New York, v. 252, p. 38-53, 1995.

URSINI, F.; MAIORINO, M.; ROVERI, A. Phospholipid hydroperoxide glutathione peroxidase (phgpx): more than an antioxidant enzyme? **Biomedical and Environmental Sciences**, Beijing, v. 10, n. 2-3, p 327-332, 1997.

URSINI, F.; MAIORINO, M.; VALENTE, M.; FERRI, L.; GREGOLIN, C. Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. **Biochimica et Biophysica Acta**, Amsterdam, v. 710, n. 2, p. 197-211, 1982.

VALKO, M.; LEIBFRITZ, D.; MONCOL, J.; CRONIN, M. T.; MAZUR, M.; TELSER, J. Free radicals and antioxidants in normal physiological functions and human disease. **International Journal of Biochemistry & Cell Biology**, Amsterdam, v. 39, n. 1, p. 44-84, 2007.

VALLET, J. L.; LEYMASTER, K. A.; CHRISTENSON, R. K. The influence of uterine function on embryonic and fetal survival. **Journal of Animal Science**, Champaign, v. 80, suppl. 2, p. E115–E125, 2002.

VAN DEN BOGAARD, A. E.; STOBBERINGH, E. E. Epidemiology of resistance to antibiotics. Links between animals and humans. **International Journal of Antimicrobial Agents**, Amsterdam, v. 14, n. 4, p. 327-335, 2000.

VAN DER MEULEN, J.; KOOPMANS, S. J.; DEKKER, R. A.; HOOGENDOORN, A. Increasing weaning age of piglets from 4 to 7 weeks reduces stress, increases post-weaning feed intake but does not improve intestinal functionality. **Animal**. v. 4, n.10, 1653-1661, 2010.

VARMA, J. K.; MARCUS, R.; STENZEL, S. A.; HANNA, S. S.; GETTNER, S.; ANDERSON, B. J.; HAYES, T.; SHIFERAW, B.; CRUME, T. L.; JOYCE, K.; FULLERTON, K. E.; VOETSCH, A. C.; ANGULO, F. J. Highly resistant salmonella newport-mdrampc transmitted through the domestic us food supply: a foodnet case-control study of sporadic salmonella newport infections, 2002-2003. **Journal of Infectious Diseases**, Chicago, v. 194, n. 2, p. 222-230, 2006.

VEAL, E. A.; DAY, A. M.; MORGAN, B. A. Hydrogen peroxide sensing and signaling. **Molecular Cell**, Cambridge, v. 26, n. 1, p. 1-14, 2007.

WEARY, D. M.; JASPER, J.; MARIA, J.; HOTZEL, M. J. Understanding weaning distress. **Applied Animal Behaviour Science**, Amsterdam, v. 110, n. 1-2, p. 24-41, 2008.

WEINBERG, S. E.; SENA, L. A.; CHANDEL, N. S. Mitochondria in the regulation of innate and adaptive immunity. **Immunity**, Cambridge, v. 42, n. 3, p. 406-417, 2015.

WESSELINK, E.; WITKAMP, R. F.; KOEKKOEK, W. A.C.; VAN ZANTEN, A. R.H.; GREFTE, S. Feeding mitochondria: Potential role of nutritional components to improve critical illness convalescence. **Clinical nutrition** (Edinburgh, Scotland), v: 38, n.3, p: 982-995, 2019.

WEST, A. P.; SHADEL, G. S.; GHOSH, S. Mitochondria in innate immune responses. **Nature Reviews Immunology**, London, v. 11, n. 6, p. 389-402, 2011.

WHITIN, J. C.; BHAMRE, S.; THAM, D. M.; COHEN, H. J. Extracellular glutathione peroxidase is secreted basolaterally by human renal proximal tubule cells. **American Journal of Physiology. Renal Physiology**, Bethesda, v. 283, n. 1, p. F20–F28, 2002.

WIJTTEN, P. J.; VAN DER MEULEN, J.; VERSTEGEN, M. W. Intestinal barrier function and absorption in pigs after weaning: a review. **British Journal of Nutrition**, Cambridge, v. 105, n. 7, p. 967-981, 2011.

WINGLER, K.; BOCHER, M.; FLOHÉ, L.; KOLLMUS, H.; BRIGELIUS-FLOHÉ, R. mRNA stability and selenocysteine insertion sequence efficiency rank gastrointestinal glutathione peroxidase high in the hierarchy of selenoproteins. **European Journal of Biochemistry**, Berlin, v. 259, n. 1, p. 149-157, 1999.

WU, G.; BAZER, F. W.; WALLACE, J. M.; SPENCER, T. E. Board-invited review: intrauterine growth retardation: implications for the animal sciences. **Journal of Animal Science**, Champaign, v. 84, n. 9, p. 2316-2337, 2006.

WUM, C.; CHENZ, Y.; JARELL, V. L.; DZIUK, P. J. Effect of initial length of uterus per embryo on fetal survival and development in the pig. **Journal of Animal Science**, Champaign, v. 67, p. 1767-1722, 1999.

XIONG, X.; TAN, B.; SONG, M.; JI, P.; KIM, K.; YIN, Y.; LIU, Y. Nutritional Intervention for the Intestinal Development and Health of Weaned Pigs. **Frontiers in Veterinary Science**. v.6, n.46, p.1-14, 2019.

YIN, J.; WU, M. M.; XIAO, H.; REN, W. K.; DUAN, J. L.; YANG, G.; LI, T. J.; YIN, Y. L. Development of an antioxidant system after early weaning in piglets. **Journal of Animal Science**, Champaign, v. 92, n. 2, p. 612-619, 2014.

YU, B. P. Cellular defenses against damage from reactive oxygen species. **Physiological Reviews**, Bethesda, v. 74, n. 1, p. 139-162, 1994.

3. OBJECTIVES

3.1 General Objective

Identify and characterize mitochondrial and cellular markers of oxidative stress and energy metabolism during pre- and post-weaning in tissues of piglets below normal weight to define cellular targets and identify the periods for nutritional intervention

3.2 Specific Objectives

Characterize biomarkers of the oxidative profile and energy metabolism in piglets in the peri-weaning period

Evaluate the levels of oxidative damage to the cellular components and mitochondria by assessing the biomarkers and oxidative damage to proteins and DNA

Characterize cell and mitochondrion antioxidant capability by assessing a variation in the expression of the main genes associated with the antioxidant defence, mitochondrial metabolism, and inflammation by quantitative RT-PCR.

4 PAPERS FOR PUBLISHING

Tissue-specific profiling reveals modulation of cellular and mitochondrial oxidative stress in normal and low birth weight piglets throughout the peri-weaning period.

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Short title: Mitochondrial oxidative stress and weaning

Abstract

Weaning is known to induce important nutritional and energetic stress in piglets. Low birth weight piglets, now frequently observed in swine production, are more likely to be affected. The weaning period is also associated with dysfunctional immune responses, uncontrolled inflammation and oxidative stress conditions that are recognized risk factors for infections and diseases. Mounting evidence indicates that mitochondria, the main cellular sources of energy in the form of adenosine triphosphate (ATP) and primary sites of reactive oxygen species (ROS) production, are related to immunity, inflammation and bacterial pathogenesis. However, no information is currently available regarding the link between mitochondrial energy production and oxidative stress in weaned piglets. The objective of this study was to characterize markers of cellular and mitochondrial energy metabolism and oxidative status in both normal- and low birth weight piglets throughout the peri-weaning period. To conduct the study, 30 multiparous sows were inseminated and litters were standardized to 12 piglets. All the piglets were weighted at day 1 and 120 piglets were selected and assigned to one of two experimental groups: normal birth weight piglets (NBW, n = 60, mean weight of 1.73 ± 0.01 kg,) and low birth weight piglets weighing less than 1.2 kg (LBW, n = 60, 1.01 ± 0.01 kg). Then, 10 piglets from each group were selected at 14, 21 (weaning), 23, 25, 29 and 35 days of age to collect plasma and organ (liver, intestine and kidney) samples. Analysis revealed that ATP concentrations were lower in liver of piglets after weaning than during lactation ($P < 0.05$) thus suggesting a significant impact of weaning stress on mitochondrial energy production. Oxidative damage to DNA (8-hydroxy-2'-deoxyguanosine, 8-OHdG) and proteins (carbonyls) measured in plasma increased after weaning and this coincides with a rise in enzymatic antioxidant activity of glutathione peroxidase (GPx) and

superoxide dismutase (SOD) ($P < 0.05$). Mitochondrial activities of both GPx and SOD are also significantly higher ($P < 0.05$) in kidney of piglets after weaning. Additionally, oxidative damage to macromolecules is more important in LBW piglets as measured concentrations of 8-OHdG and protein carbonyls are significantly higher ($P < 0.05$) in plasma and liver samples respectively than for NBW piglets. These results provide novel information about the nature, intensity and duration of weaning stress by revealing that weaning induces mitochondrial dysfunction and cellular oxidative stress conditions which last for at least 2 weeks and more severely impact smaller piglets.

Keywords: mitochondria, oxidative stress, piglets, weaning, energy metabolism

Implications

The weaning period is characterised by a high incidence of diseases in piglets leading to therapeutic use of antibiotics and substantive economic losses in swine production. This study aimed to characterize the nature and duration of weaning stress by focussing on mitochondria, the cellular sources of energy and oxidative stress. Weaning was found to perturb mitochondrial energy production and to induce oxidative stress until 35 days of age and LBW piglets were more affected. Therefore, mitochondria are identified as major actors in the establishment of weaning stress and this could set the basis for the development of alternatives to antibiotics.

Introduction

Weaning is known to impose tremendous stress on piglets and the early post-weaning period is characterised by a high incidence of metabolic disturbances and bacterial infections that are related to higher rates of morbidity and mortality (Campbell *et al.*, 2013). This problem was exacerbated over the last decades by the selection for sows with larger litter size as a means to increase profitability. This has resulted in increased variations of birth weights within litter as well as in an increased number of low birth weight (LBW) piglets (Damgaard *et al.*, 2003). These LBW piglets have impaired development of intestinal defence response compared to high birth weight piglets and are more vulnerable to the various pathologies associated with the weaning period (De Vos *et al.*, 2014, Lessard *et al.*, 2018). Unfortunately, the precarious health conditions of post-weaned piglets frequently require the utilisation of antibiotics, which ideally should be avoided (Barton, 2014). As a result, effective strategies aiming at improving metabolic health of weaned piglets and increase their defence mechanisms against bacterial infections need to be developed to limit antibiotic use and ensure profitability in swine industry.

Mitochondria are the organelles in charge of producing the energy required for all cellular functions in the form of adenosine 5' triphosphate (ATP). They are located at the interface between the environmental calorie supply and the energetic needs of cells in both normal and stressful conditions. In response to energy demands, various substrates are metabolized via several metabolic pathways to ultimately drive mitochondrial ATP synthesis by oxidative phosphorylation (Green and Tzagoloff, 1966). Mitochondrial energy metabolism is also linked to the generation of reactive oxygen species (ROS) as normal by-products. ROS have the potential to inflict

serious oxidative damage to both mitochondrial and cytoplasmic macromolecules and their concentrations are controlled by a complex network of non-enzymatic and enzymatic antioxidants. Oxidative stress can be defined as any imbalance between the production and the detoxification of ROS. In periods of high energetic demands, the production of ROS by heavily solicited mitochondria is likely to exceed the antioxidant potential, leading to such oxidative stress conditions. Recent evidence indicates that mitochondrial function and oxidative stress are closely related to innate immune response (Weinberg *et al.*, 2015), intestinal inflammation (López-Armada *et al.*, 2013) and bacterial pathogenesis (Sander and Garaude, 2018). Interestingly, mitochondria are also known to be quite responsive to a variety of nutrients and increasing evidence now suggests that directly targeting mitochondria with specific nutritional approaches could efficiently prevent and improve various conditions associated with mitochondrial dysfunction (Lapointe, 2014).

It is well recognized that weaning induces oxidative stress conditions in post-weaned piglets and this specific effect has been shown to occur whatever the weaning age (Zhu *et al.*, 2012, Buchet *et al.*, 2017). Knowledge related to the characterization of mitochondrial function and oxidative stress in normal and low-birth weight piglets during the peri-weaning period is greatly limited. Recent studies focussing on intestinal and hepatic functions of weaned piglets have reported a reduction in the activities of both mitochondrial respiratory complexes and enzymatic antioxidants during the first week following weaning (Luo *et al.*, 2016, Cao *et al.*, 2018). Other studies revealed a significant increase in the intensity of oxidative damage to lipids and proteins in the blood of piglets after weaning (Zhu *et al.*, 2012, Yin *et al.*, 2014).

Taken together, these observations indicate the importance of a more detailed analysis of mitochondrial function and oxidative stress during the peri-weaning period to better define the cellular and molecular mechanisms underlying weaning stress in piglets. Therefore, the aim of the current study is to provide for the first-time a detailed expression analysis of markers of mitochondrial energy metabolism and oxidative stress in different tissues of LBW and NBW piglets over a specific time period lasting from mid lactation (d14) until 2 weeks post-weaning (d 35). This could lay the foundations for new interventions aiming to improve health and robustness of weaned piglets.

Material and methods

Animals

During gestation and lactation, 30 conventional multiparous Yorkshire x Landrace sows were given the same feed allowances and diet formulations as previously described (Matte *et al.,* 2017). They also had free access to water throughout gestation and lactation. Sows were housed in individual pens (1.5×2.4 m) throughout gestation. Litters were standardized to 12 piglets within 48 h of birth. All piglets were weighed on day 1 and those presenting the required characteristics were assigned to one of two experimental groups: normal birth weight piglets (NBW, n = 60) and low birth weight piglets (LBW, n = 60). NBW piglets were defined as having a weight between the mean weight of all the 360 produced piglets on d 1 and a standard deviation value (1.73 ± 0.01 kg) while LBW were selected among those with a weight of less than 1.2 kg on d 1 (1.01 ± 0.01 kg). The NBW and LBW piglets have been respectively selected from 25 and 26 of the 30 generated litters. Males and females

were carefully distributed within the two groups (31 females and 29 males for LBW; 29 females and 31 males for NBW) and the sampling time points to avoid any confounding effect. Piglets had no access to creep feed and water during the lactation period and were weaned on d 21. Body weights of selected piglets were monitored during lactation on days 1, 7, 14 and 21 (weaning day) as well as at 23, 25, 29 and 35 days of age (Figure 1). After weaning, previously identified LBW and NBW piglets were kept with their original littermates and raised in pens (one litter/pen) until the end of the experimental period. Weaned piglets were fed ad libitum commercial weaning diets (3 phases feeding program) without antibiotics for the two weeks of the experimental period and had free access to drinking water. The analytical composition of these diets is described in Table 1. Within each group (LBW and NBW), 10 representative piglets were selected and sacrificed on day 14, 21 (weaning), 23, 25, 29 and 35 days of age. Samples from the left lateral lobe of the liver and the left kidney were collected along with intestinal mucosal scrapings (from the jejunum section). These samples were rinsed in phosphate-buffered saline (PBS), snap frozen in liquid nitrogen and stored at -80 °C for future measurements. Blood samples were collected from 10 LBW and 10 NBW piglets on the corresponding day of sacrifice (days 14, 21, 23, 25, 29 and 35) as well as on day 22 for the piglets sacrificed on day 29. Blood samples were collected in EDTA tubes (Becton Dickinson and Company, Rutherford, NJ, USA) and were centrifuged within 20 min at 4°C for 12 min at 1800 xg. Plasma was immediately recovered and samples were frozen at -80°C until they were assayed.

Isolation of mitochondria

Mitochondria from liver, kidney and intestinal mucosa were isolated by standard differential centrifugation. Briefly, tissues were finely minced and homogenized in 10 vol of ice-cold homogenization buffer (HEPES 20 mM pH 7.2, EGTA 1 mM, D-mannitol 210 mM, sucrose 70 mM) using a Potter (Teflon-to-glass) homogenizer. The homogenate was centrifuged at 1000 xg for 10 min for the removal of nuclei and cellular debris. A part of the supernatant corresponding to the total cellular fraction was kept for further analysis and the remaining portion was centrifuged at 10 000 xg for 10 min to pursue the isolation of the mitochondrial fraction. Pellets containing mitochondria were then washed twice with the same buffer before being finally resuspended in a minimal volume of the appropriate assay buffers. All those steps were performed at 4°C. Concentrations of mitochondrial proteins were determined using the Bicinchoninic Acid (BCA) Protein Assay Kit (Sigma-Aldrich).

Enzymatic antioxidant assays

The total cytosolic and mitochondrial extracts used for enzymatic activity analysis were obtained as described earlier and were sonicated on ice for 40 s at an amplitude of 30 % in pulse mode (2 x 20 s with a pause of 10 s) using a Q125 sonicator (Qsonica, Newtown, CT, USA). GPx activity was measured in plasma samples as well as in total and mitochondrial extracts of liver, kidney, intestinal mucosa and by using the standard indirect method of NADPH oxidation with t-butyl hydroperoxide as described elsewhere (Flohe and Gunzler, 1984). Superoxide dismutase (SOD) activity was measured in total cellular fractions and enriched mitochondrial extracts of liver, kidney and intestinal mucosa by following a previously published method (Weydert and Cullen, 2010). Total SOD activity in plasma samples was measured using a commercial kit according to instructions from the

manufacturer (Cayman Chemical, Ann Arbor, MI). All assays were performed in triplicates. For all analyses, the precision within test was assessed by calculating intra-assay coefficients of variation from all triplicated samples analysed. The inter-assay coefficients of variation was calculated from pool samples analysed in triplicates. The intra- and inter-assay coefficients of variation were respectively (3.2% and 6.2% for GPx; 4.1% and 9.8 % for SOD). All enzymatic activities were normalized to the quantity of proteins determined by using BCA Assay Kit (Sigma-Aldrich, Oakville, ON).

Determination of oxidative damage

The levels of oxidative damage to proteins (protein carbonyl content) in plasma and liver samples were determined with the OxiSelect Protein Carbonyl ELISA Kit (Cell Biolabs INC, San Diego, CA, USA) as previously described (Roy *et al.*, 2016), with minor modifications. Briefly, 100 mg of liver samples were rinsed and homogenized in ice-cold 50 mM phosphate-buffered saline solution (pH 7.4) using a Potter (Teflon-to-glass) homogenizer. Supernatant was centrifuged at 10 000 xg for 10 min at 4°C and then collected before being centrifuged at 10 000 xg for another 5 min at 4°C. The resulting supernatant was directly assayed for carbonyl content according to the provided protocol. The plasma concentration of 8-hydroxy-2-deoxyguanosine (8-OHdG), a biomarker for oxidative damage to DNA, was measured using an enzyme-linked immunoassay kit according to the manufacturer's protocol (Cedarlane Labs, Burlington, ON, USA) as previously performed (Roy *et al.*, 2016). All assays were performed in triplicates. The intra- and inter-assay coefficients of variation were respectively (4.6% and 7.6% for carbonyls; 2.7% and 10.8 % for 8-OHdG).

Quantification of ATP concentrations

Concentrations of ATP were measured in liver samples by using Molecular Probes® ATP determination kit (Thermo Fisher Scientific, Burlington, ON). Briefly, 150 mg of frozen liver were homogenized in phosphate buffer pH 7.4 (0.15 M NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4) with protease inhibitor (Sigma-Aldrich, Oakville, ON). The homogenate was then centrifuged for 5 min at 13 000 xg at 4°C, the pellet was discarded and the supernatant was centrifuged again at 13 000 xg for 5 min at 4°C. The ATP content was then measured by bioluminescence assay with recombinant firefly luciferase according to the manufacturer instructions. The light emission was quantified and recorded using the multimode microplate reader Tristar2 (Berthold Technologies, Germany). The intra- and inter-assay coefficients of variation were 3.9% and 7.2% respectively. The determined ATP concentrations were normalized to the quantity of proteins by using BCA Assay Kit (Sigma-Aldrich, Oakville, ON).

Statistical Analysis

Data were analysed using the MIXED procedure of SAS (SAS release 9.2, 2002, SAS Institute, Cary, NC, USA). Analysis of variance was performed according to a completely randomized design to test the effects of the two studied factors (birth weight, age of piglets) and their interaction on enzymatic activities, markers of oxidative damage and ATP content. Piglets were used as experimental units. The usual model for a complete randomized design used in this experiment is: $Y_{ijk} = \mu + t_I + \gamma_j + (t\gamma)_{ij} + e_{ijk}$ where Y_{ijk} is the observation for animal k in birth weight group i at age j, μ is the overall mean, t_i is the fixed effect of birth weight (i=LBW of NBW), γ_j is the fixed effect of age j of piglet, $t\gamma_{ij}$ is the interaction between birth weight and age and

e_{ijk} is the residual error. Multiple comparisons were performed with a Tukey's adjustment to compare ages and specific effect of birth weight within age was determined by multiple pairwise t-tests. Effects of the statistical model were considered significant at $P \leq 0.05$, and the tendency (trend) at $0.05 < P \leq 0.10$.

Results

Piglets

Piglets were selected and grouped according to their weight on d 1. The 60 LBW piglets have a mean birth weight of 1.01 ± 0.01 kg and were 41.6% lighter than their normal birth weight counterparts which have a mean body weight of 1.73 ± 0.01 kg (Figure 1). This difference between the two groups remains throughout the experimental period with LBW piglets being 33% lighter than NBW piglets on d 35. As expected, weight gains for both groups were modest during the first days of the post-weaning period (d 21 to 25; Figure 1).

Systemic oxidative stress

The systemic oxidative stress refers to an imbalance between oxidants and antioxidants in extracellular compartments and circulating fluids. Analysis of enzymatic activity of two major antioxidants, GPx and SOD, was first performed in blood plasma to characterize systemic oxidative stress conditions of both groups throughout the peri-weaning period (Figure 2). The activity of both enzymes was found to gradually increase following weaning ($P < 0.0001$) with the highest activities being observed on d 29 and 35 (Figure 2A, B). Overall, no differences were observed between groups for GPx and SOD activities but analysis of differences between

weight groups within age indicated higher SOD activity in LBW piglets on d 14 ($P < 0.05$; Supplementary Table S2). Circulating levels of 8-OHdG (oxidative damage to DNA) were also found to be affected by weaning with significant increases ($P < 0.05$) occurring shortly after the weaning day (Figure 2C; Supplementary Table S3). Concentrations of 8-OHdG were higher in LBW piglets ($P < 0.05$), especially during the post-weaning period (Supplementary Table S2). Analysis of oxidative damage to proteins in plasma samples was influenced by the age of piglets but did not revealed significant differences for the groups (Figure 2D).

Hepatic ATP production

Measurement of total cellular ATP concentrations in liver samples indicated that hepatic ATP production was significantly modulated during the weaning period ($P < 0.05$; Figure 3A). The hepatic ATP concentrations of both LBW and NBW piglets rapidly decreased following weaning with the lowest concentrations measured on d 23 (Supplementary Table S3). Despite the fact that no significant differences were observed between the two groups of piglets, ATP concentrations are clearly lower in liver of LBW than NBW piglets prior to weaning (d 14 and 21; Figure 2; Supplementary Table S2) and the overall values tended to be affected by birthweight for the entire experimental period ($P = 0.092$).

Mitochondrial and cellular oxidative stress in liver

Low birth weight piglets had higher oxidative damage to liver proteins than their NBW littermates as shown by measurement of protein carbonyl content ($P < 0.05$; Figure 3B). Concentrations of protein carbonyls are also significantly modulated throughout the peri-weaning period ($P < 0.0001$). GPx activity in total cellular fraction was found

to be significantly affected by the age of the piglets with the lowest activities being measured on d 14 of lactation (Figure 3C; Supplementary Table S3). This effect of the age of the piglets on GPx activity was not observed in the mitochondrial enriched fractions (Figure 3D). In contrast, SOD activity in total cellular fractions remained stable throughout the experimental period while mitochondrial SOD activity is significantly modulated by age with the highest levels observed shortly after weaning (Figure 3E, F; Supplementary Table S3). Activities of both GPx and SOD are similar between LBW and NBW groups whatever the studied liver fractions (Supplementary Table S2).

Intestinal defences against oxidative stress

A significant effect of the age of piglets was observed for GPx activity in both cellular and mitochondrial fractions obtained from the intestinal mucosa ($P < 0.05$; Figure 4A, B). The highest levels of GPx activity were measured on d 35 in both fractions and no significant effect was observed between groups. SOD activity in intestinal mucosa was also modulated throughout the peri-weaning period in the whole cellular as well as in the mitochondrial samples (Figure 4C, D; Supplementary Table S3). The observed SOD activity patterns are similar for LBW and NBW piglets in those fractions (Supplementary Table S2).

Antioxidant activities in kidney

The enzymatic activity of GPx is similar between groups in both cellular and mitochondrial fractions from kidney but it was found to be significantly modulated by the age of the piglets ($P < 0.005$; Figure 5A, B). The lowest levels of GPx activity were observed on d 14 of lactation period (Supplementary Table S3). A significant

1619 effect of the experimental period was also found for SOD activity in total cellular
1620 fractions as well as in isolated mitochondria ($P < 0.001$; Figure 5C, D). The lowest
1621 levels of SOD activity were also measured during lactation at d 14 and no significant
1622 effect of the birth weight was observed (Figure 5C, D ;Supplementary Table S3).
1623

1624 **Discussion**

1625 The weaning period is recognized as an important concern for swine producers which
1626 have to deal with high incidence of diseases affecting weaned piglets. New laws
1627 adopted by many countries restrict or regulate the use of antibiotics and specific trace
1628 minerals with antibacterial properties such as zinc oxide in swine production (Barton,
1629 2014). This has increased the need to develop alternative strategies aiming to
1630 optimize the robustness of weaned piglets. However, such a goal cannot be achieved
1631 without a better understanding of the cellular mechanisms underlying the origin, the
1632 intensity and the duration of weaning stress. Results from several studies have
1633 convincingly demonstrated that oxidative stress conditions are induced in weaned
1634 piglets whatever the weaning age (Zhu *et al.*, 2012, Buchet *et al.*, 2017, Cao *et al.*,
1635 2018). To the best of our knowledge, this is the first time where the activity of two
1636 major enzymatic antioxidant defence systems, GPx and SOD, as well as of the
1637 expression of recognized markers of oxidative damage were characterized in both
1638 cellular and mitochondrial compartments of several tissues during the peri-weaning
1639 period. Moreover, the experimental design of the current study allowed to precisely
1640 define the duration of the weaning stress period as measurements have been
1641 performed at different time points starting from d 14 in lactation until d 35 of age (14
1642 days after weaning).

Results of the present study support the notion that weaned piglets are going through a significant energetic deficit resulting from the abrupt separation from their dam and its associated stressors, especially the need to rapidly adapt to a solid and less digestible type of diet (Campbell *et al.*, 2013). Intracellular energy levels, in the form of ATP, were shown to rapidly decline following weaning in liver samples. The measured ATP concentrations on d 23 are significantly lower than the ones from mid-lactation (d 14) and immediately before weaning on d 21 for both groups of piglets. These differences are particularly obvious in the NBW piglets where the post-weaned ATP concentrations are approximately half as high as the ones measured during lactation. Afterward, the ATP content of liver cells remains lower than during the lactation period even at two weeks post-weaning. This decrease in liver ATP content following weaning is probably related to the low feed intake observed in newly weaned piglets. The average daily feed intake during the early post-weaning period is highly variable between piglets but most of them do not eat (Wijtten *et al.*, 2011). It was shown that the latency time before the first solid food intake after weaning can take up to 3 days (Bruininx *et al.*, 2001). By the end of the first week post-weaning, it was estimated that the total metabolizable energy intake of piglets is about 60-70% of the energy uptake via milk consumption in pre-weaning piglets (Spreeuwenberg *et al.*, 2001). The growth check observed in the present study, two days after weaning, is related to the transient anorexia of post-weaned piglets and is one of several features that are also known to be induced by fasting in pigs and other species (Ferraris and Carey, 2000). Intestinal disturbances frequently observed in early weaned piglets are also recognized to share common features with those caused by short fasting periods in pigs (Lallès and David, 2011). During fasting or low feed intake episodes, liver is the organ that displays the most dramatic changes due to its

crucial role in supplying energy to other tissues (Sorensen *et al.*, 2006). A study in rodents have shown that mitochondria isolated from liver of rats fasted for 18 hours presented a lower content of ATP synthase complex and decreased ATP concentrations in comparison with fed counterparts (Vendemiale *et al.*, 2001).

Fasting also induces the production mitochondrial free radical and increases oxidative damage to macromolecules in the liver of rats fasted for either 18 or 72 hours (Sorensen *et al.*, 2006). The induction of mitochondrial oxidative stress during periods of food deprivation has been associated with decreased metabolic requirements, higher mitochondrial membrane potential and increased superoxide production at the level of the complex III of the electron transport chain (Vendemiale *et al.*, 2001, Salin *et al.*, 2018). In the current study, an increase in mitochondrial SOD activity was observed immediately after weaning on d 23 and 25 where the lowest levels of liver ATP content were measured in both LBW and NBW piglets. This modulation of SOD activity was not observed in whole cellular extracts probably due to the presence of different members of the SOD family in these cellular compartments, SOD1 (CuZn-SOD) being mainly located in the cytosol while SOD2 (Mn-SOD) is exclusively retrieved in mitochondria (Zelko *et al.*, 2002). Since SOD2 activity is regulated in response to mitochondrial superoxide production, these results suggest that the decreased in liver ATP content is also associated with superoxide production in liver mitochondria of early weaned piglets. This up-regulation of mitochondrial SOD activity results in an overproduction of hydrogen peroxide as a product of the disproportionation reaction of superoxide anion catalysed by SOD (Zelko *et al.*, 2002). GPx activity is known to be triggered by H_2O_2 production in the same way as SOD is regulated by intracellular concentrations of superoxide (Brigelius-Flohé and Maiorino, 2013). GPx1, which is an intracellular GPx known to

be ubiquitously expressed, is the principal enzyme of the GPx family known to be present in liver. In normal conditions, H_2O_2 is rapidly reduced to water principally by GPx enzymes, which use the reducing equivalents from their substrate GSH (γ-glutamyl-cysteinyl-glycine). However, results from the present study did not reveal any variation of GPx activity in mitochondria isolated from liver samples and a significant decrease of activity in total cellular extracts was observed shortly after weaning, from d 21 to 23. These unexpected results could be related to observations from other studies indicating that food deprivation significantly decrease both cellular and mitochondrial GSH contents in liver (Grattagliano *et al.*, 2000, Domenicali *et al.*, 2001). GSH is known to be synthesized de novo in two sequential enzymatic ATP-dependent reactions and its binding was found to be rate-limiting for GPx catalysis. In order to have an impact on GPx activity in vivo, the magnitude of GSH decrease must be substantial considering the concentrations of GSH and hydroperoxides normally measured in cells (Brigelius-Flohé and Maiorino, 2013).

These changes in ATP production and antioxidant activities following weaning suggest the occurrence of mitochondrial dysfunction and oxidative stress conditions but they were not accompanied by an increase in the levels of oxidative damage to liver proteins. It was observed that mitochondrial dysfunction is not always related to oxidative damage to proteins in animal subjected to nutritional stress (Vendemiale *et al.*, 2001). However, results from the present study revealed the presence of higher levels of oxidative damage to proteins in liver of LBW piglets than their NBW littermates, especially during the pre-weaning period on d 14 and d 21. This higher oxidative damage in LBW piglets is related to ATP concentrations, which tended to be lower than NBW piglets for the whole experimental period with a more

pronounced difference during lactation. This indicates that LBW piglets already sustain metabolic and oxidative stress conditions during the pre-weaning period. In accordance with these results, it was recently demonstrated that the activities of the complex III of the mitochondrial respiratory chain and the ATP synthase are significantly lower in liver from intra uterine growth retarded (IUGR) piglets immediately before weaning at d 21 (Zhang *et al.*, 2017). Such deficiencies in mitochondrial function of IUGR piglets have further been observed four weeks after weaning as revealed by lower mitochondrial DNA content, ATP concentrations and oxygen consumption (Zhang *et al.*, 2016). The differences observed in the present study in terms of energy production and oxidative damage between both groups of piglets prior to weaning did not persist throughout the post-weaning period but the slower growth rate of the piglets selected in the LBW group was not as pronounced as the one reported elsewhere in IUGR piglets. However, analyses of systemic oxidative damage to DNA indicated that 8-OHdG concentrations were significantly affected by the birth weight of piglets. A sharp increase in circulating oxidative damage to DNA was observed after weaning on d 22 in both groups. However, while 8-OHdG concentrations immediately decreased on d 23 in NBW groups, they remained high until d 25 in plasma of LBW piglets. The quantification of protein carbonyls in plasma samples also suggests that weaning trigger a systemic oxidative stress in piglets with the lowest carbonyl concentrations being measured just before weaning on d 21. The highest levels of oxidative damage to proteins were observed on d 29 and 35 for both groups. As mentioned earlier, it is well known that cells respond to ROS production and oxidative stress by increasing their enzymatic antioxidant defence systems. It was therefore not surprising to observe an immediate modulation of GPx and SOD activities after weaning (Zelko *et al.*, 2002, Brigelius-

Flohé and Maiorino, 2013). In the present study, the activity of both enzymes gradually increased in parallel from d 22 to reach a maximum at d 35. Taken together, these results on the characterization of markers of systemic oxidative stress suggest that not only weaning induces oxidative stress conditions in piglets but also that this situation lasts for up to two weeks after weaning. This conclusion is in accordance with other results suggesting that metabolic and oxidative stress resulting from periods of food shortage could potentially result in medium to long-term detrimental effects (Salin *et al.*, 2018).

The anorexia occurring after weaning is known to cause major physiological changes in the structure and the function of the intestinal epithelium of weaned piglets (Wijtten *et al.*, 2011). Among these changes, modifications of villus height and crypt depth, impairment of barrier function, increased permeability, decrease in brush border enzyme activity and acute mucosal inflammation have been reported whatever the weaning age (Wijtten *et al.*, 2011). These modifications of intestinal integrity and function could be either transient or long-lasting (Moeser *et al.*, 2017). In the present study, activities of both GPx and SOD enzymes were affected by weaning in total and mitochondrial extracts from intestinal mucosa. Enzymatic activity of both antioxidants was higher during the post-weaning period in whole cellular extracts thus revealing a cellular response to oxidative stress conditions induced by weaning. This is in accordance with recently published results showing higher levels of oxidative damage in jejunum samples from early weaned piglets (Cao *et al.*, 2018). The occurrence of oxidative conditions following weaning was further supported by the gradual activation of the GPx antioxidant system observed in isolated mitochondria from intestinal mucosa. The main GPx enzyme normally retrieved in

intestinal tissue is GPx2, which is not known to be present in liver and kidney (Brigelius-Flohé and Maiorino, 2013). However, the analysis of mitochondrial SOD activity in the same samples revealed a sharp decrease in enzymatic activity immediately after weaning in both groups of piglets. The activity of mitochondrial SOD remains lower than during the lactation period even at two weeks post-weaning. An up-regulation of SOD activity should have been expected in response to oxidative stress as it was observed in liver and kidney samples. However, such decrease in mitochondrial SOD activity has previously been reported in intestinal epithelium of early-weaned piglets (Cao *et al.*, 2018). This same study further revealed that activity of complexes from the mitochondrial respiratory chain is decreased after weaning, in jejunum samples. Taken together, these results suggest that mitochondrial oxidative stress conditions occurred in the intestinal mucosa of weaned piglets and that such conditions are likely to last for up to two weeks as observed for other intestinal disorders (Moeser *et al.*, 2017). The absence of SOD activation in response to mitochondrial oxidative stress imply that the enzyme is somehow inhibited by other factors. Additional work will be necessary to better understand the mechanisms underlying the downregulation of SOD activity. Interestingly, SOD enzymes are known to be quite sensitive to intracellular concentrations of zinc and copper (Zelko *et al.*, 2002), two trace minerals frequently added in large quantities in post-weaning diets .

The consequences of weaning stress on cellular and mitochondrial oxidative stress in kidney of weaned piglets have never been studied. Results from the present study showed a significant impact of the experimental period on GPx and SOD activities in both cellular and mitochondrial extracts. In all cases, the measured enzymatic activities were at their lowest levels on d 14 of lactation, significantly

increased on d 21 and then remained relatively stable until two weeks post-weaning. This contrasts with results from other tissues, suggesting that the induction of oxidative conditions may occur before the separation of the piglets from the sows. This could somehow be related to the development of kidney since the number of mitochondria was shown to increased markedly in renal cells until the completion of the formation of nephrons at 21 days of age (Friis, 1980). The kidney is also the only of the studied tissues to express the GPx3 enzyme of the GPx family in addition of GPx1 which is also retrieved in liver and intestine. As mentioned earlier the presence of specific isoforms of GPx and SOD enzymes is likely to have a significant effect on the enzymatic activities measured in cellular and mitochondrial extracts from the targeted organs. However, as observed at systemic level and in other organs, the activities of major antioxidant systems in kidney suggest that the post-weaning mitochondrial and cellular oxidative stress conditions persist at least during two weeks.

Taken together, results from this study provide novel information about the nature, intensity and duration of metabolic and oxidative stresses known to affect post-weaned piglets by revealing that mitochondrial dysfunction and cellular oxidative stress conditions are rapidly induced following weaning and lasted for at least two weeks. They further indicate for the first time that LBW piglets sustain more severe mitochondrial energetic deficiencies and oxidative stress conditions than their normal weight counterparts during the lactation period. These findings are of particular interest considering that it is now recognized that mitochondrial function and oxidative stress are tightly associated to the innate immune response, intestinal inflammation and bacterial pathogenesis (López-Armada *et al.*, 2013, Sander and Garaude, 2018).

Additional analyses will thus be necessary to better characterize the molecular mechanisms underlying these metabolic and oxidative stresses associated to weaning as well as to determine the associated consequences for weaned piglets. It is likely that results of the present study could also apply to piglets weaned at earlier or later stage considering that it was clearly demonstrated that oxidative stress is triggered weaning independently of the weaning age but it would be very interesting to confirm this hypothesis in a near future. Results from the present study will be highly relevant for future researches aiming at developing novel approaches to improve the health and robustness of newly weaned piglets by providing targeted biomarkers to verify their efficiency at both systemic and organ levels. The increasing number of evidences indicating that mitochondria could be directly targeted by nutritional approach is highly promising (Lapointe, 2014), and this study constitutes the first indispensable step to introduce such strategies to improve resistance of newly weaning piglets.

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Declaration of interest

The authors declare that there is no conflicts of interest that could be perceived as prejudicing the impartiality of the results presented in this study.

Ethics statement

All animals involved in this project have been treated in accordance with the code of good practice in effect (Agriculture and Agri-Food Canada, 1993) and all procedures involving these animals were reviewed and approved by the Institutional Animal Care Committee (CIPA 488).

Software and data repository resources

None of the data were deposited in an official repository.

References

Agriculture and Agri-Food Canada 1993. Recommended code of practice for the care and handling of farm animals – pigs. Publ. No. 1898E. Agriculture and Agri-Food Canada, Ottawa, ON, Canada.

Barton MD 2014. Impact of antibiotic use in the swine industry. Current Opinion in Microbiology 19, 9-15.

Brigelius-Flohé R and Maiorino M 2013. Glutathione peroxidases. Biochimica et Biophysica Acta (BBA) - General Subjects 1830, 3289-3303.

Bruininx EM, van der Peet-Schwering CM, Schrama JW, Vereijken PF, Vesseur PC, Everts H, den Hartog LA and Beynen AC 2001. Individually measured feed intake characteristics and growth performance of group-housed weanling pigs: effects of sex, initial body weight, and body weight distribution within groups. Journal of Animal Science 79, 301-308.

Buchet A, Belloc C, Leblanc-Maridor MM and Merlot E 2017. Effects of age and weaning conditions on blood indicators of oxidative status in pigs. PLoS One 12.

Campbell JM, Crenshaw JD and Polo J 2013. The biological stress of early weaned piglets. Journal of Animal Science and Biotechnology 4.

Cao ST, Wang CC, Wu H, Zhang QH, Jiao LF and Hu CH 2018. Weaning disrupts intestinal antioxidant status, impairs intestinal barrier and mitochondrial function, and triggers mitophagy in piglets. Journal of Animal Science 96, 1073-1083.

Damgaard LH, Rydhmer L, Lovendahl P and Grandinson K 2003. Genetic parameters for within-litter variation in piglet birth weight and change in within-litter variation during suckling. Journal of Animal Science 81, 604-610.

De Vos M, Che L, Huygelen V, Willemen S, Michiels J, Van Cruchten S and Van Ginneken C 2014. Nutritional interventions to prevent and rear low-birthweight piglets. J Anim Physiol Anim Nutr (Berl) 98, 609-619.

Domenicali M, Caraceni P, Vendemiale G, Grattagliano I, Nardo B, Dall'Agata M, Santoni B, Trevisani F, Cavallari A, Altomare E and Bernardi M 2001. Food deprivation exacerbates mitochondrial oxidative stress in rat liver exposed to ischemia-reperfusion injury. Journal of Nutrition 131, 105-110.

Ferraris RP and Carey HV 2000. Intestinal transport during fasting and malnutrition. In Annual Review of Nutrition, pp. 195-219.

Friis C 1980. Postnatal development of the pig kidney: ultrastucure of the glomerulus and the proximal tubule. Journal of Anatomy 130, 513-526.

Grattagliano I, Vendemiale G, Caraceni P, Domenicali M, Nardo B, Cavallari A, Trevisani F, Bernardi M and Altomare E 2000. Starvation impairs antioxidant defense in fatty livers of rats fed a choline-deficient diet. Journal of Nutrition 130, 2131-2136.

Green DE and Tzagoloff A 1966. The mitochondrial electron transfer chain. Archives of Biochemistry and Biophysics 116, 293-304.

Novais AK, Roy C, Beaulieu S, Martel-Kennes Y, Lessard M, Matte JJ and Lapointe J. 2018. Evidences of mitochondrial dysfunction and oxidative stress in newly weaned piglets. In Proceedings of the Joint Annual Meeting of the American Society of Animal Science (ASAS) and the Canadian Society of Animal Science (CSAS), July 8–12th, Vancouver, Canada. Journal of Animal Science 96, 491.

Lallès JP and David JC 2011. Fasting and refeeding modulate the expression of stress proteins along the gastrointestinal tract of weaned pigs. Journal of Animal Physiology and Animal Nutrition 95, 478-488.

Lapointe J 2014. Mitochondria as promising targets for nutritional interventions aiming to improve performance and longevity of sows. J Anim Physiol Anim Nutr (Berl) 98, 809-821.

1900 Lessard M, Blais M, Beaudoin F, Deschene K, Verso LL, Bissonnette N, Lauzon K and Guay F 2018.
1901 Piglet weight gain during the first two weeks of lactation influences the immune system
1902 development. Veterinary Immunology and Immunopathology 206, 25-34.
1903 López-Armada MJ, Riveiro-Naveira RR, Vaamonde-García C and Valcárcel-Ares MN 2013.
1904 Mitochondrial dysfunction and the inflammatory response. Mitochondrion 13, 106-118.
1905 Luo Z, Zhu W, Guo Q, Luo W, Zhang J, Xu W and Xu J 2016. Weaning Induced Hepatic Oxidative
1906 Stress, Apoptosis, and Aminotransferases through MAPK Signaling Pathways in Piglets.
1907 Oxidative Medicine and Cellular Longevity 2016.
1908 Moeser AJ, Pohl CS and Rajput M 2017. Weaning stress and gastrointestinal barrier development:
1909 Implications for lifelong gut health in pigs. Animal Nutrition 3, 313-321.
1910 Roy C, Lavoie M, Richard G, Archambault A and Lapointe J 2016. Evidence that oxidative stress is
1911 higher in replacement gilts than in multiparous sows. J Anim Physiol Anim Nutr (Berl) 100, 911-
1912 919.
1913 Salin K, Villasevil EM, Anderson GJ, Auer SK, Selman C, Hartley RC, Mullen W, Chinopoulos C and
1914 Metcalfe NB 2018. Decreased mitochondrial metabolic requirements in fasting animals carry an
1915 oxidative cost. Functional Ecology 32, 2149-2157.
1916 Sander LE and Garaude J 2018. The mitochondrial respiratory chain: A metabolic rheostat of innate
1917 immune cell-mediated antibacterial responses. Mitochondrion 41, 28-36.
1918 Sorensen M, Sanz A, Gómez J, Pamplona R, Portero-Otín M, Gredilla R and Barja G 2006. Effects of
1919 fasting on oxidative stress in rat liver mitochondria. Free Radical Research 40, 339-347.
1920 Spreeuwenberg MAM, Verdonk JMAJ, Gaskins HR and Verstegen MWA 2001. Small intestine
1921 epithelial barrier function is compromised in pigs with low feed intake at weaning. Journal of
1922 Nutrition 131, 1520-1527.
1923 Vendemiale G, Grattagliano I, Caraceni P, Caraccio G, Domenicali M, Dall'Agata M, Trevisani F,
1924 Guerrieri F, Bernardi M and Altomare E 2001. Mitochondrial oxidative injury energy metabolism
1925 alteration in rat fatty liver: Effect of the nutritional status. Hepatology 33, 808-815.
1926 Weinberg SE, Sena LA and Chandel NS 2015. Mitochondria in the regulation of innate and adaptive
1927 immunity. Immunity 42, 406-417.
1928 Weydert CJ and Cullen JJ 2010. Measurement of superoxide dismutase, catalase and glutathione
1929 peroxidase in cultured cells and tissue. Nat Protoc 5, 51-66.

Wijtten PJ, van der Meulen J and Verstegen MW 2011. Intestinal barrier function and absorption in pigs after weaning: a review. Br J Nutr 105, 967-981.

Yin J, Wu MM, Xiao H, Ren WK, Duan JL, Yang G, Li TJ and Yin YL 2014. Development of an antioxidant system after early weaning in piglets. Journal of Animal Science 92, 612-619.

Zelko IN, Mariani TJ and Folz RJ 2002. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol Med 33, 337-349.

Zhang H, Li Y, Hou X, Zhang L and Wang T 2016. Medium-chain TAG improve energy metabolism and mitochondrial biogenesis in the liver of intra-uterine growth-retarded and normal-birth-weight weanling piglets. Br J Nutr 115, 1521-1530.

Zhang H, Li Y, Su W, Ying Z, Zhou L, Zhang L and Wang T 2017. Resveratrol attenuates mitochondrial dysfunction in the liver of intrauterine growth retarded suckling piglets by improving mitochondrial biogenesis and redox status. Molecular Nutrition and Food Research 61.

Zhu LH, Zhao KL, Chen XL and Xu JX 2012. Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs. Journal of Animal Science 90, 2581-2589.

Table 1 *Ingredients and nutrients provided as fed basis in weaning diets*

	Weaning Diets		
Ingredients/nutrients	d 21 to 23	d 24 to 29	d 30 to 35
Digestible energy (Kcal/kg)	3,420	3,470	3,350
Moisture (%)	9.19	9.2	10.87
Total protein (%)	18.45	18.59	20.82
Crude fibre (%)	2.35	3.01	3.02
Ash (%)	5.93	5.57	5.73
Fat (%)	5.21	6.35	4.78
Calcium (%)	0.65	0.69	0.70
Phosphorus (%)	0.67	0.75	0.68
Sodium (%)	0.52	0.51	0.38
Potassium (%)	0.75	0.66	0.96
Magnesium (%)	0.14	0.15	0.20
Copper (ppm)	114.37	132.28	121.09
Zinc (ppm)	2,850.04	2,476.49	428.38
Selenium (ppm)	0.80	0.70	0.60
Ca:P	0.98	0.92	1.03

Supplementary Table S1 *Analysis of variance (main effects and interaction) for all measured parameters in plasma, liver, intestinal mucosa and kidney (P-values).*

	Birthweight	Age	Birthweight x Age
Plasma			
GPx	0.2591	<0.0001[a]	0.3263
SOD	0.4992	<0.0001[a]	0.1234
8-OHdG	0.0415[a]	0.0175[a]	0.0725[b]
Carbonyls	0.4257	0.3330	0.8626
Liver			
ATP	0.0919[b]	0.0139[a]	0.4574
Carbonyls	0.0448[a]	<0.0001[a]	0.0701
GPx	0.7816	0.0007[a]	0.8552
GPx mito.	0.6667	0.4597	0.9641
SOD	0.9283	0.4190	0.3275
SOD mito.	0.1522	0.0383[a]	0.3046
Intestinal mucosa			
GPx	0.7432	<0.0001[a]	0.2645
GPx mito.	0.2887	0.0076[a]	0.3988
SOD	0.4526	0.0007[a]	0.4264
SOD mito.	0.3661	0.0004[a]	0.3674
Kidney			
GPx	0.1834	<0.0001[a]	0.3541
GPx mito.	0.4720	0.0025[a]	0.9974
SOD	0.4878	0.0008[a]	0.7736
SOD mito.	0.8910	0.0006[a]	0.7344

[a] Significant effect is observed at $P < 0.05$.

[b] Tendency to differ is observed at $P < 0.10$

Supplementary Table S2 *Group effect (LBW vs NBW) within age for all measured parameters in plasma, liver, intestinal mucosa and kidney (P-values).*

	Age (days)						
	14	**21**	**22**	**23**	**25**	**29**	**35**
Plasma							
GPx	0.3044	0.2792	0.3723	0.7875	0.1567	0.9464	0.0781[b]
SOD	0.0227[a]	0.1630	0.9523	0.4814	0.2432	0.7698	0.2260
8-OHdG	0.7173	0.9718	0.0716[b]	0.0546[b]	0.0081[a]	0.2003	0.9027
Carbonyls	0.4058	0.6988	0.4472	0.9261	0.9353	0.1972	0.8431
Liver							
ATP	0.0967	0.0406[a]	-	0.9592	0.8964	0.8552	0.5505
Carbonyls	0.0063[a]	0.0286[a]	-	0.2667	0.7248	0.7371	0.7933
GPx	0.4871	0.6648	-	0.2277	0.6279	0.0271[a]	0.3787
GPx mito.	0.6894	0.0517[b]	-	0.6871	0.6807	0.8156	0.2112
SOD	0.3341	0.5315	-	0.6912	0.9178	0.0327[1]	0.7617
SOD mito.	0.3675	0.9294	-	0.6181	0.1358	0.5882	0.1373
Intestinal mucosa							
GPx	0.9935	0.4267	-	0.1068	0.4689	0.3101	0.1805
GPx mito.	0.1314	0.8732	-	0.5979	0.2021	0.2112	0.4871
SOD	0.1920	0.8785	-	0.3837	0.1430	0.4784	0.5656
SOD mito.	0.3319	0.6151	-	0.0446[1]	0.3836	0.8089	0.6589
Kidney							
GPx	0.7580	0.3247	-	0.3326	0.0904[b]	0.8212	0.6114
GPx mito.	0.9107	0.6144	-	0.8501	0.5185	0.7449	0.9895
SOD	0.2290	0.3270	-	0.6930	0.7143	0.7895	0.6539
SOD mito.	0.2776	0.3688	-	0.9686	0.6101	0.9227	0.4747

[a] Values from both groups differ significantly at $P < 0.05$.

[b] Values from both groups tend to differ at $P < 0.10$

Supplementary Table S3 *Multiple comparisons with a Tukey's adjustment for age for all measured parameters in plasma, liver, intestinal mucosa and kidney.*

	Age (days)							
	14	**21**	**22**	**23**	**25**	**29**	**35**	**sem**
Plasma								
GPx (mU/mg)	18.1^{a}	18.1^{a}	20.4^{a}	21.7^{a}	24.6^{a}	24.7^{ab}	29.7^{b}	0.9
SOD (mU/mg)	142.1^{a}	108.4^{a}	115.2^{a}	132.1^{ab}	164.3^{ac}	214.4^{bc}	202.7^{c}	12.1
8-OHdG (pg/ml)	12.9^{ab}	11.2^{a}	15.9^{b}	13.2^{ab}	12.3^{ab}	11.9^{ab}	11.9^{ab}	0.9
Carb. (nmol/mg)	4.99	4.66	4.82	4.55	4.79	5.31	5.25	0.27
Liver								
ATP (nmol/mg)	4.45^{a}	4.13^{ab}	-	2.36^{b}	2.73^{ab}	2.89^{ab}	3.42^{ab}	0.58
Carb. (nmol/mg)	1.28^{a}	1.09^{ab}	-	1.04^{ab}	0.97^{b}	0.91^{b}	0.94^{b}	0.07
GPx (mU/mg)	166.2^{a}	212.3^{bc}	-	178.3^{b}	198.2^{bc}	194.2^{abc}	196.1^{c}	8.8
GPx m. (mU/mg)	117.1	123.1	-	120.9	130.1	129.6	120.2	6.4
SOD (mU/mg)	32.2	35.1	-	36.2	37.8	36.2	33.6	2.1
SOD m. (mU/mg)	10.5^{ab}	10.3^{ab}	-	11.2^{b}	11.2^{b}	10.5^{ab}	9.5^{a}	0.4
Int. mucosa								
GPx (mU/mg)	26.1^{a}	30.9^{a}	-	32.4^{a}	28.3^{a}	29.1^{a}	45.9^{b}	2.7
GPx m. (mU/mg)	35.4^{ab}	33.2^{b}	-	31.9^{b}	36.4^{b}	38.1^{b}	43.8^{b}	2.3
SOD (mU/mg)	9.3^{a}	9.9^{ab}	-	11.8^{c}	11.1^{bc}	10.7^{abc}	9.9^{ab}	0.4
SOD m. (mU/mg)	5.2^{a}	5.3^{a}	-	3.8^{b}	4.2^{ab}	4.2^{ab}	3.9^{b}	0.3
Kidney								
GPx (mU/mg)	90.1^{a}	121.3^{b}	-	127.8^{b}	125.9^{b}	118.2^{b}	130.5^{b}	5.6
GPx m. (mU/mg)	71.6^{a}	87.4^{b}	-	86.6^{b}	87.5^{b}	84.1^{ab}	92.9^{b}	3.6
SOD (mU/mg)	37.8^{a}	44.5^{ab}	-	43.8^{ab}	47.6^{b}	43.3^{ab}	48.3^{b}	1.8
SOD m. (mU/mg)	11.2^{a}	13.1^{b}	-	14.2^{b}	14.5^{b}	14.6^{ab}	15.4^{b}	0.7

^{abc} LS- means with different superscript differ significantly at $P < 0.05$.

Carb., protein carbonyls; GPx m., GPx mitochondria; SOD m., SOD mitochondria.

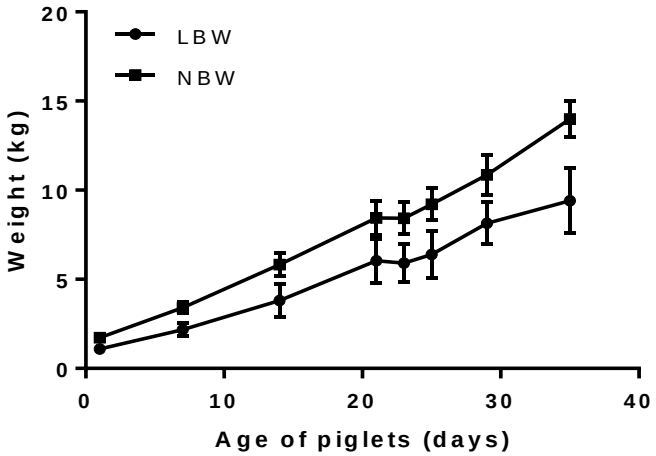

Figure 1 Evolution of the bodyweights of low birthweight piglets (LBW) and normal birthweight piglets (NBW) throughout the lactation and the post-weaning period. Each points in the graph represents means ± standard deviation (SD). The number of LBW and NBW piglets is 60 for d 1, 7 and 14 and then it decreased by 10 piglets per day from d 21 to d 35 (10 piglets per group were slaughtered at each time point starting from d 14).

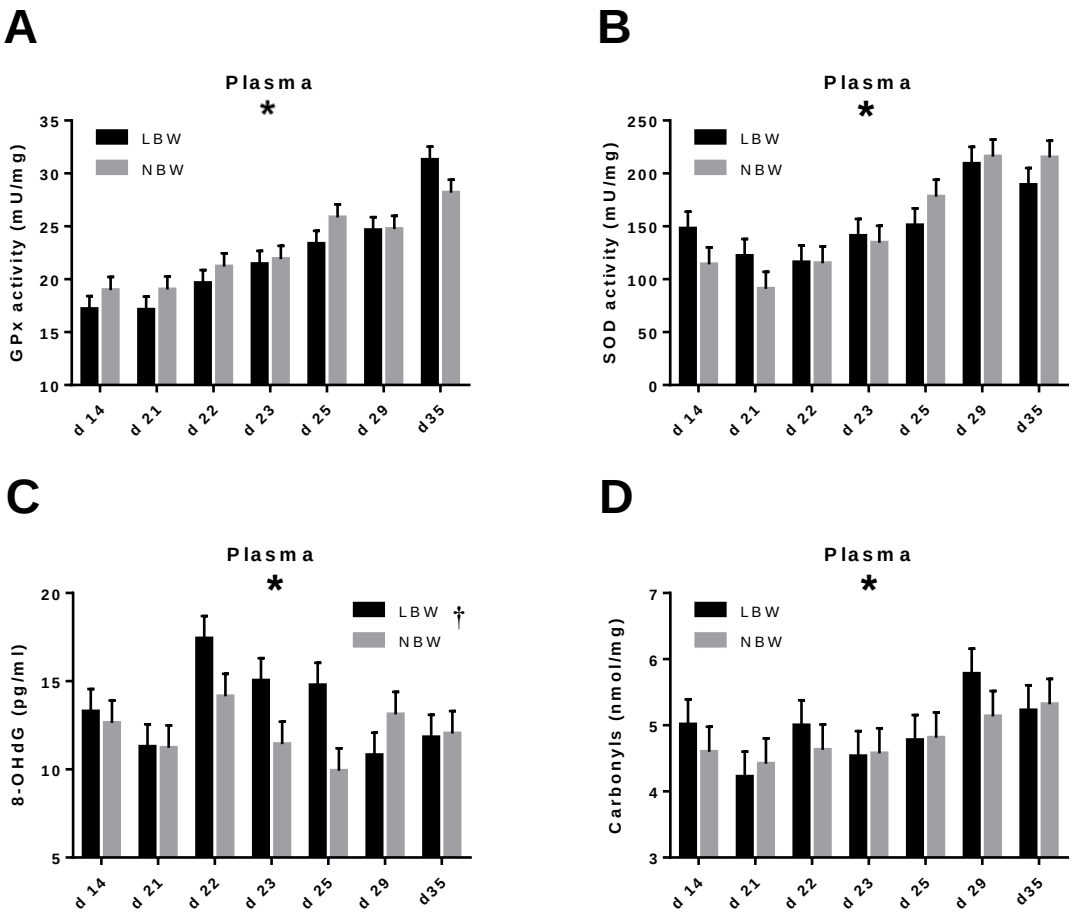

Figure 2 Systemic oxidative stress in LBW and NBW piglets of different ages throughout the peri-weaning period. Specific enzymatic activities of glutathione peroxidase (A) and superoxide dismutase (B) have been measured in plasma samples from the two experimental groups of piglets from different ages. Biomarkers of oxidative damage to DNA (8-hydroxy-2'-deoxyguanosine) (C) and proteins (protein carbonyls) (D) were quantified in plasma samples from the same piglets. Bars in the graphs represent least-square means ± standard error of the estimates of 10 piglets. Asterisk (*) indicates a significant effect of the age on means at $P \leq 0.05$. Dagger (†) indicates a significant effect of the birthweight on means at $P \leq 0.05$. In all cases the interaction between age and birthweight is not significant $P > 0.05$ (Supplementary Table S1 for detailed *P*-values).

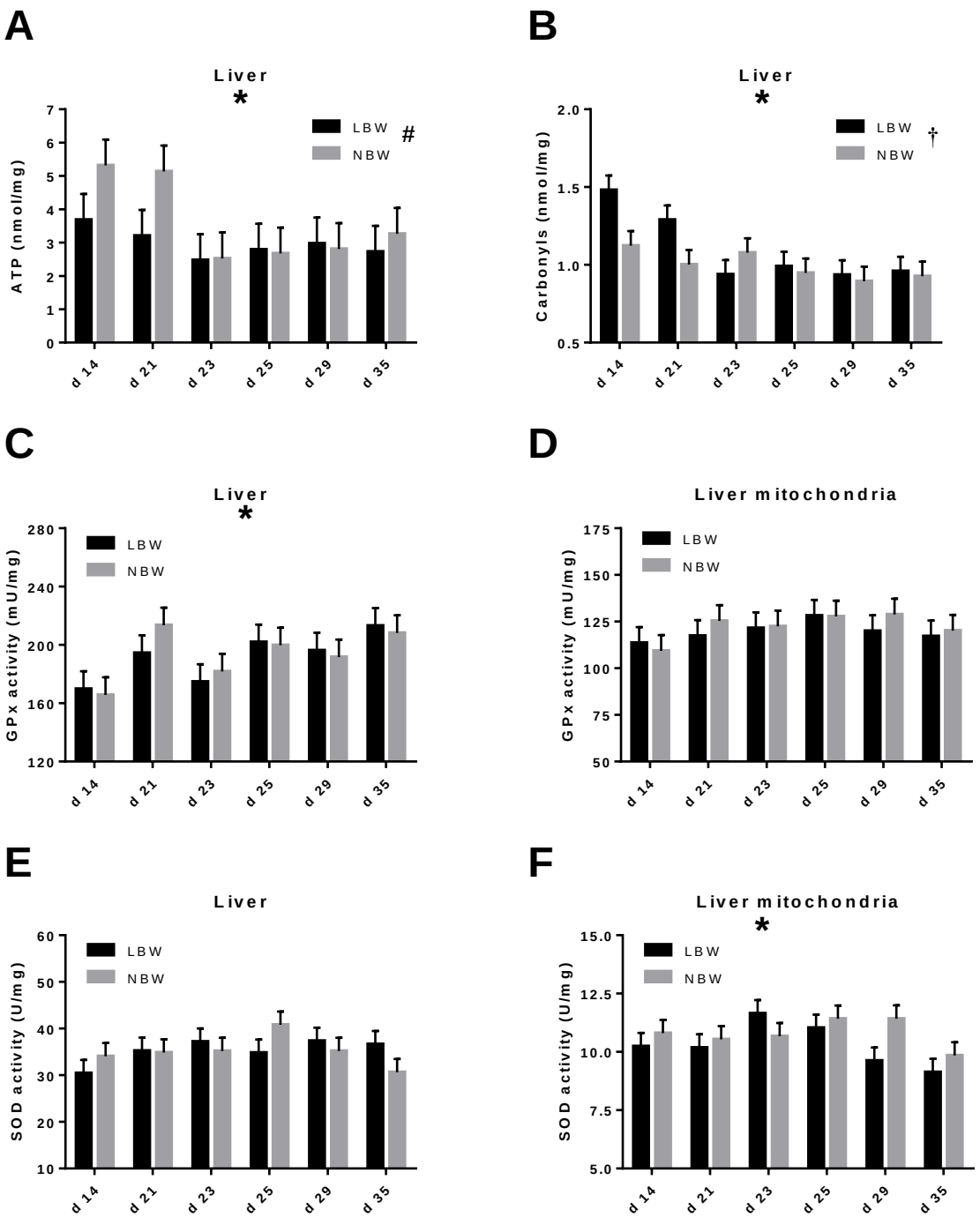

Figure 3 Mitochondrial function and oxidative stress conditions in liver of LBW and NBW piglets of different ages throughout the peri-weaning period. Adenosine-5'-Triphosphate (ATP) concentrations were measured in total liver samples from LBW and NBW piglets (A). Biomarkers of oxidative damage to proteins (protein carbonyls)

(B) were quantified in total liver samples from the same piglets. Specific enzymatic activity of glutathione peroxidase (GPx) was measured in both cellular (C) and mitochondrial (D) extracts from liver of LBW and NBW piglets. Specific enzymatic activity of superoxide dismutase (SOD) was measured in both cellular (E) and mitochondrial (F) extracts from the two groups of piglets. Bars in the graphs represent least-square means ± standard error of the estimates of 10 piglets. Asterisk (*) indicates a significant effect of the age on means at $P \leq 0.05$. Dagger (†) indicates a significant effect of the birthweight on means at $P \leq 0.05$. Pound (#) indicates that means tended to be affected by birthweight at $P < 0.01$. In all cases the interaction between age and birthweight is not significant $P > 0.05$ (Supplementary Table S1 for detailed *P*-values).

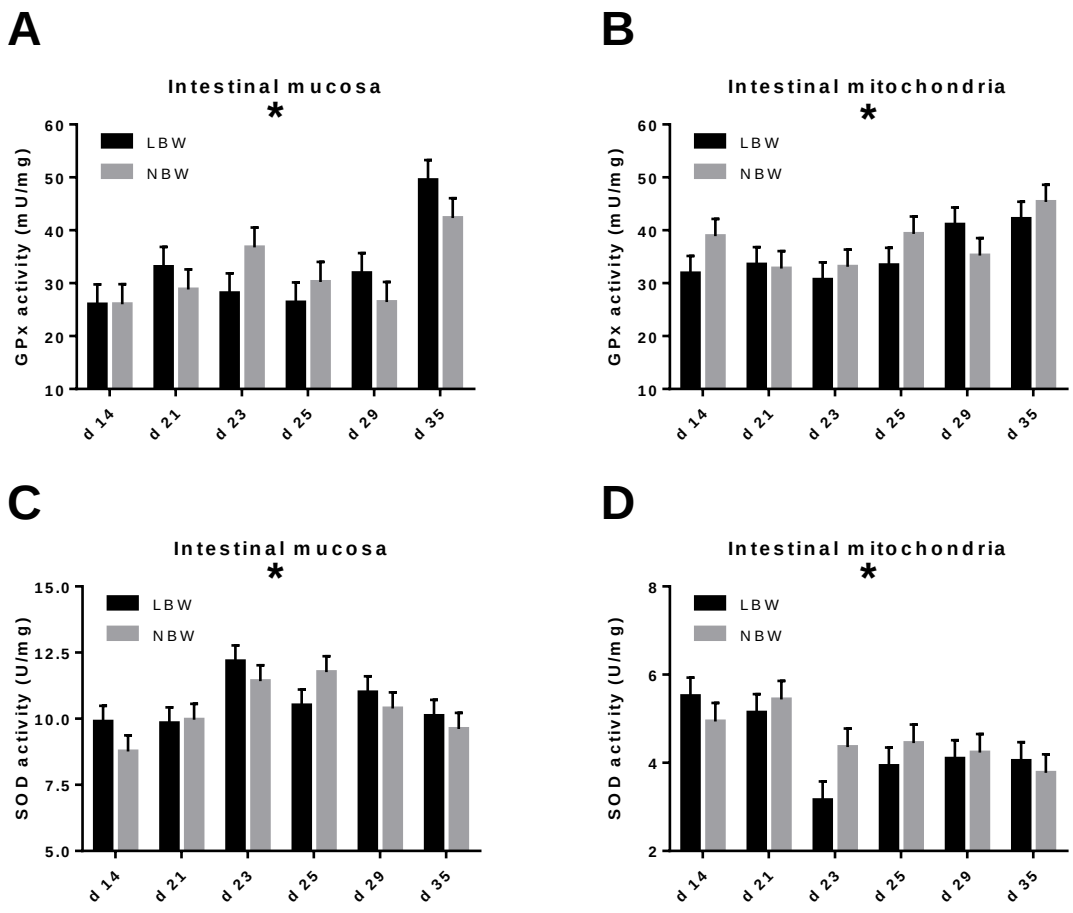

Figure 4 Major antioxidant activity in intestinal mucosa of LBW and NBW piglets of different ages throughout the peri-weaning period. Specific enzymatic activity of glutathione peroxidase (GPx) was measured in both cellular (A) and mitochondrial (B) extracts from mucosa of the jejunum of LBW and NBW piglets. Specific enzymatic activity of superoxide dismutase (SOD) was measured in both cellular (C) and mitochondrial (D) extracts from mucosa of the jejunum of the two groups of piglets. Bars in the graphs represent least-square means ± standard error of the estimates of 10 piglets. Asterisk (*) indicates a significant effect of the age on means at $P \leq 0.05$. In all cases the interaction between age and birthweight is not significant $P > 0.05$ (Supplementary Table S1 for detailed *P*-values).

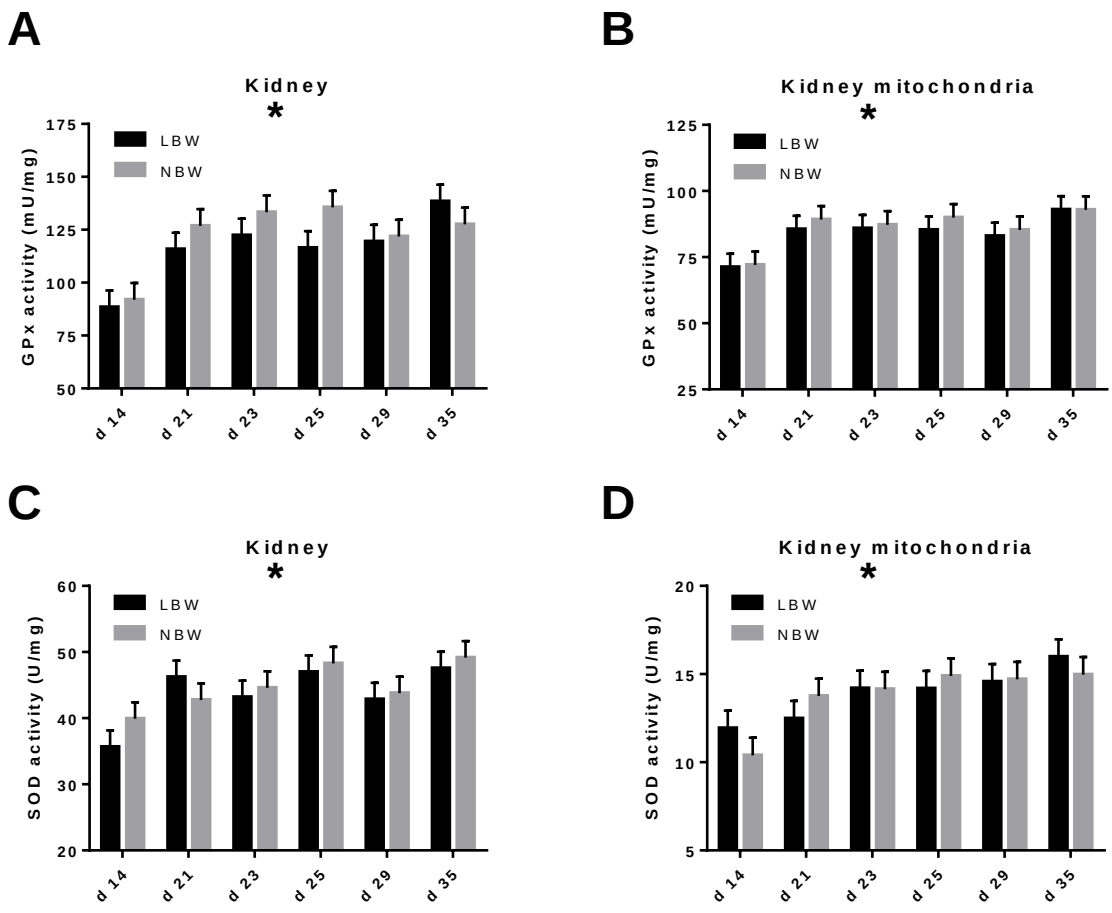

Figure 5 Major antioxidant activity in kidney of LBW and NBW piglets of different ages throughout the peri-weaning period. Specific enzymatic activity of glutathione peroxidase (GPx) was measured in both cellular (A) and mitochondrial (B) extracts from kidney of LBW and NBW piglets. Specific enzymatic activity of superoxide dismutase (SOD) was measured in both cellular (C) and mitochondrial (D) extracts from kidney of the two groups of piglets. Bars in the graphs represent least-square means ± standard error of the estimates of 10 piglets. Asterisk (*) indicates a significant effect of the age on means at $P \leq 0.05$. In all cases the interaction between age and birthweight is not significant $P > 0.05$ (Supplementary Table S1 for detailed *P*-values).

Weaning differentially affects mitochondrial function, oxidative stress, inflammation and apoptosis in normal and low birth weight piglets

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Abstract

Weaning is associated with dysfunctional immune responses and inflammatory conditions which contribute to increase the occurrence of infections and diseases in swine production. Mounting evidence indicates that mitochondria, the main cellular sources of energy, are closely related to immunity, inflammation and antioxidant defenses. Recent findings indicate that weaning induces mitochondrial dysfunction and cellular oxidative stress conditions which last for at least 2 weeks and more severely impact smaller piglets. However, the molecular mechanisms underlying these physiological consequences of weaning and the relation with systemic inflammatory status are unknown. The objective of this study was to further characterize these mechanisms in both normal and low birth weight (NBW and LBW) piglets throughout the peri-weaning period. To conduct the study, 30 sows were inseminated, and specific piglets from their litters were assigned to one of two experimental groups: NBW (n = 60, 1.73 ± 0.01 kg,) and LBW piglets weighing less than 1.2 kg (n = 60, 1.01 ± 0.01 kg). Then, 10 piglets from each group were selected at 14, 21 (weaning), 23, 25, 29 and 35 days of age to collect organs (liver, intestinal mucosa and kidney) and plasma samples. Specific porcine RT2 Profiler™ PCR Arrays related to mitochondrial function, oxidative stress, inflammation and apoptosis processes were first used to target genes that are modulated following weaning in NBW piglets (d 23 and d 35 vs. d 14). Expression of selected genes was evaluated by quantitative PCR. These analyses revealed that mRNA expression of inflammatory molecules (*IL-8* and *CCL19*) increased after weaning in intestinal mucosa, while expression of genes encoding subunits of the mitochondrial respiratory chain (*NDUFA2* and *NDUFA5*) was downregulated in the liver of both groups ($P < 0.05$). Interestingly, major modulators of mitochondrial apoptosis

(*BCL2A1* and *BNIP3*) and antioxidant defense system (*TXNRD2*, *GPx3*, *HMOX1*) were found to be highly expressed in NBW than LBW piglets ($P < 0.05$). Furthermore, the systemic levels of inflammatory molecules (*TNF-α* and *IL1-β*) significantly increased in the days following weaning and are higher in NBW piglets ($P < 0.05$). These results provide novel information about the molecular origin of the mitochondrial dysfunction and oxidative stress observed in weaned piglets and suggest that apoptosis, antioxidant defenses and inflammatory response are compromised in LBW piglets.

Introduction

The separation of piglets from sows at weaning is known to induce many simultaneous stressors resulting in low feed intake, intestinal disturbances, inflammation and increased occurrence of diseases [1]. The post-weaning period is characterized by higher morbidity and mortality rates that causes substantial economic losses for producers. This is particularly true for low birthweight (LBW) piglets, which have become common in modern swine production with the advent of genetic selection for sows producing large litters [2, 3]. During the post-weaning period, these LBW piglets are recognized to have a lower feed intake than their normal birthweight (NBW) counterparts and to suffer from an incomplete development of both digestive and immune systems [4, 5]. These conditions result in an increase vulnerability of LBW piglets to bacterial infections and various pathologies known to be associated with the post-weaning period. This situation is highly problematic for swine producers which have to conform their practices with new and upcoming regulations regarding the utilisation of antibiotics and zinc oxide

(ZnO) [6, 7]. As a result, alternatives to these products commonly used to promote health and growth of weaned piglets needed to be developed.

It was convincingly demonstrated by several studies that oxidative stress conditions occur in weaned piglets whatever the weaning age [8-10]. Oxidative stress is normally defined as any imbalance between the generation of reactive oxygen species (ROS) and their neutralization by antioxidants. Several evidences indicate that resistance to diseases and integrity of intestinal barrier function could all be negatively affected by oxidative stress in mammals [11, 12]. In all cells, mitochondria play a crucial role in producing energy production but are also the main sources of ROS as by-products of cellular respiration. Recent studies have clearly revealed that mitochondria constitute essential hubs controlling innate immune response [13], programmed cell death or apoptosis [14], inflammatory processes [15] and bacterial pathogenesis [16]. The links between mitochondrial energy metabolism, oxidative stress and development of adverse physiological outcomes might constitute important issues in swine production for animals facing high energetic demands [17]. Studies on the specific relation between mitochondrial function, oxidative stress and weaning stress are greatly limited but it was reported that the activities of mitochondrial respiratory complexes decrease in intestinal and hepatic tissues during the first week following weaning [9, 18]. Interestingly, we have recently demonstrated that mitochondrial dysfunction and cellular oxidative stress conditions are rapidly induced after weaning in piglets of 21 days of age and lasted for at least two weeks [19]. Results from this study also revealed that LBW piglets are affected by more severe mitochondrial energetic deficiencies and sustain higher oxidative damage than NBW piglets throughout the peri-weaning period.

Taken together, these results support the implication of mitochondrial dysfunction and oxidative stress conditions in the establishment of post-weaning stress, especially in LBW piglets. However, a more detailed analysis of the molecular mechanisms underlying these detrimental effects of weaning on mitochondria is needed in the optic of developing new molecular approaches to increase the robustness of weaning piglets. Mitochondria are at the interface between energetic substrate provided by the diet and cellular functions and an increasing number of evidence suggest that mitochondrial function can be modulated by nutrigenomics approaches [17]. In the same vein, the molecular consequences of weaning on the many physiological processes relying on mitochondrial function such as inflammation and programmed cell death also need to be better characterized throughout the peri-weaning period. Therefore, the objective of the present study is to provide for the first time a detailed molecular characterization of markers of mitochondrial energy metabolism, oxidative stress, inflammation and apoptosis in different tissues of both LBW and NBW piglets throughout a determined time period lasting from mid-lactation (day 14) until 2 weeks after weaning (day 35). This could contribute to establish the foundations for the development of novel targeted interventions aiming to improve health and resistance of weaned piglets.

Materials and methods

Animals

All animals involved in this research project have been treated in accordance with the code of good practice in effect (Agriculture and Agri-Food Canada, 1993) and all procedures involving these animals were approved by the Institutional Animal Care Committee of the Sherbrooke Research and Development Centre (CIPA 488).

For this study, 30 conventional multiparous Yorkshire x Landrace sows were given the same feed allowances and diet formulations during gestation and lactation as previously described (Matte et al., 2017) and had free access to water. Litters were standardized to 12 piglets within 48 h of birth. The piglets were all weighed on day 1 and those presenting the required characteristics were assigned to one of two experimental groups: low birth weight piglets (LBW, n = 60) and normal birth weight piglets (NBW, n = 60). The selection criteria for NBW piglets was having a weight between the mean weight of the 360 produced piglets on d 1 and a standard deviation value (1.73 ± 0.01 kg) while the LBW piglets were selected among those presenting a body weight of less than 1.2 kg on d 1 (1.01 ± 0.01 kg). The NBW and LBW piglets have been respectively selected from 25 and 26 out of the 30 litters. Both males and females were selected but they were carefully distributed within the two experimental groups (31 females and 29 males for LBW; 29 females and 31 males for NBW) as well as between the different sampling time points in order to avoid any confounding effect. Piglets had no access to feed and water during the lactation period and were weaned on d 21. All the selected piglets were also weighted during lactation on days 1, 7, 14 and 21 (weaning day) as well as at 23, 25, 29 and 35 days of age. After weaning, previously identified LBW and NBW piglets were kept with their original littermates and raised in pens (one litter/pen) until weaning. Weaned piglets were fed ad libitum commercial weaning diets (3 phases feeding program) without antibiotics for the two weeks of the experimental period and had free access to drinking water. The analytical composition of these diets is described in Table S1. Within each group (LBW and NBW), 10 representative piglets were selected and sacrificed on day 14, 21 (weaning), 23, 25, 29 and 35 days of age. Samples from the left lateral lobe of the liver and the left kidney were collected along

with intestinal mucosal scrapings from the jejunum section. These samples were rinsed in phosphate-buffered saline (PBS), snap frozen in liquid nitrogen and stored at -80 °C for future analyses. Blood samples were collected from 10 LBW and 10 NBW piglets on the corresponding day of sacrifice (days 14, 21, 23, 25, 29 and 35) as well as on day 22 for the piglets sacrificed on day 29. Blood samples were collected in EDTA tubes (Becton Dickinson and Company, Rutherford, NJ, USA) and were centrifuged within 20 min at 4°C for 12 min at 1800 xg. Plasma was immediately recovered and samples were frozen at -80°C until they were assayed.

RNA extraction and cDNA synthesis

Total RNA was extracted from 50 mg of liver, intestinal mucosa and kidney samples using RNeasy spin columns (Qiagen, Mississauga, ON, Canada). The extracted RNA was dissolved in water and quantified by spectrophotometry using a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA). An RNA aliquot was taken to verify its integrity using the Bioanalyzer RNA 6000 Nano assay (Agilent Technologies, Mississauga, ON, Canada). RNA integrity number (RIN) index was used as a numerical assessment of the integrity of RNA and only samples with a RIN greater than 9 were kept for further analyses. For each sample, 1 µg of total RNA was treated with DNase I (Life Technologies) to remove contaminating genomic DNA and first-strand cDNA was synthesized from 1 µg of total RNA using either the RT^2 First Strand Kit (Qiagen) for array analyses or SuperScript™ IV First-Strand Synthesis System (Life Technologies) for quantitative real-time PCR amplifications of specific genes.

RT2 Profiler™ qPCR Array

Specific RT2 profiler PCR Array (Qiagen, Mississauga, ON, Canada) were used to assess the expression of porcine genes involved in mitochondrial energy metabolism (PASS-008Z-24), oxidative stress (PASS-065Z-24), apoptosis (PASS-012Z-24) and cytokines and chemokines (PASS-150ZC-24) in intestinal mucosa samples. For liver, only mitochondrial energy metabolism and oxidative stress arrays were performed. In order to profile the expression of genes involved in these processes throughout the peri-weaning period, these specific arrays were conducted on previously synthetized cDNA from intestinal and liver samples from NBW piglets of 14, 23 and 35 days of age following instructions provided by the manufacturer. Using SYBR Green ROX qPCR Mastermix (Qiagen), the samples were run on an Applied Biosystems™ 7500 Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA). Each array screened for 84 pathway-focussed porcine genes and five housekeeping genes and the data obtained were analyzed with the online Qiagen analysis software (RT2 profiler PCR array data analysis V3.5). Expression of genes that required more than 35 cycles to reach the threshold was determined as below the detection limit and discarded. Relative gene expression for days 23 and 35 were expressed as fold changes on a log2 scale compared to the expression on day 14 using the $2^{-\Delta\Delta Ct}$ method. Statistically significant differences between piglets from different ages were calculated using a student-t-test on the log-transformed values of the expression normalized for the selected reference genes. A log2 fold change of 1 represents a two fold increase in gene expression, whereas a log2 fold change of -1 represents a two fold reduction in expression.

Quantitative RT-PCR

The relative mRNA abundance of targeted genes in liver, intestinal mucosa and kidneys was determined using real time PCR amplifications on an Applied Biosystems™ 7500 Fast Real-Time PCR System (Applied Biosystems). Primers were designed using the Primer Express software 3.0 (PE Applied Biosystems, Foster City, CA, USA) according to the National Center for Biotechnology Information (NCBI) database. Primer sequences for studied and reference genes are shown in Table 2. Quantitative real-time PCR were performed in a 10-µL reaction volume using Power SYBR-Green Master Mix (PE Applied BioSystems) with the following cycling conditions: 10 min at 95ºC followed by 40 cycles at 95ºC for 15 s and at 60ºC for 45 s. Specificity of amplified fragments was verified with the melting curve analysis (Dissociation Curves v1.0; PE Applied Biosystems). Amplifications were performed in triplicate. The cycle threshold (Ct), the number of cycles required for the fluorescent signal to cross the threshold, was determined for all studied and reference genes. The relative expression of each gene was then calculated using $2^{-\Delta Ct}$, where ΔCt is the difference between the Ct of the genes of interest and the reference genes which were the least affected by treatment according to the NormFinder statistical algorithm (Andersen et al., 2004). These reference genes are RPL4 (Ribosomal Protein L4) and RPL13 (Ribosomal Protein L13A) for intestinal mucosa samples and liver samples as well as B2M (beta-2-Microglobulin) and GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase) for kidney samples.

Cytokines assays

Quantification of pro-inflammatory cytokines as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were assayed in duplicate in plasma samples using commercial

porcine ELISA kits according to the protocols provided by the manufacturer (R&D Systems, Minneapolis MN, USA). Briefly, standard curves were made using seven serial dilutions of the recombinant porcine cytokine for TNF-α were 2000 pg/ml and 4000 pg/ml for IL-1β for the first point, respectively. The readings were measured by a spectrophotometer at an OD of 450 nm and the correction made at 540 nm. The intra-assay coefficients of variation for TNF-α and IL-1β were 3.1% and 3.8% respectively, and the inter-assay coefficients of variation for TNF-α and IL-1β were 3.38% and 4.6%, respectively. The results were expressed in picograms per millilitre based on a standard curve.

Statistical Analysis

Data were analysed using the MIXED procedure of SAS (SAS release 9.2, 2002, SAS Institute, Cary, NC, USA). Analysis of variance was performed according to a completely randomized design to test the effects of the two studied factors (birthweight, age of piglets) and their interaction on genes expression and cytokines concentrations. Piglets were used as experimental units. The usual model for a complete randomized design used in this experiment is: $Y_{ijk} = \mu + t_I + \gamma_j + (t\gamma)_{ij} + e_{ijk}$, where Y_{ijk} is the observation for animal k in birthweight group i at age j, μ is the overall mean, ti is the fixed effect of birthweight (i = LBW of NBW), γj is the fixed effect of age j of piglet, tγij is the interaction between birthweight and age and eijk is the residual error. Effects of the statistical model were considered significant at $P \leq 0.05$, and the tendency (trend) at $0.05 < P \leq 0.10$.

Results

Expression of mitochondrial energy metabolism and oxidative stress-associated genes in liver of pre- and post-weaned NBW piglets

We have selected the NBW piglets to first determine if some genes related to mitochondrial energy metabolism and oxidative stress in liver that are differentially expressed between the pre and post-weaning period. The mean body weight of these piglets was 1.73 ± 0.01 kg at d 1 and they were 41.6% heavier than the LBW piglets. The weight gains for both groups were modest during the first days of the post-weaning period and the weight difference between them remains until d 35 (S1 Fig.). The decision to target the NBW piglets and to specifically use the mitochondrial energy metabolism and oxidative stress arrays is based on previous results showing that energy production and antioxidant response are compromises in liver of both LBW and NBW piglets. Thus, by performing these PCR arrays, we found that the number of differently expressed genes, compared to d 14, is greater two weeks after weaning at d 35 days than at d 23 (Tables 1 and 2). Almost all the differentially expressed genes in liver were found to be down-regulated. Among the ones related to the mitochondrial energy metabolism, the majority were genes encoding subunits of the mitochondrial respiratory chain such as the NADH dehydrogenase and succinate dehydrogenase complexes. Results from the oxidative stress arrays indicate that only a few genes encoding proteins implicated in the antioxidant response are differentially expressed between pre- and post-weaning periods. These ones are mainly related to the glutathione and thioredoxine systems (*GPx3, TXN* and *TXNRD2*). The complete results obtained with these profiler arrays are provided in S3 and S4 tables.

Table 1. Differently expressed genes identified by RT2-profilers arrays in the liver of 23 versus 14 days old normal birth weight (NBW) piglets

Arrays/ Genes[a]	Description	Fold[b]	P- value[c]
Oxidative stress			
ALB	Albumin	-2.60	0.040
ALOX12	Arachidonate 12-lipoxygenase	-5.82	0.013
PRNP	Prion protein	-2.17	0.046
Mito. energy			
ATP5A1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1	-2.69	<0.01
ATP6V1G3	V-type proton ATPase subunit G 3-like	-3.52	<0.01
NDUFB6	NADH dehydrogenase 1 beta subcomplex 6	-2.55	0.020
NDUFA1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 1-like	-2.31	<0.01
NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa	-6.55	0.020
NDUFS2	NADH dehydrogenase (ubiquinone) (NADH-coenzyme Q reductase)	-6.47	0.044
SDHB	Succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	-3.18	0.026

[a]RT^2-profilers used were oxidative stress and mitochondrial energy metabolism (mito. energy) and each array included 84 genes.[b]Fold change ($2^{-\Delta\Delta Ct}$) is the normalized gene expression ($2^{-\Delta Ct}$) at d 23 divided the normalized gene expression ($2^{-\Delta Ct}$) at d 14. Fold-change values greater than one indicates a positive- or an up-regulation and values less than one indicate a negative or down-regulation.
[c]The p values are calculated based on a Student's t-test of the replicate ($2^{-\Delta Ct}$) values for each gene from NBW piglets of both ages.

Table 2. Differently expressed genes identified by RT^2-profilers arrays in the liver of 35 versus 14 days old normal birth weight (NBW) piglets

Arrays/ Genes[a]	Description	Fold[b]	pvalue[c]
Oxidative stress			
ALB	Albumin	-3.78	<0.01
ALOX12	Arachidonate 12-lipoxygenase	-5.02	<0.01
APOE	Apolipoprotein E	-2.26	<0.01
CCL5	Chemokine (C-C motif) ligand 5	-2.24	<0.01
FOXM1	Forkhead box M1	-3.62	0.049
GPX3	Glutathione peroxidase 3 (plasma)	-4.57	0.015
MT-III	Metallothionein-III	-5.96	<0.01
PTGR1	Prostaglandin reductase 1	3.61	0.020
TTNLOC100620261	Titin	-37.57	0.038
TXN	Thioredoxin	2.80	0.012
TXNRD2	Thioredoxin reductase 2	-2.06	<0.01
Mito. energy			
ATP6V1C2	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C2	-3.49	0.031
ATP6V1G3	V-type proton ATPase subunit G 3-like	-2.92	<0.01
DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	-2.93	0.035
HSP70.2	Heat shock protein 70.2	-6.42	0.015
LOC100154992	Mitochondrial inner membrane protein OXA1L-like	-2.84	0.035
NDUFA10	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial-like	-2.18	0.037
NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa	-88.59	0.012
NDUFS2	NADH dehydrogenase (ubiquinone) (NADH-coenzyme Q reductase)	-16.72	0.036
NDUFS3	NADH dehydrogenase (ubiquinone) (NADH-coenzyme Q reductase)	-2.28	0.019
NDUFS7	NADH dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial-like	-2.02	0.038
SDHA	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	-2.68	0.036
SDHB	Succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	-2.94	0.030

[a]RT^2-profilers used were oxidative stress and mitochondrial energy metabolism (mito. energy) and each array included 84 genes.
[b]Fold change ($2^{-\Delta\Delta Ct}$) is the normalized gene expression ($2^{-\Delta Ct}$) at d 23 divided the normalized gene expression ($2^{-\Delta Ct}$) at d 14. Fold-change values greater than one indicates a positive- or an up-regulation and values less than one indicate a negative or down-regulation.
[c]The p values are calculated based on a Student's t-test of the replicate ($2^{-\Delta Ct}$) values for each gene from NBW piglets of both ages.

Expression of mitochondrial energy metabolism, oxidative stress, inflammation and apoptosis-associated genes in intestinal mucosa of pre and post-weaned NBW piglets

As for the liver, intestinal mucosa samples from NBW piglets of 23 and 35 days of age were used to identify genes associated to mitochondrial energy metabolism and oxidative stress that are differentially expressed between the pre and post-weaning period. We first observed that the expression of only 3 genes related to mitochondrial energy metabolism, subunits of ATPase and NADH dehydrogenase, was modulated between d 14 and the post-weaning period (Tables 3 and 4). In the oxidative stress PCR array, we found that several genes encoding enzymes implicated in the antioxidant response were highly expressed at d 23 and d 35 when compared to d 14 in the lactation period. Similarly to our observations in the liver, these genes are related to the glutathione (*GPx1, GPx2* and *GST*) and thioredoxin (*TXNRD2*) systems which are known to be crucial in intestinal cellular defenses against oxidative stress. We performed the cytokines and chemokines PCR array due to the recognized inflammatory conditions known to occurred within the digestive system of weaned piglets and we observed that many genes encoding inflammatory factors such as members of CC and CXC chemokines families as well as some interleukins are up-regulated in intestinal mucosa of NBW piglets at both d 23 and d 35 in comparison to d 14 (Tables 3 and 4). The apoptosis PCR array was initially chosen because of the established link between programmed cell death and mitochondrial dysfunction which

2457 we have previously observed in both NBW and LBW piglets. Results from this
2458 specific array indicate that only 3 genes associated to apoptosis were induced at d
2459 23, including *BCL2A1* which is known for its anti-apoptotic action. More genes
2460 implicated in programmed-cell death and cell survival were found to be differentially
2461 expressed two weeks after weaning at d 35 (Tables 3 and 4). The complete results
2462 obtained with these profiler arrays are provided in S5, S6, S7 and S8 tables.

2463 **Table 3. Differently expressed genes identified by RT^2-profilers arrays in the**
2464 **intestinal mucosa of 23 versus 14 days old normal birth weight (NBW) piglets**

Arrays/ Genes[a]	Description	Fold[b]	p-value[c]
Oxidative stress			
GP91-PHOX	NADPH oxidase heavy chain subunit	5.92	<0.01
GPX1	Glutathione peroxidase 1	2.51	0.023
LOC100049683	Solute carrier family 7, member 11	2.84	0.046
LOC100739163	Glutathione S-transferase P	2.26	0.047
MPO	Myeloperoxidase	24.58	0.010
NCF1	Neutrophil cytosolic factor 1	5.04	0.017
TXNRD2	Thioredoxin reductase 2	2.31	0.031
Mito. energy			
ATP6V0D2	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d2	22.45	0.019
ATP6V1E2	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E2	8.50	0.038
NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa	11.93	0.033
Cytokines			
CCL1	CCL1	8.53	<0.01
CCL19	Chemokine (C-C motif) ligand 19	7.93	<0.01
CCL22	C-C motif chemokine 22-like	9.30	0.021
CCL8	Chemokine (C-C motif) ligand 8	5.21	<0.01
CSF3	Colony stimulating factor 3 (granulocyte)	3.35	<0.01
IFNB1	Interferon beta	14.52	<0.01
IL12A	Interleukin 12A (natural killer cell stimulatory factor 1)	9.60	<0.01
IL12B	Interleukin 12B (natural killer cell stimulatory factor 2)	33.47	0.035
IL13	Interleukin 13	4.74	0.014
IL16	Interleukin 16	2.57	<0.01
IL17A	Interleukin 17A	2.09	<0.01
IL4	Interleukin 4	2.93	0.016
IL6	Interleukin 6 (interferon, beta 2)	10.14	<0.01
LTA	Lymphotoxin alpha (TNF superfamily, member 1)	22.81	<0.01
LTB	Lymphotoxin beta (TNF superfamily, member 3)	10.71	<0.01
TGFB1	Transforming growth factor, beta 1	3.68	<0.01
TNFSF13B	Tumor necrosis factor (ligand) superfamily, member 13b	11.50	<0.01
Apoptosis			
BCL2A1	Apoptosis-associated speck-like protein containing a CARD-like	5.27	0.049
CD40	CD40 ligand	4.82	<0.01

[a]RT^2-profilers used were oxidative stress, mitochondrial energy metabolism (mito. energy), cytokines and chemokines (cytokines) and apoptosis. Each array included 84 genes.
[b]Fold change ($2^{-\Delta\Delta Ct}$) is the normalized gene expression ($2^{-\Delta Ct}$) at d 23 divided the normalized gene expression ($2^{-\Delta Ct}$) at d 14. Fold-change values greater than one indicates a positive- or an up-regulation and values less than one indicate a negative or down-regulation.

[c]The p values are calculated based on a Student's t-test of the replicate ($2^{-\Delta Ct}$) values for each gene from NBW piglets of both ages.

Table 4. Differently expressed genes identified by RT²-profilers arrays in the intestinal mucosa of 35 versus 14 days old normal birth weight (NBW) piglets

Arrays/ Genes[a]	Description	Fold[b]	p-value[c]
Oxidative stress			
AOX1	Aldehyde oxidase-like	-2.17	0.017
CCL5	Chemokine (C-C motif) ligand 5	2.10	<0.01
FTH1	Ferritin, heavy polypeptide 1	-2.48	<0.01
GPX2	Glutathione peroxidase 2 (gastrointestinal)	4.82	<0.01
GPX4	Glutathione peroxidase 4 (phospholipid hydroperoxidase)	-2.19	<0.01
HMOX1	Heme oxygenase (decycling) 1	-5.31	0.036
SFTPD	Surfactant protein D	2.57	0.023
SIRT2	Sirtuin 2	-2.19	<0.01
UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	-2.62	0.046
Mito. energy			
ATP4B	ATPase, H+/K+ exchanging, beta polypeptide	9.00	<0.01
EDN1	Endothelin 1	-2.21	0.025
NDUFB4	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 4-like	-2.27	<0.01
Cytokines			
BMP3	Bone morphogenetic protein 3	-2.58	<0.01
C5	Complement component 5	2.42	<0.01
CCL19	Chemokine (C-C motif) ligand 19	2.38	0.015
CCL21	Chemokine (C-C motif) ligand 21	-2.98	0.028
CCL28	Chemokine (C-C motif) ligand 28	3.37	0.027
CXCL10	Chemokine (C-X-C motif) ligand 10	6.36	<0.01
CXCL9	Chemokine (C-X-C motif) ligand 9	8.18	<0.01
IFN-ALPHA-4	Interferon-alpha-4	-2.69	<0.01
IFN-ALPHA-5	Interferon, alpha 5	-2.06	<0.01
IL12B	Interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)	10.41	<0.01
IL17A	Interleukin 17A	4.68	<0.01
IL17F	Interleukin 17F	5.66	0.028
IL18	Interleukin 18 (interferon-gamma-inducing factor)	2.10	<0.01
IL2	Interleukin 2	18.86	0.021
IL21	Interleukin 21	264.39	<0.01
IL22	Interleukin 22	10.10	0.022
INHBA	Inhibin, beta A	-2.98	<0.01
CNTF	Ciliary neurotrophic factor-like	-2.27	<0.01
TNFSF13B	Tumor necrosis factor (ligand) superfamily, member 13b	29.53	<0.01
Apoptosis			
APAF1	Apoptotic peptidase activating factor 1	-2.58	0.044
BCL2A1	Apoptosis-associated speck-like protein containing a CARD-like	2.94	<0.01
LOC100154044	BCL2-like 2	-2.83	0.029
CASP1	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	2.09	<0.01
CASP10	Caspase 10, apoptosis-related cysteine peptidase	-2.06	0.012
CIDEB	Cell-death-inducing DNA-fragmentation-factor-like effector B	-2.63	<0.01
IGF1R	Insulin-like growth factor 1 receptor	-4.88	<0.01
LOC100522011	Apoptosis-associated speck-like protein containing a CARD-like	2.09	<0.01
LTA	Lymphotoxin alpha (TNF superfamily, member 1)	-4.11	0.042
MTL5	Metallothionein-like 5, testis-specific (tesmin)	2.24	0.032

[a]RT²-profilers used were oxidative stress, mitochondrial energy metabolism (mito. energy), cytokines and chemokines (cytokines) and apoptosis. Each array included 84 genes.

[b]Fold change ($2^{-\Delta\Delta Ct}$) is the normalized gene expression ($2^{-\Delta Ct}$) at d 23 divided the normalized gene expression ($2^{-\Delta Ct}$) at d 14. Fold-change values greater than one indicates a positive- or an up-regulation and values less than one indicate a negative or down-regulation.
[c]The p values are calculated based on a Student's t-test of the replicate ($2^{-\Delta Ct}$) values for each gene from NBW piglets of both ages.

Expression of targeted genes in liver of both LBW and NBW piglets throughout the peri-weaning period

The results obtained with the PCR arrays and others from previous studies allowed us to target some specific genes associated with mitochondrial energy metabolism and oxidative that are differentially expressed before and after weaning. In order to confirm the PCR array data and to characterize the molecular mechanisms associated with mitochondrial dysfunction and oxidative stress during the post-weaning period we have analyzed these specific genes throughout the peri-weaning period in liver samples of LBW and NBW piglets. Results from these analyses first indicated that genes encoding *BNIP3*, known to be implicated in degradation of defective mitochondria, the mitochondrial antioxidant thioredoxin reductase 2 (*TXNRD2*) and the antioxidant enzyme *GPx3* are highly expressed in NBW than LBW piglets (Figure 1). We also observed that the expression of heme oxigenase 1 (*HMOX1*), methionine sulfoxide reductase system A (*MSRA*) and peroxiredoxin 3 (*PRDX3*) is modulated during the peri-weaning period with the lowest expression levels being measured at d 23 and d 25 (Figure 1C and D). These 3 genes are known to be up-regulated in presence of mitochondrial oxidative stress. Finally, the mRNA expression of subunits of the NADH dehydrogenase, *NDUFA2* and *NDUFA5*, is also significantly modulated throughout the peri-weaning period. We noted that the expression of these genes decreased sharply after weaning before increasing at d 29 (Figure 1F and G).

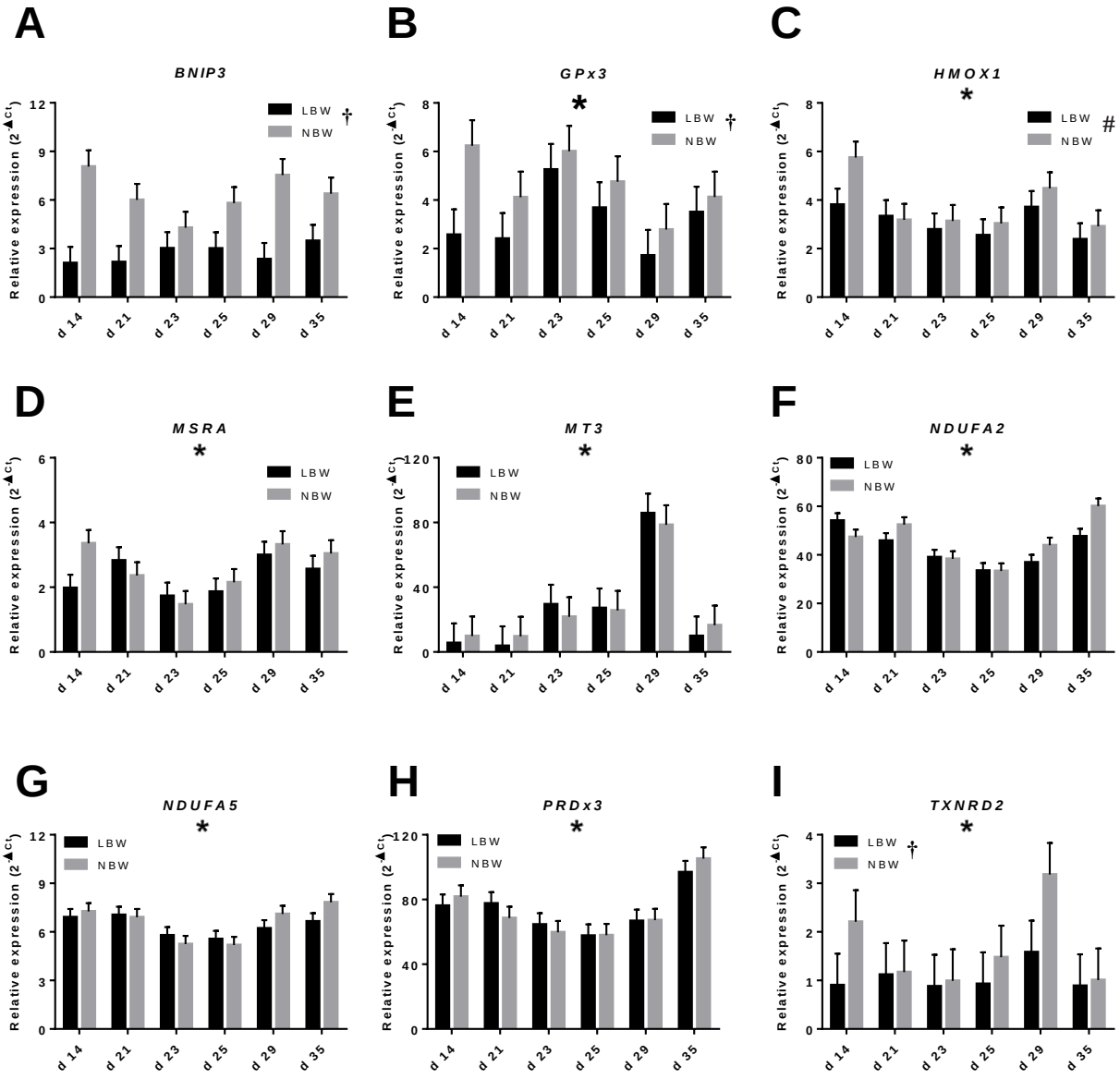

Figure 1. Relative mRNA levels of *BNIP3*, *GPx3*, *HMOX1*, *MSRA*, *MT3*, *NDUFA2*, *NDUFA5*, *PRDx3* and *TXNRD2* were analyzed in liver of LBW and NBW piglets of different ages throughout the peri-weaning period. Bars in the graphs represent least-square means ± Sy.x of 10 piglets. Asterisk (*) indicates a significant effect of the age on means at P ≤ 0.05. Dagger (†) indicates a significant effect of the birthweight on means at P ≤ 0.05. In all cases, the interaction between age and birthweight is not significant P > 0.05. LBW = low-birthweight piglets; NBW = normal-birthweight piglets; (A) *BNIP3* = BCL2 interacting protein 3; (B) *GPx3* = glutathione

peroxidase 3; (C) *HMOX1* = heme oxygenase 1; (D) *MRSA* = methionine sulfoxide reductase A, (E) *MT3* = metallothionein 3; (F) *NDUFA2* = NADH ubiquinone oxidoreductase subunit A2; (G) *NDUFA5* = NADH ubiquinone oxidoreductase subunit A5; (H) *PRDx3* = peroxideroxin 3; (I) *TXNRD2* = thioredoxin reductase 2.

Expression of targeted genes in intestinal mucosa of both LBW and NBW piglets throughout the peri-weaning period

As for the liver, the expression of the mitochondrial thioredoxin reductase (*TXNRD2*) in the intestinal mucosa of NBW piglets was found to be significantly higher than in their LBW counterparts. Similar observation was made for the *BCL2A1* gene which promotes cell survival and was also found to be highly expressed in NBW piglets (Figure 2A). The expression of the genes encoding the pro-inflammatory chemokine *CCL19* and the interleukin 8 was significantly modulated between piglets from different ages with the highest levels being measured after weaning as observed with the PCR arrays. The mRNA expression of major intestinal antioxidant *GPx2* and the metallothionein 3 which binds metals such as zinc and copper is also significantly regulated throughout the peri-weaning period. The results of the PCR arrays revealing that genes encoding subunits of the mitochondrial electron transport chain are down-regulated after weaning were confirmed by quantitative PCR for both *NDUFB2* and *NDUFB7* genes (Figure 2G and H).

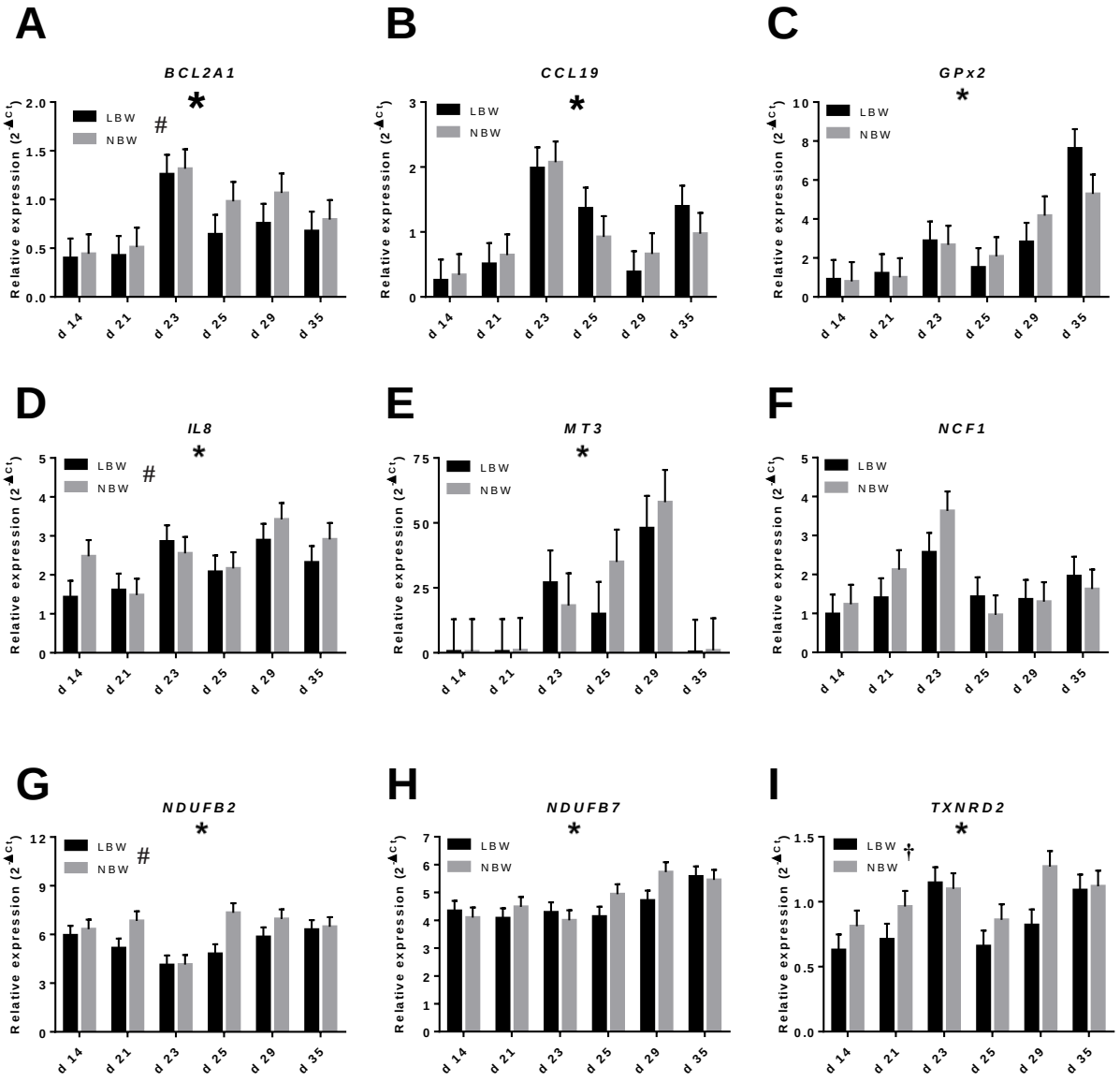

Figure 2. Relative mRNA levels of *BCL2A1*, *CCL19*, *GPx2*, *IL8*, *MT3*, *NCF1*, *NDUFB2*, *NDUFB7* and *TXNRD2* were analyzed in mucosa of the jejunum from the two experimental groups of piglets from different ages throughout the peri-weaning period. Bars in the graphs represent least-square means ± Sy.x of 10 piglets. Asterisk (*) indicates a significant effect of the age on means at P ≤ 0.05. Dagger (†) indicates a significant effect of the birthweight on means at P ≤ 0.05. In all cases, the interaction between age and birthweight is not significant P > 0.05. Pound (#) indicates that means tended to be affected by birthweight at P < 0.10. LBW = low-

birthweight piglets; NBW = normal-birthweight piglets; (A) *BCL2A1* = B-cell lymphoma 2-related protein A1; (B) *CCL19* = c-c motif chemokine ligand 19; (C) *GPx2* = glutathione peroxidase 2; (D) *IL8* = interleukin 8; (E) *MT3* = metallothionein 3; (F) *NCF1*= neutrophil cytosol factor 1; (G) *NDUFB2* = NADH ubiquinone oxidoreductase subunit B2; (H) *NDUFB7* = NADH ubiquinone oxidoreductase subunit B7; (I) *TXNRD2* = thioredoxin reductase 2.

Expression of targeted genes in kidney of both LBW and NBW piglets throughout the peri-weaning period

For the kidney, targeted genes were selected based on the array results obtained for the liver samples as well as from previous study. The quantitative PCR results indicated that the expression of the *BNIP3* genes is regulated by the ages of the piglets but, in contrast to what was observed in liver, is not influenced by the birth weight (Figure 3A). The expression of the intracellular *GPx1* was not found to be modulated during the peri-weaning period but the extracellular *GPx3* is differentially expressed throughout the same period with the highest expression levels being measured at d 23 for both groups (Figure 3C). In opposite to the liver, no difference of mRNA expression was detected for the MSRA gene but the metallothioneins 1 and 2 are highly expressed during the post-weaning period as it is also the case for *MT3* in the intestinal mucosa. Finally, as it was observed for the liver and the intestine, the expression of genes encoding subunits of the mitochondrial respiratory chain (*NDUFA2* and *NDUFA5*) is significantly affected by the age of the piglets from both groups with the lowest levels observed immediately after weaning (Figure 3G and H).

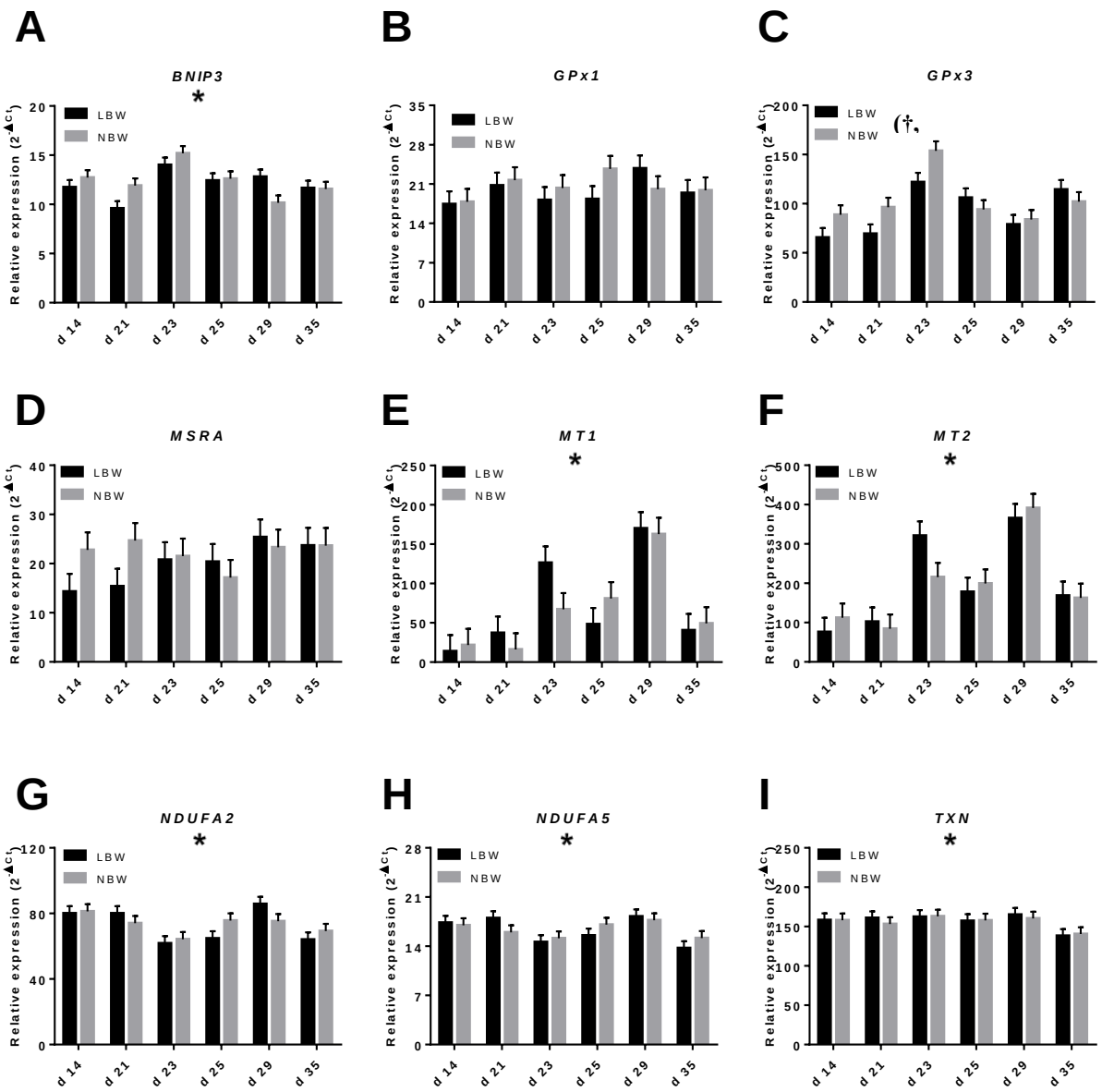

Figure 3. Relative mRNA levels of *BNIP3*, *GPx1, GPx3*, *MSRA*, *MT1*, *MT2*, *NDUFA2, NDUFA5* and *TXN* were analyzed in kidney of LBW and NBW piglets of different ages throughout the peri-weaning period. Bars in the graphs represent least-square means ± Sy.x of 10 piglets. Asterisk (*) indicates a significant effect of the age on means at P ≤ 0.05. Dagger (†) indicates a significant effect of the birthweight on means at P ≤ 0.05. Pound (#) indicates that means tended to be affected by birthweight at P < 0.10. Dagger and asterisk (†, *) indicates a significant

interaction between age and birthweight at $P < 0.01$. LBW = low-birthweight piglets; NBW = normal-birthweight piglets; (A) *BNIP3* = BCL2 interacting protein 3; (B) *GPx1* = glutathione peroxidase 1; (C) *GPx3* = glutathione peroxidase 3; (D) *MRSA*= methionine sulfoxide reductase A; (E) *MT1* = metallothionein 1A; (F) *MT2* = metallothionein 2B; (G) *NDUFA2* = NADH ubiquinone oxidoreductase subunit A2; (H) *NDUFA5* = NADH ubiquinone oxidoreductase subunit A5; (I) *TXN* = thioredoxin.

Expression of inflammatory cytokines in plasma of both LBW and NBW piglets throughout the peri-weaning period

The inflammatory status of LBW and NBW piglets during the peri-weaning period was assessed by performing ELISA analyses to measure the levels of tumor necrosis factor (TNF-α) and interleukin 1 (IL-1β) in plasma samples (Figure 4). Results from these experiments indicate that the age of the piglets have a significant impact on the plasma levels of both inflammatory cytokines. The lowest levels of TNF-α and IL-1β are detected at the end of the experimental period at d 29 and 35. We further observed that these inflammatory cytokines are highly expressed in NBW piglets when compared with their LBW counterparts.

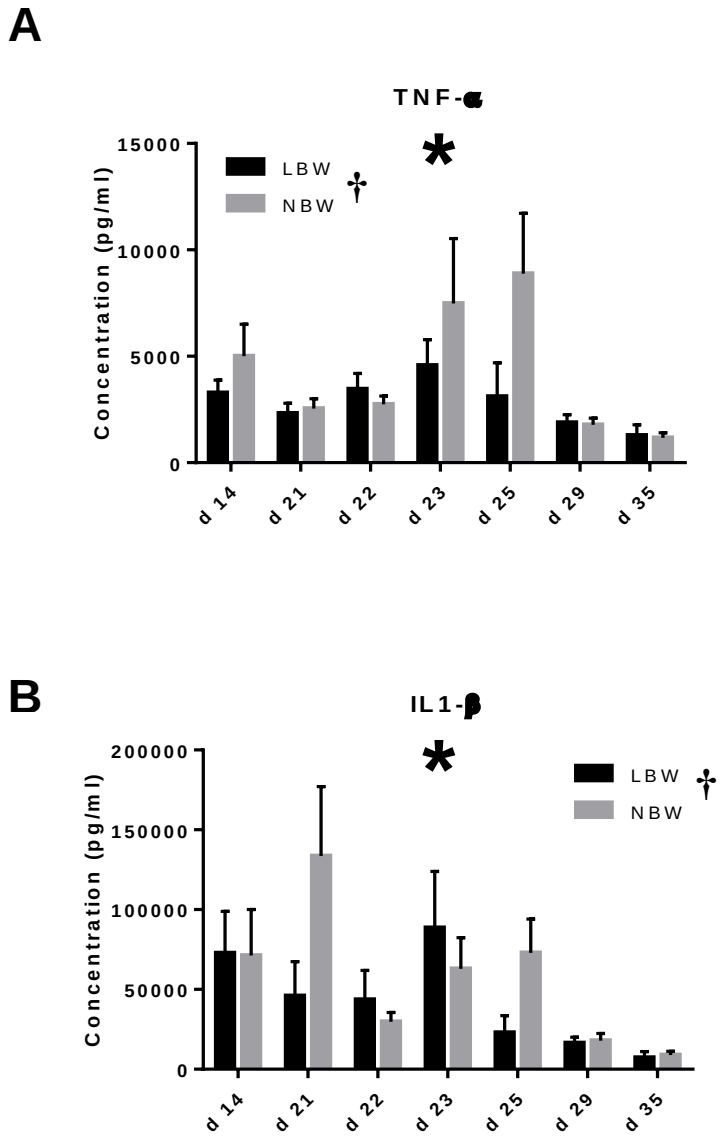

Figure 4. Expression of inflammatory cytokines in plasma of both LBW and NBW piglets throughout the peri-weaning period. Bars in the graphs represent least-square means ± Sy.x of 10 piglets. Asterisk (*) indicates a significant effect of the age on means at P ≤ 0.05. Dagger (†) indicates a significant effect of the birthweight on means at P ≤ 0.05.

Discussion

Weaning is one of the most stressful periods in the life of piglets that result in physiological, immunological and behavioral changes that often induce energy deficit and oxidative stress [20, 21]. In our recent study we demonstrated that weaning induced mitochondrial dysfunction and oxidative stress conditions in piglets aged 21 days, for at least two weeks. Interestingly, LBW piglets sustain more severe mitochondrial energetic deficiencies and oxidative stress conditions than NBW [19]. The current study allowed a detailed analysis of the expression of genes associated with mitochondrial oxidative phosphorylation, oxidative stress, inflammation and apoptosis that were performed at different times from the 14 day of lactation until the 35 day of age (two weeks after weaning) in LBW and NBW piglets.

Results of the present study for hepatic genes encoding subunits of the mitochondrial transport chain located at complex I such as *NDUFA2* and *NDFUA5* showed significantly decreased of *NDUFA2* mRNA expression in the liver of both groups after weaning at d 23 remaining down-regulated until d 29. Thereafter, was observed a significant up-regulation in expression in d 35 similar to lactation. The hepatic mRNA abundance of *NDUFA5* decreases as well after weaning d 23, then was significantly modulated at d 25. As a subsequent increase was observed at d 29, remaining up-regulated until d 35. No differences were observed of both subunits for LBW and NBW piglets. These results have implications for the process carried out by the complex I, fundamental in the regulation of mitochondrial biogenesis, requiring the interaction of multiple transcriptional factors, for a complex biological process called oxidative phosphorylation resulting in the ATP production by mitochondria. Therefore, our previous results related the low concentrations of ATP, observed in the liver of piglets in both groups in the post-weaning period. We investigated ATP

concentrations on d 23 which were significantly lower when compared during lactation on d 14 and before weaning on d 21 for both groups of piglets. This decrease in post-weaning ATP concentrations was even more evident in NBW piglets, which reduced concentrations by half the values observed during lactation. However, ATP content of liver cells remains lower in piglets of both groups even at 2 weeks post weaning [19]. Furthermore, the expression of both genes subunits remained lower post weaning as during the lactation period throughout d 29. Therefore, this decrease probably resulted from impaired mitochondrial biogenesis and low capacity for energy metabolism in complex I, which is a complex biological process [22]. It has been reported that weaning substantially causes oxidative stress in weaned piglets [23].

Oxidative stress is generally considered an imbalance between the production and elimination of ROS. An imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage [24]. Our results indicate a up-regulation in mRNA expression GPX3 rapidly after weaning, starting from d 23. These classic antioxidant enzymes, such as SOD and GSH-Px, comprise the first line of defense against ROS. Further, glutathione peroxidase 3 was first identified in the renal tubules and subsequently secreted into the plasma, however it has been identified in several tissues. Especially in conditions of inflammation, there is an increase in the relative expression of GPx3 being considered as a result of oxidative stress [25]. However, the results obtained in our previous work did not reveal variation in GPx 1 activity in mitochondria isolated from liver samples and a significant decrease in total cell extract activity was observed shortly after weaning, from day 21 to day 23 [19]. The abundance of GPX3 mRNA showed a significant difference between LBW and NBW piglets, with lower

2647 expression in LBW piglets. These results suggest to a lower antioxidant capacity of
2648 LBW compared to NBW piglets.

2649 Our findings of expression level of mitochondrial antioxidant thioredoxin
2650 reductase 2 (*TXNRD2*) was particularly lower in piglets LBW than NBW with increase
2651 the expression levels in both groups at d 29. In addition, the expression of
2652 peroxiredoxin 3 (*PRx3*) tended to decrease at d 23 and lowest expression at d 25,
2653 following by up-regulation at d 35. Peroxiredoxin 3 *(Prx 3)* is the isoform identified in
2654 mitochondria [26]. The induction of mitochondrial oxidative stress could modulated
2655 these genes with up-regulation in the presence of mitochondrial ROS. Moreover, the
2656 enzyme Trx reductase (TrxR) scavenges ROS using NADPH through the
2657 thioredoxins (*Trx*) system. Further, the antioxidant system of thioredoxins interacts
2658 with peroxiredoxins (*Prx*), which constitute a family of thiol-specific peroxidases that
2659 depend on Trx as a donor of hydrogen to reduce H_2O_2 [27]. In addition, changes at
2660 *TXNRD2* mRNA levels are related mitochondrial DNA integrity. It has been reported
2661 a lower expression of *TXNRD2* in LBW, who suggested that LBW showed
2662 mitochondrial damage to the liver [28].

2663 Expression of heme oxygenase-1 (*HO-1*) genes can improve the antioxidant
2664 capacity of cells and alleviate oxidative damage. The expression of hepatic HO-1
2665 mRNA was lowest in LBW piglets. However, there was a tendency for greater
2666 expression of this gene at d 14, with lowest expression observed at d 25, however in
2667 any case these results were not significant. Previous studies showed that weaning
2668 causes dysfunction in the hepatic antioxidant system and induces oxidative stress
2669 [21, 29]. These results showed that in piglets LBW, showed reduced antioxidant
2670 capacity in the body. Taken together, these defense system expression of *HMOX1*

and peroxiredoxin 3 *PRDX3* is modulated during the peri-weaning period with up-regulated at d 29.

Therefore, the increase in pro oxidants that cause oxidative stress contributes to mitochondrial dysfunction, including disruption of the mitochondrial respiratory chain and mutation of mitochondrial DNA (mtDNA). However, our results for the levels of *BNIP3* showed a significant highly expressed regulation in NBW compared to LBW piglets. Moreover, *BNIP3* is located in the mitochondrial membrane, can induce mitochondrial turnover. This process is vital, such as normal cell renewal induced by oxidative stress [30]. Therefore, our results suggest that LBW piglets showed less capacity to eliminate dysfunctional mitochondria. It has been also observed in rodents *BNIP3* absent led to an increase in mitochondrial mass in the liver due to a defect in mitochondria turnover by mitophagy [31].

It has been reported that weaning is one of the most stressful periods in piglets life. As a consequence, feed intake during the first days after weaning does not meet high energy demand [32]. After weaning, the piglets go through a period of fasting, that causes a decrease on energy levels [33]. The main source of cellular energy is produced by mitochondria throughout oxidative phosphorylation [34]. Furthermore, in the current study, we demonstrated the expression of genes coding of mitochondrial respiratory chain subunits, such as *NDUFB2* and *NDFUB7* were affected in LBW and NBW piglets throughout weaning period. Consistently, we showed a significantly decrease in the levels of *NDUFB2* mRNA after weaning at d 23. Moreover, the levels of *NDUFB2* exhibited tendency to lowest expression in LBW compared to NBW piglets. These results indicate that post weaning, this period of high energy demand, LBW indicate lower expression in genes of energy metabolism compared to NBW. Further, the mRNA abundance of *NDUFB7* exhibited no

differences between LBW and NBW piglets. Moreover, the mRNA abundance showed to be significant upregulated at d 29 in the piglets intestinal mucosa. The intestine has a high demand for ATP to maintain intestinal integrity and therefore the deficiency in energy is critical for intestinal barrier dysfunction [36, 37]. Both subunits are located in complex I *NADH: Ubiquinone reductase* which is involved in electron transport and proton pumping that contributes to the synthesis of ATP in complex V. Therefore, an imbalance in the electron and proton transport complexes can lead to a decrease in the production of ATP and also in the flow of electrons that can result in the excessive generation of ROS [35]. Moreover, damage to complex I can cause disturbances in energy metabolism. Recent study revealed that the activity of complexes in the mitochondrial respiratory chain decreases after weaning in jejunum samples [38]. These results showed that weaning could lead to a decline of energy production by declined oxidative phosphorylation complexes [39].

Post-weaning anorexia and growth check has been reported as many factors to induce morphological and functional changes in intestinal epithelial, and these changes lead to the induction of intestinal barrier dysfunction [40, 41]. In the current study the expression of *GPx2* exhibited gradual activation from intestinal mucosa. Further, a significant increase in expression at d 29 remained until d 35. The gradual activation in the expression of *GPx2* observed throughout may be related to the dysfunction in the intestinal epithelial barrier caused by weaning, negatively affecting intestinal epithelial cells [32]. The enzyme *GPx2* is mainly expressed in the crypt bases, which coincides with proliferation and turnover of intestinal cells regulated by the Wnt pathway [42]. This gradual activation in the levels of expression at d 29 may occur due long-lasting response to oxidative stress even 2 weeks post weaning. The antioxidant enzyme GSH-Px act to protect the body against the pro oxidants that can

cause damage [25]. In our recent study, we identified that the activity of GPx enzyme were affected by weaning in the total and mitochondrial fractions of the intestinal mucosa [19].

The expression of the mitochondrial thioredoxin reductase 2 showed up-regulation, following weaning on d 23 remained to the levels observed during the lactation period at d 25. Interestingly, the expression in LBW piglets demonstrated to be significantly lowest compared to NBW. The consequences of weaning induces to oxidative stress that cause increase of ROS [39]. *TXRND2* acts in the redox control that is essential to eliminate ROS and protect cells from damage caused by free radicals [27]. Thioredoxin and GSH comprise the main intracellular redox system in the intestinal epithelial cells [43]. This increase observed subsequent to weaning suggests an increase in ROS caused by the oxidative stress occurring shortly after weaning. Furthermore, we observed that LBW may has lowest capacity in the antioxidant response compared to NBW. *TXNRD2* levels was also found to be lower in IUGR piglets in d0 and d19, it showed that IUGR have a lower capacity to mount an antioxidant response even in the early postnatal period [44].

Such results observed for the variable birth weight, it has been demonstrated in some studies that the postnatal growth and intestinal development of LBW piglets were delayed [45, 46]. Taken together, the results from the expression of genes encoding subunits of the oxidative phosphorylation complex associated with antioxidant enzymes, indicate weaning period up-regulated genes of antioxidant system in the piglet's intestine. The effects cause by post weaning anorexia can result in the inhibition of oxidative phosphorylation complexes, resulting in the decline of ATP synthesis.

Therefore, changes in the epithelial barrier that occurred or weaning were caused by a high production of cytokines, revealing an activation of the TGI immune system after weaning [47] .Our results indicate that weaning induced inflammation by up-regulation of interleukin 8 (*IL8*) and chemokines *CCL19* chemokines observed at d 23. *IL8* expression during the entire period after weaning was up-regulated compared to d 14. Interestingly, LBW demonstrated tendency of highest expression pointed at d 23 compared to NBW. *CCL19* expression has highest levels being measured after weaning. The inflammation process is the consequence of oxidative stress, and the pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins, are all induced by oxidative stress. It has been described that the activity of intestinal genes and serum inflammatory cytokines in LBW piglets tended to increase compared to NBW piglets, indicating that LBW piglets developed a stronger inflammatory response after the LPS challenge [48]. Our results support that weaning induced inflammatory intestinal response by up-regulation of pro-inflammatory cytokines.

These modulation in the levels of pro-inflammatory cytokines were also indicated in plasma *TNF-α* increase shortly after weaning in d 23. This concentrations remained with a significant decrease in d 35. Levels of cytokine IL1b indicate a significant decrease in d 29 and d 35. Results regarding the groups, both cytokines there was increase in d 25 in NBW piglets. These results suggest that NBW piglets showed a greater response to the production of these cytokines. These results coincide with these observed by inflammation status indicate that weaning has a significant impact oh the levels of pro-inflammatory cytokines. Our results indicate a significant impact on the plasma levels of both inflammatory cytokines. Is accordance with study published associated weaning with modulation blood plasma IL-1

concentration in piglets [49]. The reduced *TNF-α* expression in piglets LBW post weaning compared to NBW may could increased disease susceptibility in this period.

However, post weaning could induce a long lasting oxidative stress and inflammation and consequent apoptosis. The main regulatory protein involved in the intrinsic cell survival pathway is *BCL2A1* (B-cell lymphoma-2 related protein A1). In the current study, a significant increase in BCL2A1 was observed shortly after weaning at d 23. Regardless the group effect, lowest expression was observed in LBW piglets. Therefore, *BCL2A1* is an anti-apoptosis gene, that has functions in the survival cells. The expression of *BCL2A1* gene plays important role in immune system protects cells from various death stimuli [50]. However, the increase in *BCL2A1* expression after weaning can be correlated to the neutralizing the pro-apoptotic members. It has been showed that relationship between BAX (pro-apoptosis) and *BCL2* members is a determining factor in cellular apoptosis and the decrease in this relationship is fundamental in suppressing apoptosis [51]. Thereby, our results support that LBW with lowest expression of *BCL2A1* could be affected the cellular survival compared to NBW.

Lastly, new laws adopted by many countries restrict or regulate the use of antibiotics and specific trace minerals with antibacterial properties such as zinc oxide in swine production [6]. In the current study, the increase observed in the expression of MT-III (metallothionein-3), *MT-1a* and *MT-2b* immediately after weaning at d 23, then remaining greater until the d 29, and a significant decrease at d 35, may be due to high Zn supplementation in the diet of the piglets supplied at d 21 to d 29. Zinc is an important cofactor of gene expression mechanisms MT expression is regulated by the amount of cytosolic free zinc ions [52]. Interessently, supplemental Zn in the diet is an important regulator in the expression of CuZn-SOD and metallothionein content

in piglets liver [53]. An increase in mRNA expression and protein of MT and also in zinc concentration in IPEC-J2 cells was observed in vitro with 2425ppm of supplementation, and this regulation can be observed within 24 h after exposure of the different Zn concentrations [54]. Expression of metallothione increased in the jejunal, liver and kidney tissues, reflecting increased tissue zinc-binding capacity. Further, metallothioneins may have an antioxidant role due to the ability to sequester oxygen and nitrogen due to their being rich in thiol groups [55]. Expression of *MT-1a*, *MT-2b* and *MT-3* were significantly higher post weaning most pronounced effects for MT-2b > MT-1a > MT-3 observed in different tissues. These results may be directly response to related to the level of Zn in the post-weaning diet.

The consequences of stress at weaning in the expression of energy metabolism and oxidative stress genes in the kidney of weaned piglets have never been studied. The results obtained from genes encoding mitochondrial respiratory chain as observed a modulate response by weaning in liver and intestine, this patterns was observed in the kidney. Both subunits of mitochondrial chain were modulated by weaning, *NDUFA2* and *NDFUA5* were modulate at d 23 after weaning. Results for *GPx3*, was regulated by weaning with significant increase at d 23 followed following by a decrease at d 29. Regardless, the expression for the groups, a lowest expression was observed in LBW piglets. These results pointed to a lower antioxidant capacity of weaned LBW piglets over the ROS neutralization capacity through the expression of antioxidants [46]. The kidney is known to express the *GPx3* enzyme of the GPx family [56]. Immediately after weaning, at d 23 an increase in BNIP3 expression was observed, with a significant decrease at d 29. This gene plays in regulation in mitochondrial function and autophagy [57]. Our findings indicated a modulation throughout the weaning period. Taken together these results, compared

as observed at in other organs, the gene expression in kidney suggest that the post-weaning induce a oxidative stress and energy deficit in both groups.

Conclusion

Taken together, results from this study provide information about molecular mechanisms underlying metabolic and oxidative stresses associated post-weaning piglets. These findings indicate weaning causes decrease in mitochondrial energy in liver, intestine and kidney after weaning associated with oxidative stress conditions. Further, these findings suggest that antioxidant defenses, inflammatory response and apoptosis, are compromised in LBW piglets. These results are important to develop new approaches to improve health of newly weaned piglets.

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Ethics statement

All animals involved in this project have been treated in accordance with the code of good practice in effect (Agriculture and Agri-Food Canada, 1993) and all procedures involving these animals were reviewed and approved by the Institutional Animal Care Committee (CIPA 488).

Reference

1. Campbell JM, Crenshaw JD, Polo J. The biological stress of early weaned piglets. Journal of Animal Science and Biotechnology. 2013;4(1). doi: 10.1186/2049-1891-4-19.
2. Collins CL, Pluske JR, Morrison RS, McDonald TN, Smits RJ, Henman DJ, et al. Post-weaning and whole-of-life performance of pigs is determined by live weight at weaning and the complexity of the diet fed after weaning. Animal Nutrition. 2017;3(4):372-9. doi: 10.1016/j.aninu.2017.01.001.
3. Damgaard LH, Rydhmer L, Lovendahl P, Grandinson K. Genetic parameters for within-litter variation in piglet birth weight and change in within-litter variation during suckling. J Anim Sci. 2003;81(3):604-10. PubMed PMID: 12661639.
4. De Vos M, Che L, Huygelen V, Willemen S, Michiels J, Van Cruchten S, et al. Nutritional interventions to prevent and rear low-birthweight piglets. J Anim Physiol Anim Nutr (Berl). 2014;98(4):609-19. doi: 10.1111/jpn.12133. PubMed PMID: 24118084.
5. Lessard M, Blais M, Beaudoin F, Deschene K, Verso LL, Bissonnette N, et al. Piglet weight gain during the first two weeks of lactation influences the immune system development. Vet Immunol Immunopathol. 2018;206:25-34. Epub 2018/12/07. doi: 10.1016/j.vetimm.2018.11.005. PubMed PMID: 30502909.
6. Barton MD. Impact of antibiotic use in the swine industry. Curr Opin Microbiol. 2014;19:9-15. Epub 2014/06/25. doi: 10.1016/j.mib.2014.05.017. PubMed PMID: 24959754.
7. Record V. Pig industry fears withdrawal of zinc oxide from this summer. Vet Rec. 2017;180(13):315. doi: 10.1136/vr.j1602. PubMed PMID: 28364061.
8. Buchet A, Belloc C, Leblanc-Maridor MM, Merlot E. Effects of age and weaning conditions on blood indicators of oxidative status in pigs. PLoS ONE. 2017;12(5). doi: 10.1371/journal.pone.0178487.
9. Cao ST, Wang CC, Wu H, Zhang QH, Jiao LF, Hu CH. Weaning disrupts intestinal antioxidant status, impairs intestinal barrier and mitochondrial function, and triggers mitophagy in piglets. J Anim Sci. 2018;96(3):1073-83. doi: 10.1093/jas/skx062.
10. 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. doi: 10.2527/jas.2011-4444.
11. Agarwal A, Gupta S, Sikka S. The role of free radicals and antioxidants in reproduction. Curr Opin Obstet Gynecol. 2006;18(3):325-32. PubMed PMID: 16735834.
12. John LJ, Fromm M, Schulzke JD. Epithelial barriers in intestinal inflammation. Antioxid Redox Signal. 2011;15(5):1255-70. doi: 10.1089/ars.2011.3892. PubMed PMID: 21294654.
13. Weinberg SE, Sena LA, Chandel NS. Mitochondria in the regulation of innate and adaptive immunity. Immunity. 2015;42(3):406-17. doi: 10.1016/j.immuni.2015.02.002. PubMed PMID: 25786173; PubMed Central PMCID: PMCPMC4365295.
14. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med. 2010;48(6):749-62. Epub 2010/01/05. doi: S0891-5849(09)00793-X [pii]

10.1016/j.freeradbiomed.2009.12.022. PubMed PMID: 20045723.
15. López-Armada MJ, Riveiro-Naveira RR, Vaamonde-García C, Valcárcel-Ares MN. Mitochondrial dysfunction and the inflammatory response. Mitochondrion. 2013;13(2):106-18. doi: 10.1016/j.mito.2013.01.003.
16. Garaude J, Acín-Pérez R, Martínez-Cano S, Enamorado M, Ugolini M, Nistal-Villán E, et al. Mitochondrial respiratory-chain adaptations in macrophages contribute to antibacterial host defense. Nat Immunol. 2016;17(9):1037-45. doi: 10.1038/ni.3509.
17. Lapointe J. Mitochondria as promising targets for nutritional interventions aiming to improve performance and longevity of sows. J Anim Physiol Anim Nutr (Berl). 2014;98(5):809-21. doi: 10.1111/jpn.12160. PubMed PMID: 24438174.
18. Luo Z, Zhu W, Guo Q, Luo W, Zhang J, Xu W, et al. Weaning Induced Hepatic Oxidative Stress, Apoptosis, and Aminotransferases through MAPK Signaling Pathways in Piglets. Oxidative Medicine and Cellular Longevity. 2016;2016. doi: 10.1155/2016/4768541.
19. Novais AK, Martel-Kennes Y, Roy C, Deschene K, Beaulieu S, Bergeron N, et al. Tissue-specific profiling reveals modulation of cellular and mitochondrial oxidative stress in normal- and low-birthweight piglets throughout the peri-weaning period. Animal. 2019:1-11. Epub 2019/11/26. doi: 10.1017/S1751731119002829. PubMed PMID: 31760964.

20. Zhu LH ZKL, Chen X L, Xu J X Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs. American Society of Animal Science 2012:2581–9 doi: 10.2527/jas2012-4444.
21. Xu J, Xu C, Chen X, Cai X, Yang S, Sheng Y, et al. Regulation of an antioxidant blend on intestinal redox status and major microbiota in early weaned piglets. Nutrition. 2014;30(5):584-9. Epub 2014/04/05. doi: 10.1016/j.nut.2013.10.018. PubMed PMID: 24698350.
22. Guerrero-Castillo S, Baertling F, Kownatzki D, Wessels HJ, Arnold S, Brandt U, et al. The Assembly Pathway of Mitochondrial Respiratory Chain Complex I. Cell Metab. 2017;25(1):128-39. Epub 2016/10/11. doi: 10.1016/j.cmet.2016.09.002. PubMed PMID: 27720676.
23. 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. Epub 2012/08/17. doi: 10.2527/jas.2012-4444. PubMed PMID: 22896732.
24. Sies H. Role of metabolic H2O2 generation: redox signaling and oxidative stress. J Biol Chem. 2014;289(13):8735-41. Epub 2014/02/12. doi: 10.1074/jbc.R113.544635. PubMed PMID: 24515117; PubMed Central PMCID: PMCPMC3979367.
25. Brigelius-Flohe R, Maiorino M. Glutathione peroxidases. Biochim Biophys Acta. 2013;1830(5):3289-303. Epub 2012/12/04. doi: 10.1016/j.bbagen.2012.11.020. PubMed PMID: 23201771.
26. Chang TS, Cho CS, Park S, Yu S, Kang SW, Rhee SG. Peroxiredoxin III, a mitochondrion-specific peroxidase, regulates apoptotic signaling by mitochondria. J Biol Chem. 2004;279(40):41975-84. Epub 2004/07/29. doi: 10.1074/jbc.M407707200. PubMed PMID: 15280382.
27. Netto LE, Antunes F. The Roles of Peroxiredoxin and Thioredoxin in Hydrogen Peroxide Sensing and in Signal Transduction. Mol Cells. 2016;39(1):65-71. Epub 2016/01/28. doi: 10.14348/molcells.2016.2349. PubMed PMID: 26813662; PubMed Central PMCID: PMCPMC4749877.
28. Feng C, Bai K, Wang A, Ge X, Zhao Y, Zhang L, et al. Effects of dimethylglycine sodium salt supplementation on growth performance, hepatic antioxidant capacity, and mitochondria-related gene expression in weanling piglets born with low birth weight1. J Anim Sci. 2018;96(9):3791-803. Epub 2018/06/23. doi: 10.1093/jas/sky233. PubMed PMID: 29931075; PubMed Central PMCID: PMCPMC6127790.
29. 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. Epub 2012/11/28. doi: 10.1017/S0007114512004321. PubMed PMID: 23176743.
30. Ney PA. Mitochondrial autophagy: Origins, significance, and role of BNIP3 and NIX. Biochim Biophys Acta. 2015;1853(10 Pt B):2775-83. Epub 2015/03/11. doi: 10.1016/j.bbamcr.2015.02.022. PubMed PMID: 25753537.
31. Glick D, Zhang W, Beaton M, Marsboom G, Gruber M, Simon MC, et al. BNip3 regulates mitochondrial function and lipid metabolism in the liver. Mol Cell Biol. 2012;32(13):2570-84. Epub 2012/05/02. doi: 10.1128/MCB.00167-12. PubMed PMID: 22547685; PubMed Central PMCID: PMCPMC3434502.
32. Pie´ S LsJ, Blazy F, Laffitte J, Se`ve B, Oswald I P Weaning Is Associated with an Upregulation of Expression of Inflammatory Cytokines in the Intestine of Piglets. Nutritional Immunology. 2004.
33. Lalles JP, David JC. Fasting and refeeding modulate the expression of stress proteins along the gastrointestinal tract of weaned pigs. J Anim Physiol Anim Nutr (Berl). 2011;95(4):478-88. Epub 2010/11/26. doi: 10.1111/j.1439-0396.2010.01075.x. PubMed PMID: 21091542.
34. A GDaT. The Mitochondrial Electron Transfer Chain Archives of Biochemistry and Biophysics. 1966;116: 293–304.
35. Barros MH, McStay GP. Modular biogenesis of mitochondrial respiratory complexes. Mitochondrion. 2020;50:94-114. Epub 2019/11/02. doi: 10.1016/j.mito.2019.10.008. PubMed PMID: 31669617.
36. Blachier F, Boutry C, Bos C, Tome D. Metabolism and functions of L-glutamate in the epithelial cells of the small and large intestines. Am J Clin Nutr. 2009;90(3):814S-21S. Epub 2009/07/03. doi: 10.3945/ajcn.2009.27462S. PubMed PMID: 19571215.
37. Van Der Schoor SR, Reeds PJ, Stoll B, Henry JF, Rosenberger JR, Burrin DG, et al. The high metabolic cost of a functional gut. Gastroenterology. 2002;123(6):1931-40. Epub 2002/11/28. doi: 10.1053/gast.2002.37062. PubMed PMID: 12454850.
38. Cao ST, Wang CC, Wu H, Zhang QH, Jiao LF, Hu CH. Weaning disrupts intestinal antioxidant status, impairs intestinal barrier and mitochondrial function, and triggers mitophagy in piglets. J Anim

Sci. 2018;96(3):1073-83. Epub 2018/04/05. doi: 10.1093/jas/skx062. PubMed PMID: 29617867; PubMed Central PMCID: PMCPMC6093500.
39. Yang H, Xiong X, Wang X, Tan B, Li T, Yin Y. Effects of Weaning on Intestinal Upper Villus Epithelial Cells of Piglets. PLoS One. 2016;11(3):e0150216. Epub 2016/03/31. doi: 10.1371/journal.pone.0150216. PubMed PMID: 27022727; PubMed Central PMCID: PMCPMC4811545.
40. Wijtten PJ, van der Meulen J, Verstegen MW. Intestinal barrier function and absorption in pigs after weaning: a review. Br J Nutr. 2011;105(7):967-81. Epub 2011/02/10. doi: 10.1017/S0007114510005660. PubMed PMID: 21303573.
41. Moeser AJ, Pohl CS, Rajput M. Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. Anim Nutr. 2017;3(4):313-21. Epub 2018/05/17. doi: 10.1016/j.aninu.2017.06.003. PubMed PMID: 29767141; PubMed Central PMCID: PMCPMC5941262.
42. Pinto D, Gregorieff A, Begthel H, Clevers H. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. Genes Dev. 2003;17(14):1709-13. Epub 2003/07/17. doi: 10.1101/gad.267103. PubMed PMID: 12865297; PubMed Central PMCID: PMCPMC196179.
43. Circu ML, Aw TY. Intestinal redox biology and oxidative stress. Semin Cell Dev Biol. 2012;23(7):729-37. Epub 2012/04/10. doi: 10.1016/j.semcdb.2012.03.014. PubMed PMID: 22484611; PubMed Central PMCID: PMCPMC3396776.
44. Wang W, Degroote J, Van Ginneken C, Van Poucke M, Vergauwen H, Dam TM, et al. Intrauterine growth restriction in neonatal piglets affects small intestinal mucosal permeability and mRNA expression of redox-sensitive genes. FASEB J. 2016;30(2):863-73. Epub 2015/10/31. doi: 10.1096/fj.15-274779. PubMed PMID: 26514167.
45. Wang J J CL, Li DF , Yin YL, Wang X Q, Li P, Dangott L J, Hu WX, Wu G. Intrauterine Growth Restriction Affects the Proteomes of the Small Intestine, Liver, and Skeletal Muscle in Newborn Pigs. The Journal of Nutrition Genomics, Proteomics, and Metabolomics. 2007:60-6.
46. Zhang H, Li Y, Wang T. Antioxidant capacity and concentration of redox-active trace mineral in fully weaned intra-uterine growth retardation piglets. J Anim Sci Biotechnol. 2015;6:48. Epub 2015/11/21. doi: 10.1186/s40104-015-0047-7. PubMed PMID: 26587234; PubMed Central PMCID: PMCPMC4652383.
47. McCracken B A SME, Roos M A, Zuckermann F A, Gaskins H. R Weaning Anorexia May Contribute to Local Inflammation in the Piglet Small Intestine. Biochemical and Molecular Action of Nutrients. 1998.
48. Bissonnette N, Jiang XR, Matte JJ, Guay F, Talbot G, Bontempo V, et al. Effect of a post-weaning diet supplemented with functional feed additives on ileal transcriptome activity and serum cytokines in piglets challenged with lipopolysaccharide. Vet Immunol Immunopathol. 2016;182:136-49. Epub 2016/11/20. doi: 10.1016/j.vetimm.2016.10.004. PubMed PMID: 27863544.
49. McCRACKEN BA GH, RUWE-KAISER PJ, KLASING KC, JEWELL DE. Diet-Dependent and Diet-Independent Metabolic Responses underlie Growth Stasis of Pigs at Weaning. Nutrient Metabolism. 1995:2838-45.
50. Mandal M, Borowski C, Palomero T, Ferrando AA, Oberdoerffer P, Meng F, et al. The BCL2A1 gene as a pre-T cell receptor-induced regulator of thymocyte survival. J Exp Med. 2005;201(4):603-14. Epub 2005/02/25. doi: 10.1084/jem.20041924. PubMed PMID: 15728238; PubMed Central PMCID: PMCPMC2213063.
51. Berberich I, Hildeman DA. The Bcl2a1 gene cluster finally knocked out: first clues to understanding the enigmatic role of the Bcl-2 protein A1. Cell Death Differ. 2017;24(4):572-4. Epub 2017/03/25. doi: 10.1038/cdd.2017.25. PubMed PMID: 28338657; PubMed Central PMCID: PMCPMC5384030.
52. Saydam N, Adams TK, Steiner F, Schaffner W, Freedman JH. Regulation of metallothionein transcription by the metal-responsive transcription factor MTF-1: identification of signal transduction cascades that control metal-inducible transcription. J Biol Chem. 2002;277(23):20438-45. Epub 2002/03/30. doi: 10.1074/jbc.M110631200. PubMed PMID: 11923282.
53. Wang MQ TW, Ye SS, Du YJ , Wang C, Shen SX, . Effects of Dietary Pharmacological Zinc on Growth, Liver Metallothionein, Cu, Zn -SOD concentration and Serum Parameters in Piglets Journal of Animal and Veterinary Advances 2012:1390-4.
54. Martin L, Lodemann U, Bondzio A, Gefeller EM, Vahjen W, Aschenbach JR, et al. A high amount of dietary zinc changes the expression of zinc transporters and metallothionein in jejunal epithelial cells in vitro and in vivo but does not prevent zinc accumulation in jejunal tissue of piglets. J Nutr. 2013;143(8):1205-10. Epub 2013/06/14. doi: 10.3945/jn.113.177881. PubMed PMID: 23761649.

55. Ruttkay-Nedecky B, Nejdl L, Gumulec J, Zitka O, Masarik M, Eckschlager T, et al. The role of metallothionein in oxidative stress. Int J Mol Sci. 2013;14(3):6044-66. Epub 2013/03/19. doi: 10.3390/ijms14036044. PubMed PMID: 23502468; PubMed Central PMCID: PMCPMC3634463.

56. Olson GE, Whitin JC, Hill KE, Winfrey VP, Motley AK, Austin LM, et al. Extracellular glutathione peroxidase (Gpx3) binds specifically to basement membranes of mouse renal cortex tubule cells. Am J Physiol Renal Physiol. 2010;298(5):F1244-53. Epub 2009/12/18. doi: 10.1152/ajprenal.00662.2009. PubMed PMID: 20015939; PubMed Central PMCID: PMCPMC2867408.

57. Rikka S, Quinsay MN, Thomas RL, Kubli DA, Zhang X, Murphy AN, et al. Bnip3 impairs mitochondrial bioenergetics and stimulates mitochondrial turnover. Cell Death Differ. 2011;18(4):721-31. Epub 2011/02/01. doi: 10.1038/cdd.2010.146. PubMed PMID: 21278801; PubMed Central PMCID: PMCPMC3058880.

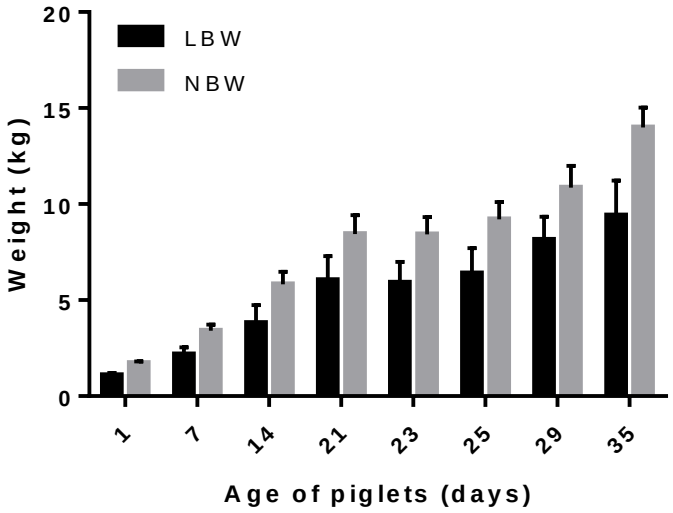

Figure S1. Evolution of the bodyweights of low birth weight piglets (LBW) and normal birth weight piglets (NBW) throughout the lactation and the post-weaning period. Each points in the graph represents means ± standard deviation (SD). The number of LBW and NBW piglets is 60 for d 1, 7 and 14 and then it decreased by 10 piglets per day from d 21 to d 35 (10 piglets per group were slaughtered at each time point starting from d 14).

Table S1. Ingredients and nutrients provided as fed basis in weaning diets for piglets

Ingredients/nutrients	Weaning Diets		
	d 21 to 23	d 24 to 29	d 30 to 35
Digestible energy (Kcal/kg)	3,420	3,470	3,350
Moisture (%)	9.19	9.2	10.87
Total protein (%)	18.45	18.59	20.82
Crude fibre (%)	2.35	3.01	3.02
Ash (%)	5.93	5.57	5.73
Fat (%)	5.21	6.35	4.78
Calcium (%)	0.65	0.69	0.70
Phosphorus (%)	0.67	0.75	0.68
Sodium (%)	0.52	0.51	0.38
Potassium (%)	0.75	0.66	0.96
Magnesium (%)	0.14	0.15	0.20
Copper (ppm)	114.37	132.28	121.09
Zinc (ppm)	2 850.04	2 476.49	428.38
Selenium (ppm)	0.80	0.70	0.60
Ca:P	0.98	0.92	1.03

Table S2. Primers sequence used for quantitative reverse transcription (RT PCR)

Gene	Gene Bank	Name	Sequence 5' to 3'	Product(bp)
BCL2A1	NM_001164511.2	B-cell lymphoma 2-related protein A1	F:GGATAAGGCAAAACGGAGGCT R:TAACTTCCACAAAGGTCAGCCA	84
BNIP3	XM_003359404.4	BCL2 interacting protein 3	F:GGAACACGAGCGTGATGAAGA R:GACGCCTCCCGATGTAGATC	122
CCL19	NM_001170516.1	C-C motif chemokine ligand 19	F:AGTCACACTACTGGCCCTCA R:CAGACAGGCAGCAGTCTTCA	89
GPx1	NM_214201.1	glutathione peroxidase 1	F:CAGGTACAGCCGTCGCTTTC R:AAAATCCCGAGAGTAGCACTGTAAC	137
GPx2	NM_001115136.1	glutathione peroxidase 2	F: TGCAACCAATTTGGACATCAG R: TTCACGTCACACTTCTGGATAAGG	122
GPx3	NM_001115155.1	glutathione peroxidase 3	F:AAACAGGAACCGGGAGACAA R:AGGACAGGCGTTCTTCAGGAA	156
HMOX1	NM_001004027.1	heme oxygenase 1	F:GGTCACCCGAGAAGGCTTTA R:GACCGGGTTCTCCTTGTTGT	97
IL-8	NM_213867.1	interleukin 8	F:AGAACTGAGAAGCAACAACAACAG R:CACAGGAATGAGGCATAGATGTAG	131
MSRA	XM_021072635.1	methionine sulfoxide reductase A	F:GCATCACGTCAATGGCAACA R:TCGGACTACTTCTGCATGGC	94
MT1A	NM_001001266.2	metallothionein 1A	F:GCTTGGTCTCACCTGCCTC R:CTCTTCTTGCAGGAGGTGCAT	119
MT2B	XM_003355808.4	metallothionein 2B	F:GCCTGAAGTTGGGGAGACC R:TAGCAAACGGGTCAGGTTGTAT	89
MT3	NM_214056.1	metallothionein 3	F:CAAGTGCGAGGGATGCAAAT R:TTACACACGCAATCCTTGGC	95
NCF1	NM_001113220.1	neutrophil cytosol factor 1	F:GCGCTTCACTGAGATCTACGA R:CTGCCCATCAAACCAACGTG	127
NDUFA2	XM_003124046.4	NADH ubiquinone oxidoreductase subunit A2	F:TCTGATGTGCAGCCCAAGC R:TTTGCCACTTAGCACATTCTCC	120
NDUFA5	XM_003134727.3	NADH ubiquinone oxidoreductase subunit A5	F:GGTTAAAGCGGAACCAGATGTTA R: GGCTCCCATGGCTTCCA	132
NDUFB2	NM_001244885.1	NADH ubiquinone oxidoreductase subunit B2	F:CGCTTTTGGCACGACTCAGA R:GCCTAATTCTTCATCCGTCCAC	81
NDUFB7	XM_003480775.4	NADH ubiquinone oxidoreductase subunit B7	F:CCACCTTCCCGCCTGACTA R:ATCAGCTGTGCGTCGTTCA	88
PRDx3	NM_001244531.1	peroxideroxin 3	F:TCTGGCTTGGATAAATACACC R:GACCTTCTAACAGCACACC	115
TXN	XM_021083263.1	thioredoxin	F: ATTCCAATGTCGTGTTCCTTGAA R:TCCTTGTTAGCTCCAGAAAATTCAC	127
TXNRD2	NM_001168702.1	thioredoxin reductase 2	F:CGAAAGGCGGAAAAGAGATTC R:TTCCATATTCCACAGCACCTTC	104
B2M	NM_213978.1	beta-2-Microglobulin	F:CTTTCAGCAAGGACTGGTCTTTCTAC R:GTGGTCTCGATCCCACTTAACTATC	134
GAPDH	NM_001206359.1	glyceraldehyde-3-phosphate dehydrogenase	F:CCCCAACGTGTCGGTTGT R:CTCGGACGCCTGCTTCAC	91
RPL13A	NM_001244068.1	ribosomal protein L13A	F:GTGGCCAAGCAGGTACTTCTG R:GGGACGGGTTGGTATTCATG	136
RPL4	XM_005659862.3	ribosomal protein L4	F:AGGAGGCTGTTCTGCTTCTG R:TCCAGGGATGTTTCTGAAGG	185

Table S3. Relative expression levels of the genes of the oxidative stress in liver in post-weaning (D23 and D35) in relation to the pre-weaning period (D14)

Symbol	Description	D23	D35
ALB	Albumin	-2.60*	-3.77*
ALOX12	Arachidonate 12-lipoxygenase	-5.82*	-5.01*
AOX1	Aldehyde oxidase-like	1.05	1.70
APOE	Apolipoprotein E	-1.68*	-2.26*
BAG2	BAG family molecular chaperone regulator 2-like	-1.31	-1.97^{A}
BNIP3	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like	-1.53	-1.88^{A}
CAT	Catalase	-1.61	-1.29
CCL5	Chemokine (C-C motif) ligand 5	-2.01	-2.24*
CYGB	Cytoglobin-like	-4.23	-3.68^{A}
DHCR24	24-dehydrocholesterol reductase	-2.10	-1.93
DUOX1	Dual oxidase 1	-2.02^{B}	-4.06^{B}
DUOX2	Dual oxidase 2	-7.34^{B}	-12.7^{B}*
DUSP1	Dual specificity phosphatase 1	-1.80	-1.94
EPHX2	Epoxide hydrolase 2, cytoplasmic	-1.32	1.06
EPX	Eosinophil peroxidase	1.03^{B}	-1.48^{B}
FHL2	Four and a half LIM domains protein 2-like	-2.37^{B}	-9.65^{B}
FOXM1	Forkhead box M1	-8.15^{B}	-3.62*
FTH1	Ferritin, heavy polypeptide 1	-1.35	-1.37
GCLC	Glutamate-cysteine ligase, catalytic subunit	-1.68	-1.73
GCLM	Glutamate-cysteine ligase, modifier subunit	-1.78	1.62
GLA	Alpha-galactosidase A	-3.46*	-2.20*
GP91-PHOX	NADPH oxidase heavy chain subunit	-3.82	-6.86*
GPX1	Glutathione peroxidase 1	-1.76	1.22
GPX2	Glutathione peroxidase 2 (gastrointestinal)	-2.92^{B}	-14.5^{B}*
GPX3	Glutathione peroxidase 3 (plasma)	-3.04	-4.57*
GPX4	Glutathione peroxidase 4 (phospholipid hydroperoxidase)	-1.27	-1.30
GPX5	Glutathione peroxidase 5 (epididymal androgen-related protein)	-3.40^{B}	-2.20^{B}*
GSS	Glutathione synthetase	-2.98	-3.88
GSTZ1	Glutathione S-transferase zeta 1	-3.07	-3.00
HMOX1	Heme oxygenase (decycling) 1	-2.35	-3.40*
HSP70.2	Heat shock protein 70.2	-1.80	-4.02
HSP90AA1	90-kDa heat shock protein	-1.27	-1.59
KRT1	Keratin 1	1.64^{B}	-1.13
LHPP	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	-3.30	-3.86*
LOC100049683	Solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11	-1.63^{B}	-1.01^{B}
LOC100516395	Lactoperoxidase-like	-6.30^{B}	1.88^{B}
TTN	Titin-like	-2.87^{B}	-9.85^{B}
LOC100622575	Pancreatic secretory trypsin inhibitor-like	-5.51^{B}	-42.37^{B}
LOC100739163	Glutathione S-transferase P	1.12	1.68
LOC396625	Glutathione reductase	-1.40	-1.45
LOC733635	Aldo-keto reductase family 1 member C2-like	-2.76	-5.29
MB	Myoglobin	-1.86^{C}	-1.13
MBL2	Mannose-binding lectin (protein C) 2, soluble	-2.53	-1.10
MGST3	Microsomal glutathione S-transferase 3	-1.75	1.14
MPO	Myeloperoxidase	-5.20^{B}	-2.70^{B}
MPV17	MpV17 mitochondrial inner membrane protein	-2.27^{B}	-3.17^{B}
MSRA	Peptide methionine sulfoxide reductase	-5.37	-2.95*
MT-III	Metallothionein-III	-2.12	-5.96*
NCF1	Neutrophil cytosolic factor 1	-3.54^{A}	-7.16*
NCF2	Neutrophil cytosolic factor 2	-2.77^{B}	-5.57^{B}
NCOA7	Nuclear receptor coactivator 7	-1.95	-3.09
NOS2	Nitric oxide synthase 2, inducible	-3.30^{B}	-2.64^{B}
NOX4	NADPH oxidase 4	-20.07^{B}	-61.9^{B}*
NOX5	NADPH oxidase, EF-hand calcium binding domain 5	1.68	-2.82
NQO1	NAD(P)H dehydrogenase, quinone 1	-1.02	2.75
NUDT1	Nudix (nucleoside diphosphate linked moiety X)-type motif 1	-2.79	-1.30
OXR1	Oxidation resistance 1	-1.61	-1.65
OXSR1	Oxidative-stress responsive 1	-2.88	-3.79*
PDLIM1	PDZ and LIM domain 1	-1.37	-1.38
PRDX1	Peroxiredoxin-1-like	-1.08	1.74*
PRDX2	Peroxiredoxin 2	-1.10	1.02
PRDX3	Peroxiredoxin 3	1.10	1.75*
PRDX4	Peroxiredoxin 4	-1.20	-1.09
PRDX5	Peroxiredoxin 5	-1.05	1.30
PRDX6	Peroxiredoxin 6	1.18	1.70*
PRNP	Prion protein	-2.16*	-1.97*
PTGR1	Prostaglandin reductase 1	1.04	3.61*
PTGS1	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and	-3.07	-3.55*

	cyclooxygenase)		
PTGS2	Prostaglandin G/H synthase-2	-2.23[B]	-2.56[B]
RNF7	Ring finger protein 7	-1.28	-1.07
SCARA3	Scavenger receptor class A, member 3	-4.99[B]	-14.1[B]*
SEPP1	Selenoprotein P, plasma, 1	1.52	2.12
SFTPD	Surfactant protein D	-2.46[B]	-9.03[B]
SIRT2	Sirtuin 2	-2.20	-4.07*
SOD1	Superoxide dismutase 1, soluble	1.05	-1.14
SOD3	Extracellular superoxide dismutase	-1.60	-2.23*
SQSTM1	Sequestosome-1-like	-1.29	-1.54*
TRAPPC6B	Trafficking protein particle complex subunit 6B-like	-1.15[A]	-1.09[B]
TTNLOC100620261	Titin	-4.28[B]	-37.57*
TXN	Thioredoxin	1.33	2.79*
TXNRD1	Thioredoxin reductase 1	1.29	1.24
TXNRD2	Thioredoxin reductase 2	-1.62	-2.06*
UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	-2.08[B]	-3.81[B]
VIMP	Selenoprotein S	1.24	1.65

A: This gene's average threshold cycle is relatively high (> 30) in either the control or the test sample, and is reasonably low in the other sample (< 30). B: This gene's average threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and test samples, and the p-value for the fold-change is either unavailable or relatively high (p > 0.05). C: This gene's average threshold cycle is either not determined or greater than the defined cut-off (default 35), in both samples meaning that its expression was undetected, making this fold-change result erroneous and un-interpretable. *The *P-values* (p < 0.05) are calculated based on a Student's t-test of the replicate 2^(- Delta CT) values for each gene in the control and treatment groups.

Table S4 - Relative expression levels of the genes of the energy metabolism in liver in post-weaning (D23 and D35) in relation to the pre-weaning period (D14)

Symbol	Description	D23	D35
ARRDC3	Arrestin domain containing 3	-1.06	-1.42
ASB1	Ankyrin repeat and SOCS box protein 1-like	-3.26[A]	-4.20[A]
ATP12A	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	-1.32[B]	-1.11[B]
ATP4A	ATPase, H+/K+ exchanging, alpha polypeptide	-3.97[B]	-11.6[B]
ATP4B	ATPase, H+/K+ exchanging, beta polypeptide	-14.5[B]	-2.24[B]
ATP5A1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle	-2.69*	-1.66*
ATP5B	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide	-1.54*	-1.49
ATP5E	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit	-1.50	1.02
ATP5F1	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit B1	-1.02	-1.48
ATP5G1	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit C1 (subunit 9)	-2.34	-1.68
LOC100037988	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit C2 (subunit 9)	-1.67[B]	-1.21[B]
ATP5G3	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit C3 (subunit 9)	54.32	73.04
ATP5H	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit d	-1.05	-1.19
ATP5I	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit E	-1.23	-1.16
ATP5L	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit G	-1.10	1.02
ATP6V0A2	ATPase, H+ transporting, lysosomal V0 subunit a2	-2.37	-2.27
ATP6V0D2	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d2	-8.64[A]	-1.42[A]
ATP6V1C2	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C2	-2.79	-3.49*
ATP6V1E2	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E2	-2.12[B]	-12.07[B]
ATP6V1G3	V-type proton ATPase subunit G 3-like	-3.51*	-2.92*
BCS1L	BCS1-like (S. cerevisiae)	-2.64	-4.86[A]
Cox5b	Mitochondrial cytochrome c oxidase subunit Vb	-2.11	-1.34
COX6A1	Cytochrome c oxidase subunit VIa polypeptide 1	1.07	-1.04
COX6C	COX6C protein	-1.00	1.02
COX7A2	COX7A2 protein	-1.12	-1.18
DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	-1.50	-2.92*
EDN1	Endothelin 1	-2.50[B]	-3.38[B]
GADD45B	Growth arrest and DNA-damage-inducible, beta	-2.21[A]	-2.73[A]
HSP70.2	Heat shock protein 70.2	-2.73	-6.41*
LHPP	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	-5.13	-6.45
LOC100154992	Mitochondrial inner membrane protein OXA1L-like	-1.87	-2.83*
LOC100156375	COX15 homolog, cytochrome c oxidase assembly protein	-3.69	-1.81
LOC100156879	Ubiquinol--cytochrome c reductase	-2.00	-2.49
LOC100156967	Cytochrome c oxidase subunit 5A, mitochondrial-like	-2.48	-1.25
LOC100157935	Cytochrome c oxidase subunit 8C, mitochondrial-like	-4.50[B]	-6.26[B]
LOC100511690	Cytochrome c1, heme protein, mitochondrial-like	-2.38	-2.34

LOC100517408	Cytochrome c oxidase subunit 7B2, mitochondrial-like	2.11B	-14.40[B]
LOC100519366	Cytochrome c oxidase subunit 6B1-like	1.51B	1.95[B]
LOC100519594	Cytochrome c oxidase subunit 6A2, mitochondrial-like	-2.56*[A]	-3.26[A]
LOC100522725	Cytochrome c oxidase subunit 8A, mitochondrial-like	-2.48	-2.57
LOC100624067	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial-like	-1.94*	-1.51*
LOC100624950	Cytochrome b561 domain-containing protein 1-like	-1.31	-1.89*
LOC100739238	Low-density lipoprotein receptor-related protein 5-like	-3.19	-7.24
NDUFB6	NADH dehydrogenase 1 beta subcomplex 6	-2.55*	-1.34
ATP5O	ATP synthase H+-transporting mitochondrial F1 complex O subunit	1.07	1.03
NDUFA1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 1-like	-2.30*	-1.57*
NDUFA10	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial-like	-1.27	-2.17*
NDUFA11	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 11, 14.7kDa	-1.65	-1.84
NDUFA2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8kDa	-6.73[A]	1.78
NDUFA3	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 3-like	-2.16	-2.28
NDUFA4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4, 9kDa	-1.24	-1.22
NDUFA5	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5-like	-1.59*	-1.04
NDUFA6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14kDa	-4.09*[A]	-7.78*[A]
NDUFA7	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 7-like	-1.51	-1.01
NDUFA8	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8, 19kDa	-1.63	-1.41
NDUFA9	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial-like	-3.55[B]	-2.99[B]
NDUFAB1	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1, 8kDa	-1.42	-1.56
NDUFB10	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10-like	-1.19	-1.33
NDUFB2	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2, 8kDa	-1.31	-1.07
NDUFB3	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa	-1.38[B]	1.11*
NDUFB4	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 4-like	-1.54*	-1.59*
NDUFB5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5, 16kDa	-66.13*[A]	-1.11
NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa	-6.55*	-88.59*
NDUFB8	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8, 19kDa	-1.40	1.13
NDUFB9	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9, 22kDa	-1.11	-1.00
NDUFC1	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1, 6kDa	-1.45	1.07
NDUFS1	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase)	-2.72	-2.31
NDUFS2	NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase)	-6.47*	-16.72*
NDUFS3	NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30kDa (NADH-coenzyme Q reductase)	-1.78	-2.27*
NDUFS4	NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase)	1.04	1.08
NDUFS5	NADH dehydrogenase (ubiquinone) Fe-S protein 5, 15kDa (NADH-coenzyme Q reductase)	-1.65*	-1.33*
NDUFS6	NADH dehydrogenase [ubiquinone] iron-sulfur protein 6, mitochondrial-like	-2.05	-2.03
NDUFS7	NADH dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial-like	-1.34	-2.02*
NDUFS8	NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial-like	-3.16[B]	-2.31[B]
NDUFV1	NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial-like	-3.97[A]	-5.14[A]
PPA1	Pyrophosphatase 1	-1.58	1.30
PPA2	Inorganic pyrophosphatase 2, mitochondrial-like	-1.30	-177.6[A]
SDHA	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	-3.01	-2.67*
SDHB	Succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	-3.17*	-2.93*
SDHC	Succinate dehydrogenase cytochrome b560 subunit, mitochondrial-like	-1.28	-1.19
SDHD	Succinate dehydrogenase complex, subunit D, integral membrane protein	-1.24	-1.35*
SLC25A25	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 25	-3.33[A]	-7.44[A]
UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	-7.34[B]	-16.55[B]
UQCR10	Ubiquinol-cytochrome c reductase complex 7.2 kDa protein	24.94	30.59

A: This gene's average threshold cycle is relatively high (> 30) in either the control or the test sample, and is reasonably low in the other sample (< 30). B: This gene's average threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and test samples, and the p-value for the fold-change is either unavailable or relatively high (p > 0.05). *The P-values (p < 0.05) are calculated based on a Student's t-test of the replicate 2^(- Delta CT) values for each gene in the control and treatment groups.

Table S5- Relative expression levels of the genes of the oxidative stress in intestinal mucosa in post-weaning (D23 and D35) in relation to the pre-weaning period (D-14)

Symbol	Description	D23	D35
ALB	Albumin	-4.35[B]	4.39[B]
ALOX12	Arachidonate 12-lipoxygenase	2.50[A]	-1.93[B]
AOX1	Aldehyde oxidase-like	-1.00[B]	-2.17*
APOE	Apolipoprotein E	3.52	1.00
BAG2	BAG family molecular chaperone regulator 2-like	2.68	1.65*
BNIP3	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like	1.34	-1.35

CAT	Catalase	1.34	-1.13
CCL5	Chemokine (C-C motif) ligand 5	1.76	2.09*
CYGB	Cytoglobin-like	1.46	-1.85*
DHCR24	24-dehydrocholesterol reductase	1.85	1.05
DUOX1	Dual oxidase 1	2.22[B]	1.34[B]
DUOX2	Dual oxidase 2	15.3[B]	8.12[B]
DUSP1	Dual specificity phosphatase 1	1.20	-2.17
EPHX2	Epoxide hydrolase 2, cytoplasmic	-1.08	1.79
EPX	Eosinophil peroxidase	46.7[B]	1.19[B]
FHL2	Four and a half LIM domains protein 2-like	1.95	-1.84*[A]
FOXM1	Forkhead box M1	3.24*[A]	1.03[B]
FTH1	Ferritin, heavy polypeptide 1	1.07	-2.47*
GCLC	Glutamate-cysteine ligase, catalytic subunit	1.85	-1.38
GCLM	Glutamate-cysteine ligase, modifier subunit	-1.02	1.96
GLA	Alpha-galactosidase A	-2.42	-2.72
GP91-PHOX	NADPH oxidase heavy chain subunit	5.91*	-1.32
GPX1	Glutathione peroxidase 1	2.50*	1.06
GPX2	Glutathione peroxidase 2 (gastrointestinal)	3.60	4.82*
GPX3	Glutathione peroxidase 3 (plasma)	-3.89	-21.73
GPX4	Glutathione peroxidase 4 (phospholipid hydroperoxidase)	-1.92*	-2.18*
GPX5	Glutathione peroxidase 5 (epididymal androgen-related protein)	2.61[B]	1.45[B]
GSS	Glutathione synthetase	1.23	-1.69*
GSTZ1	Glutathione S-transferase zeta 1	-1.06	-3.80*[A]
HMOX1	Heme oxygenase (decycling) 1	-1.43	-5.30*
HSP70.2	Heat shock protein 70.2	3.51	1.75
HSP90AA1	90-kDa heat shock protein	1.92	-1.44
KRT1	Keratin 1	1.56[B]	-1.25[B]
LHPP	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	3.24	1.22
LOC100049683	Solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11	2.83*	5.72[B]
LOC100516395	Lactoperoxidase-like	2.29[B]	4.83[B]
TTN	Titin-like	2.52[B]	-1.24[B]
LOC100622575	Pancreatic secretory trypsin inhibitor-like	1.03	1.65
LOC100739163	Glutathione S-transferase P	2.25*	1.44*
LOC396625	Glutathione reductase	1.22	1.67*
LOC733635	Aldo-keto reductase family 1 member C2-like	-1.05	1.05
MB	Myoglobin	-1.58[B]	-1.64[B]
MBL2	Mannose-binding lectin (protein C) 2, soluble	2.80[B]	1.65[B]
MGST3	Microsomal glutathione S-transferase 3	-1.56*	1.40*
MPO	Myeloperoxidase	24.57*	4.30[B]
MPV17	MpV17 mitochondrial inner membrane protein	2.55[B]	1.85[B]
MSRA	Peptide methionine sulfoxide reductase	-1.40	-1.03
MT-III	Metallothionein-III	29.99	1.14
NCF1	Neutrophil cytosolic factor 1	5.03*	-1.34
NCF2	Neutrophil cytosolic factor 2	3.12[A]	1.21[A]
NCOA7	Nuclear receptor coactivator 7	1.58	-1.57
NOS2	Nitric oxide synthase 2, inducible	7.61[A]	7.78[A]
NOX4	NADPH oxidase 4	1.62[B]	-3.79*[A]
NOX5	NADPH oxidase, EF-hand calcium binding domain 5	1.09[B]	-2.87[B]
NQO1	NAD(P)H dehydrogenase, quinone 1	3.58	1.61
NUDT1	Nudix (nucleoside diphosphate linked moiety X)-type motif 1	1.22	1.17
OXR1	Oxidation resistance 1	-1.39	-1.45
OXSR1	Oxidative-stress responsive 1	1.24	-1.74*
PDLIM1	PDZ and LIM domain 1	1.64	-1.25
PRDX1	Peroxiredoxin-1-like	-1.29	1.08
PRDX2	Peroxiredoxin 2	-1.33	1.00
PRDX3	Peroxiredoxin 3	-1.75*	1.03
PRDX4	Peroxiredoxin 4	-1.30	1.30
PRDX5	Peroxiredoxin 5	-1.03	-1.20
PRDX6	Peroxiredoxin 6	-1.76*	-1.46*
PRNP	Prion protein	1.46	-1.32[A]
PTGR1	Prostaglandin reductase 1	2.23	2.23

PTGS1	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	2.71	-1.96[A]
PTGS2	Prostaglandin G/H synthase-2	4.40[A]	-1.15[B]
RNF7	Ring finger protein 7	-1.51	-1.52
SCARA3	Scavenger receptor class A, member 3	2.95[B]	-1.23[B]
SEPP1	Selenoprotein P, plasma, 1	-3.26	-1.67*
SFTPD	Surfactant protein D	1.86	2.57*
SIRT2	Sirtuin 2	1.19	-2.19*
SOD1	Superoxide dismutase 1, soluble	1.01	-1.43
SOD3	Extracellular superoxide dismutase	5.91	-1.07
SQSTM1	Sequestosome-1-like	1.69	-1.44
TRAPPC6B	Trafficking protein particle complex subunit 6B-like	1.05	-1.07
TTNLOC100620261	Titin	-1.25[B]	-2.16[B]
TXN	Thioredoxin	1.12	1.25
TXNRD1	Thioredoxin reductase 1	2.18	1.21
TXNRD2	Thioredoxin reductase 2	2.31*	-1.08
UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	2.92[B]	-2.62*
VIMP	Selenoprotein S	-1.39	-1.06

A: This gene's average threshold cycle is relatively high (> 30) in either the control or the test sample, and is reasonably low in the other sample (< 30). B: This gene's average threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and test samples, and the p-value for the fold-change is either unavailable or relatively high (p > 0.05). *The *P-values* (p < 0.05) are calculated based on a Student's t-test of the replicate 2^(- Delta CT) values for each gene in the control and treatment groups.

Table S6. Relative expression levels of the genes of the energy metabolism in intestinal mucosa in post-weaning (D23 and D35) in relation to the pre-weaning period (D14)

Symbol	Description	D23	D35
ARRDC3	Arrestin domain containing 3	-1.74	-2.71*
ASB1	Ankyrin repeat and SOCS box protein 1-like	2.16	-2.67*[A]
ATP12A	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	7.91[B]	-1.52*
ATP4A	ATPase, H+/K+ exchanging, alpha polypeptide	5.23[B]	-1.12[B]
ATP4B	ATPase, H+/K+ exchanging, beta polypeptide	2.41[B]	9.00*
ATP5A1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle	-1.28	-1.01
ATP5B	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide	-1.08	1.28
ATP5E	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit	-1.84	1.16
ATP5F1	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit B1	-1.34	1.58
ATP5G1	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit C1 (subunit 9)	-1.04	1.34
LOC100037988	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit C2 (subunit 9)	2.45[B]	-3.34*[B]
ATP5G3	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit C3 (subunit 9)	-2.03*	1.01
ATP5H	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit d	-2.12*	-1.19
ATP5I	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit E	-2.14*	-1.62
ATP5L	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit G	-1.35	-1.56
ATP6V0A2	ATPase, H+ transporting, lysosomal V0 subunit a2	1.34	-1.31*
ATP6V0D2	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d2	22.4*	-1.66[B]
ATP6V1C2	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C2	1.07	-1.81
ATP6V1E2	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E2	8.49*	1.17[B]
ATP6V1G3	V-type proton ATPase subunit G 3-like	2.16[B]	-1.82[B]
BCS1L	BCS1-like (S. cerevisiae)	5.07[A]	2.41[A]
Cox5b	Mitochondrial cytochrome c oxidase subunit Vb	-1.28	1.25
COX6A1	Cytochrome c oxidase subunit VIa polypeptide 1	-1.65	1.14
COX6C	COX6C protein	-3.18*	-1.21
COX7A2	COX7A2 protein	-2.36*	-1.53
DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	1.13	-1.41*
EDN1	Endothelin 1	2.73[A]	-2.20*
GADD45B	Growth arrest and DNA-damage-inducible, beta	1.82	-1.49
HSP70.2	Heat shock protein 70.2	3.11	1.39
LHPP	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	2.61	-1.26
LOC100154992	Mitochondrial inner membrane protein OXA1L-like	1.92	1.312
LOC100156375	COX15 homolog, cytochrome c oxidase assembly protein	-1.42	-1.16
LOC100156879	Ubiquinol--cytochrome c reductase	2.04	1.40
LOC100156967	Cytochrome c oxidase subunit 5A, mitochondrial-like	-1.49	-1.22
LOC100157935	Cytochrome c oxidase subunit 8C, mitochondrial-like	-4.16*[B]	-3.23*[B]
LOC100511690	Cytochrome c1, heme protein, mitochondrial-like	1.54	1.32
LOC100517408	Cytochrome c oxidase subunit 7B2, mitochondrial-like	-15.74*[B]	-1.31[B]

LOC100519366	Cytochrome c oxidase subunit 6B1-like	3.70[B]	-1.01[B]
LOC100519594	Cytochrome c oxidase subunit 6A2, mitochondrial-like	1.92	-1.02
LOC100522725	Cytochrome c oxidase subunit 8A, mitochondrial-like	-1.03	-1.26
LOC100624067	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial-like	-1.26	-1.32
LOC100624950	Cytochrome b561 domain-containing protein 1-like	-1.73	.'-3.*
LOC100739238	Low-density lipoprotein receptor-related protein 5-like	1.61	-3.31*
NDUFB6	NADH dehydrogenase 1 beta subcomplex 6	-1.87	-1.01
ATP5O	ATP synthase H+-transporting mitochondrial F1 complex O subunit	-2.61*	-1.12
NDUFA1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 1-like	-1.92	-1.85
NDUFA10	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial-like	1.36	1.02
NDUFA11	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 11, 14.7kDa	-1.29	1.27
NDUFA2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8kDa	-1.76	1.26
NDUFA3	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 3-like	1.43	-1.24*
NDUFA4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4, 9kDa	-2.63*	-1.56
NDUFA5	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5-like	-2.13*	-1.27
NDUFA6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14kDa	2.22*[A]	-1.49[B]
NDUFA7	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 7-like	-1.64	-1.00
NDUFA8	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8, 19kDa	-1.77	1.00
NDUFA9	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial-like	1.04[B]	1.76[B]
NDUFAB1	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1, 8kDa	1.04	-1.21
NDUFB10	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10-like	-1.28	-1.27
NDUFB2	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2, 8kDa	-3.09*	-1.23
NDUFB3	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa	-1.88[B]	1.78*[A]
NDUFB4	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 4-like	-1.32	-2.26*
NDUFB5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5, 16kDa	-1.02	1.55
NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa	11.9*	1.33[B]
NDUFB8	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8, 19kDa	-1.85*	1.01
NDUFB9	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9, 22kDa	-1.70	-1.06
NDUFC1	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1, 6kDa	-2.88*	-1.34
NDUFS1	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase)	1.01	1.40
NDUFS2	NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase)	3.69[B]	-1.34[B]
NDUFS3	NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30kDa (NADH-coenzyme Q reductase)	1.29	1.16
NDUFS4	NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase)	-1.79	-1.02
NDUFS5	NADH dehydrogenase (ubiquinone) Fe-S protein 5, 15kDa (NADH-coenzyme Q reductase)	-2.52*	-1.01
NDUFS6	NADH dehydrogenase [ubiquinone] iron-sulfur protein 6, mitochondrial-like	-1.15	-1.08
NDUFS7	NADH dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial-like	-1.20	-1.82*
NDUFS8	NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial-like	1.16[B]	1.10[B]
NDUFV1	NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial-like	2.58[A]	1.49[A]
PPA1	Pyrophosphatase 1	-1.58	1.37
PPA2	Inorganic pyrophosphatase 2, mitochondrial-like	-1.41	-1.10
SDHA	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	1.23	-1.21
SDHB	Succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	-1.37	1.05
SDHC	Succinate dehydrogenase cytochrome b560 subunit, mitochondrial-like	-1.86*	-1.23
SDHD	Succinate dehydrogenase complex, subunit D, integral membrane protein	-1.24	-1.35
SLC25A25	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 25	2.53	-1.10
UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	5.59[B]	-1.36[B]
UQCR10	Ubiquinol-cytochrome c reductase complex 7.2 kDa protein	-2.14*	1.38

A: This gene's average threshold cycle is relatively high (> 30) in either the control or the test sample, and is reasonably low in the other sample (< 30). B: This gene's average threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and test samples, and the p-value for the fold-change is either unavailable or relatively high (p > 0.05. *The *P-values* (p < 0.05) are calculated based on a Student's t-test of the replicate 2^(- Delta CT) values for each gene in the control and treatment groups.

Table S7. Relative expression levels of the genes of cytokines in intestinal mucosa in post-weaning (D23 and D35) in relation to the pre-weaning period (D14)

Symbol	Description	D23	D35
ADIPOQ	Adiponectin, C1Q and collagen domain containing	3.49[B]	-1.62[B]
AMCF-II	Alveolar macrophage-derived chemotactic factor-II	1.14	2.71
BMP2	Bone morphogenetic protein 2	1.51*	-1.28
BMP3	Bone morphogenetic protein 3	1.45	-2.57*
BMP4	Bone morphogenetic protein 4	1.13	-1.23*
BMP6	Bone morphogenetic protein 6	1.14[B]	-2.95[B]
Bmp7	Bone morphogenetic protein 7	1.89[A]	-1.24[B]
C5	Complement component 5	1.92	2.42*
CCL1	Chemokine (C-C motif) ligand 1	8.52*	1.22[B]
CCL11	CCL11	-1.87	1.04
CCL17	Chemokine ligand 17-like protein	29.41[*A]	1.54[B]

CCL19	Chemokine (C-C motif) ligand 19	7.92*	2.38*
CCL2	Chemokine (C-C motif) ligand 2	1.80*	1.25
CCL20	Chemokine (C-C motif) ligand 20	-2.09	-2.93
CCL21	Chemokine (C-C motif) ligand 21	1.15	-2.97*
CCL22	C-C motif chemokine 22-like	9.30*	-1.53[B]
CCL25	Chemokine (C-C motif) ligand 25	-5.21	-1.39*
CCL27	Chemokine (C-C motif) ligand 27	1.80[A]	1.51*[A]
CCL28	Chemokine (C-C motif) ligand 28	1.04	3.37*
CCL3L1	Chemokine (C-C motif) ligand 3-like 1	8.28*[A]	3.63[A]
CCL4	Chemokine (C-C motif) ligand 4	9.14*[A]	4.56*[A]
CCL5	Chemokine (C-C motif) ligand 5	-1.03	1.48*
CCL8	Chemokine (C-C motif) ligand 8	5.21*	2.38
CD40LG	CD40 ligand	1.35*[A]	1.12[B]
CSF1	Colony stimulating factor 1 (macrophage)	3.34[A]	1.24[B]
CSF2	Colony stimulating factor 2 (granulocyte-macrophage)	-1.42[B]	-1.83*
CSF3	Colony stimulating factor 3 (granulocyte)	3.34*	-1.71*
CXCL10	Chemokine (C-X-C motif) ligand 10	6.25	6.35*
CXCL11	Chemokine (C-X-C motif) ligand 11	20.45[A]	12.9*[A]
CXCL12	Chemokine (C-X-C motif) ligand 12	1.08	-1.17
LOC396594	Growth-regulated protein homolog gamma	-1.48	-1.48
CXCL9	Chemokine (C-X-C motif) ligand 9	10.4	8.17*
FASLG	Fas ligand (TNF superfamily, member 6)	-1.31[B]	1.07[B]
IFN-ALPHA-4	Interferon-alpha-4	1.55[B]	-2.69*
IFN-ALPHA-5	Interferon, alpha 5	2,76[A]	-2.05*
IFNB1	Interferon beta	14.52*	1.98[B]
IFNG	Interferon-gamma	2.31[B]	19.16*[A]
IL10	Interleukin 10	-1.00	-1.15
IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)	9.60*	-1.64[B]
IL12B	Interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)	33.47*	10.40*
IL13	Interleukin 13	4.74*	-1.55[B]
IL15	Interleukin 15	-1.45*	1.35
IL16	Interleukin 16	2.57*	1.06
IL17A	Interleukin 17A	2.09*	4.67*
IL17F	Interleukin 17F	-1.39[B]	5.66*
IL18	Interleukin 18 (interferon-gamma-inducing factor)	1.21	2.09*
IL1A	Interleukin 1, alpha	1.97[A]	1.67[A]
IL1B1	Interleukin 1, beta 1	3.54*[A]	1.35[B]
IL2	Interleukin 2	25.63[B]	18.86*
IL21	Interleukin 21	52.72[B]	264.38*
IL22	Interleukin 22	1.04[B]	10.10*
IL23A	Interleukin 23, alpha subunit p19	1.99[B]	-1.10[B]
IL27	Interleukin 27	5.50[B]	1.32[B]
IL4	Interleukin 4	2.93*	-1.68[B]
IL5	Interleukin 5	-1.03[A]	-1.54[B]
IL6	Interleukin 6 (interferon, beta 2)	10.14*	2.74[B]
IL7	Interleukin 7	-1.23*	1.12*
CXCL8	Interleukin 8	-1.36	1.46
IL9	Interleukin 9	-1.38[B]	-1.15[B]
INHA	Inhibin, alpha	-1.92*[C]	-1.31*[C]
INHBA	Inhibin, beta A	1.00[A]	-2.98*
LIF	Leukemia inhibitory factor (cholinergic differentiation factor)	1.91[A]	-1.40[B]
OSM	Oncostatin-M-like	2.85[B]	-1.31*[C]
LOC100515857	C-C motif chemokine 3-like	-1.23[B]	1.80*
CCL23	C-C motif chemokine 23-like	10.41*[A]	2.18[B]
CCL16	C-C motif chemokine 16-like	-45.74[A]	-9.86[A]
CNTF	Ciliary neurotrophic factor-like	1.21[A]	-2.26*
CXCL13	C-X-C motif chemokine 13-like	12.55[A]	4.15*[A]
THPO	Thrombopoietin	1.61[B]	2.07[B]
LOC100621682	Uncharacterized LOC100621682	1.62[B]	-1.32[B]
TNFSF9	Tumor necrosis factor ligand superfamily member 9-like	1.99*	1.26[B]
CCL24	Chemokine ligand 24-like protein	10.22[A]	11.47*[A]
LTA	Lymphotoxin alpha (TNF superfamily, member 1)	22.80*	-2.25[B]
LTB	Lymphotoxin beta (TNF superfamily, member 3)	10.70*	1.52
MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	1.02	-1.23
MSTN	Myostatin	1.19[B]	-1.66[B]
SPP1	Secreted phosphoprotein 1	141.50*[A]	10.97[A]
TGFB1	Transforming growth factor, beta 1	3.68*	-1.01
TGFB2	Transforming growth factor, beta 2	-1.34	-2.73*[A]
TNF	Tumor necrosis factor	1.50*	-1.34
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	-1.04	1.65*
TNFSF13B	Tumor necrosis factor (ligand) superfamily, member 13b	11.49*	29.53*
TNFSF4	Tumor necrosis factor (ligand) superfamily, member 4	1.52[B]	-1.22[B]

VEGFA	Vascular endothelial growth factor A	1.09	-1.87

A: This gene's average threshold cycle is relatively high (> 30) in either the control or the test sample, and is reasonably low in the other sample (< 30). B: This gene's average threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and test samples, and the p-value for the fold-change is either unavailable or relatively high (p > 0.05). *The *P-values* (p < 0.05) are calculated based on a Student's t-test of the replicate 2^(- Delta CT) values for each gene in the control and treatment groups.

Table S8. Relative expression levels of the genes of the apoptosis in intestinal mucosa in post-weaning (D23 and D35) in relation to the pre-weaning period (D14)

Symbol	Description	D23	D35
ABL1	C-abl oncogene 1, non-receptor tyrosine kinase	1.76	-3.15*[A]
AIFM1	Apoptosis-inducing factor, mitochondrion-associated, 1	1.03	-1.22*
AIFM3	Apoptosis-inducing factor, mitochondrion-associated, 3	1.25[B]	-1.74[B]
AKT1	V-akt murine thymoma viral oncogene homolog 1	1.75	-1.86*
APAF1	Apoptotic peptidase activating factor 1	-1.17	-2.57*
BAD	BCL2-associated agonist of cell death	1.69	-1.34[A]
BAG3	BCL2-associated athanogene 3	-1.54	-6.69*[A]
BAK1	Bak protein	1.39	-1.19*
BAX	BCL2-associated X protein	-1.31	-1.34
BCL2	B-cell CLL/lymphoma 2	2.32	-1.29*
BCL2A1	BCL2-related protein A1	5.27*	2.94*
BCL2L1	BCL2-like 1	1.39	-1.15
BCL2L10	BCL2-like 10 (apoptosis facilitator)	3.18[B]	-2.09[B]
LOC100154044	BCL2-like 2	1.18	-2.82*
BID	BH3 interacting domain death agonist	-1.08	-1.48*
LOC100622859	Baculoviral IAP repeat containing 2	1.08	-1.40
BIRC3	Baculoviral IAP repeat containing 3	-1.09	1.15
BIRC5	Baculoviral IAP repeat containing 5	1.21	1.84*
BNIP2	BCL2/adenovirus E1B 19kDa interacting protein 2	-1.02[B]	-1.14[B]
BNIP3L	BCL2/adenovirus E1B 19kDa interacting protein 3-like	-1.56*	-1.58*
BRAF	V-raf murine sarcoma viral oncogene homolog B1	1.05	-1.64*
CASP1	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	-1.17	2.08*
CASP10	Caspase 10, apoptosis-related cysteine peptidase	-1.20	-2.06*
CASP14	Caspase 14, apoptosis-related cysteine peptidase	4.06[B]	1.00[C]
CASP2	Caspase 2, apoptosis-related cysteine peptidase	2.34	-1.26
CASP3	Caspase 3, apoptosis-related cysteine peptidase	-1.51*	-1.25
LOC100522887	Caspase 4, apoptosis-related cysteine peptidase	-1.02	1.47*
CASP6	Caspase 6, apoptosis-related cysteine peptidase	-1.20	1.05
CASP8	Caspase 8, apoptosis-related cysteine peptidase	-1.29*	1.23*
CBX4	Chromobox homolog 4	1.32	-2.58*[A]
CD40	CD40 molecule, TNF receptor superfamily member 5	4.81*	1.34*
CD40LG	CD40 ligand	1.37[A]	-1.06[B]
CD70	CD70 molecule	7.03[B]	1.99[B]
CFDP1	Craniofacial development protein 1	-1.02	-1.32
CIDEA	Cell death-inducing DFFA-like effector a	-1.32[B]	-1.78[B]
CIDEB	Cell-death-inducing DNA-fragmentation-factor-like effector B	-1.02	-2.62*
CUL2	Cullin 2	1.17	1.18
CYCS	Cytochrome c, somatic	-1.51	-1.42
LOC100516103	Death-associated protein kinase 1	3.41	-1.47*
DDX20	DEAD (Asp-Glu-Ala-Asp) box polypeptide 20	1.64	-1.57*
DIABLO	Diablo, IAP-binding mitochondrial protein	-1.18	-1.01
DPF2	D4, zinc and double PHD fingers family 2	1.84*	-1.03
ERC1	ELKS/RAB6-interacting/CAST family member 1	-1.11	-5.05[A]
ERN2	Endoplasmic reticulum to nucleus signaling 2	1.86[A]	-1.62[B]
FAS	Fas (TNF receptor superfamily, member 6)	1.98*	1.76*
FASLG	Fas ligand (TNF superfamily, member 6)	-1.11[B]	1.60*
FEM1B	Fem-1 homolog b (C. elegans)	1.02	-1.79*
GADD45A	Growth arrest and DNA-damage-inducible, alpha	-1.17	-1.45
IGF1R	Insulin-like growth factor 1 receptor	1.46	-4.88*
IL10	Interleukin 10	-1.95[A]	1.01
LALBA	Lactalbumin, alpha-	-2.64[B]	-2.40[B]
HRK	Activator of apoptosis harakiri-like	-1.12[B]	-4.60[B]
LOC100156777	Caspase 7	1.00	1.03
TNFRSF21	Tumor necrosis factor receptor superfamily member 21-like	1.39	-1.87*
RIPK2	Receptor-interacting serine/threonine-protein kinase 2-like	-1.49	-1.22
CASP9	Caspase-9-like	1.37[B]	-1.94[B]
LTBR	Tumor necrosis factor receptor superfamily member 3-like	1.56	-1.35

LOC100522011	Apoptosis-associated speck-like protein containing a CARD-like	-1.38*	2.09*
DFFA	DNA fragmentation factor subunit alpha-like	-1.00	-1.48
LOC100523672	Nucleolar protein 3-like	-1.25[B]	-1.79[B]
BNIP3	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like	-1.35[A]	-3.16*[A]
LOC641352	Caspase-15	1.82	-1.73*
LTA	Lymphotoxin alpha (TNF superfamily, member 1)	10.33[B]	-4.11*
MCL1	Myeloid cell leukemia sequence 1 (BCL2-related)	1.05	-1.07
MTL5	Metallothionein-like 5, testis-specific (tesmin)	3.47[B]	2.24*
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	2.15	-1.12
NOD1	Nucleotide-binding oligomerization domain containing 1	6.1B[B]	1.17[B]
PAK7	P21 protein (Cdc42/Rac)-activated kinase 7	-5.87[B]	-7.39[B]
PPP2R1A	Protein phosphatase 2, regulatory subunit A, alpha	2.10	-2.75*[A]
PPP2R1B	Protein phosphatase 2, regulatory subunit A, beta	-1.03	-1.70*
PROP1	PROP paired-like homeobox 1	1.31[B]	-1.08[B]
RFWD2	Ring finger and WD repeat domain 2	-1.05	-1.49*
SART1	Squamous cell carcinoma antigen recognized by T cells	2.33	-1.96*
LOC100517325	Signal-induced proliferation-associated 1	3.76[B]	-1.91[B]
STAMBP	STAM binding protein	-1.17	-1.34
TNF	Tumor necrosis factor	1.61	-1.95
TNFRSF11B	Tumor necrosis factor receptor superfamily, member 11b	3.03[B]	-1.31[B]
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A	1.36	-1.73*[A]
TNFRSF1B	Tumor necrosis factor receptor superfamily, member 1B	4.43	-1.15
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	-1.46	1.24
TP53	Tumor protein p53	1.84	-1.07
TP53BP2	Tumor protein p53 binding protein, 2	1.61	-1.69*
TRAF2	TNF receptor-associated factor 2	2.34	-1.58
XIAP	X-linked inhibitor of apoptosis	-1.16	-1.16

A: This gene's average threshold cycle is relatively high (> 30) in either the control or the test sample, and is reasonably low in the other sample (< 30). B: This gene's average threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and test samples, and the p-value for the fold-change is either unavailable or relatively high ($p > 0.05$). *The *P-values* ($p < 0.05$) are calculated based on a Student's t-test of the replicate 2^(- Delta CT) values for each gene in the control and treatment groups.