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Anti-oxidative stress system cooperating with IgA promotes the low temperature adaptation of Rongchang piglets

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Abstract

Young animals are more susceptible to temperature; mild cold stress results in body damage and decreased productivity. Some studies have demonstrated that minor damage caused by hypothermia can be self-recovering, but this mechanism remains to be explored. Thirty Rongchang piglets were randomly divided into a control group (N = 15, temperature = 22.80–25.70 °C, humidity = 46– 66%) and a cold stress group (N = 15, temperature = 13.20–16.20 °C, humidity = 77–93 %) and were fed normal diets for 30 days. Blood samples were collected on 15th and 30th days to detect immune system and oxidative stress indicators. Aspartate aminotransferase, alanine aminotransferase, and malondialdehyde content in the serum of Rongchang piglets increased by 50%, 110%, and 30% in the cold stress group. The interleukin and immunoglobulins in serum in the cold stress group were lower than those in the control group. However, the levels of IgA, glutathione peroxidase, and CAT gradually recovered with the extension of the cold treatment time. Mild cold stress leads to liver damage and decreased immunity and the development of oxidative stress in Rongchang piglets. However, Rongchang piglets achieve cold acclimation via the synergistic effects of immune regulation involving IgA and the involvement of glutathione peroxidase and catalase in hydrogen peroxide catabolism. This study provides the theoretical and experimental basis for exploring cold adaptation in Rongchang piglets.

Keywords: Rongchang piglets, cold stress, oxidative stress, immunoglobulin A, glutathione peroxidase
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Introduction

Low temperature is usually a negative external factor that affects the growth of livestock and poultry (Xue *et al*., 2018; Igoshin *et al*., 2019; Windsor, 2021). Piglets are more sensitive to temperature, causing high mortality, as they have a well-developed thermoneutral zone. Even if they do survive, further growth and health are impacted (Zhang *et al*., 2020; Yu *et al*., 2021). To overcome those problems, a piglet incubator and a constant temperature incubator are usually used to increase the ambient temperature (Windsor, 2021). The latter is not susceptible to the surrounding conditions and provides a stable temperature and humidity but is more expensive. Therefore, there is no doubt that more open piglet incubators are chosen by numerous small and free-range farmers.

Cold stress can limit productive performance because low temperatures result in decreased immunity, intestinal bleeding, fear, and mental stress (Carroll *et al*., 2012; Browne *et al*., 2017; Kong *et al*., 2020). The body secretes cortisol to increase heat production, maintain temperature, and adapt to the environment via the hypothalamic–pituitary–adrenal axis (HPA axis), but this causes a decrease in feed conversion rate (Uetake *et al*., 2018; Law & Clow, 2020). However, other studies have shown that

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persistent mild cold stress causes the organism to acclimatize to the low temperature, i.e., cold adaptation or cold acclimation (Castellani & Young, 2016; Wang *et al*., 2022), which helps the body to adapt to low temperature and even enhances immunity (Guo *et al*., 2021; Zhang *et al*., 2023). These contradictory reports indicate the complex nature of the animal's adaptation to cold stress. Whether Rongchang piglets in an open piglet incubator will adopt a cold acclimation mechanism remains unclear.

Since the liver contains a large number of enzymes used for energy metabolism, we first investigated the effect of the liver on the cold stress (Ji *et al*., 2020). Some studies indicate that alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are markedly enhanced under cold stress and are released from hepatocyte mitochondria when the liver is damaged, accompanying an inflammatory response (Ji *et al*., 2020; Loomba *et al*., 2021). How Rongchang piglets with an immature immunity system will adapt to the cold environment and if they recover gradually under a lower immunity is important to determine.

Other studies have reported that cold stress also triggers oxidative stress (OS) in the body and causes tissue damage (Rathwa *et al*., 2017; Xue *et al*., 2018). The reason is that the cold stress causes damage to mitochondria and elevates the levels of reactive oxygen species (ROS) (Mateu-Sanz *et al*., 2021), such as the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-). However, a set of antioxidant systems such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) from *Micrococcus* can reduce oxidation in the body and further relieve oxidative damage (Cabello-Verrugio *et al*., 2017; He *et al*., 2021). The relationship between cold adaptation and antioxidant systems in Rongchang piglets remains unclear.

Therefore, this study hypothesized that in the early phase of cold stress, low temperatures would induce liver damage and oxidative stress in Rongchang piglets, such that their immature immune systems would not exert a primary protective role. However, in the later phase of cold stress, Rongchang piglets are hypothesized to adapt to the cold, which is probably related to anti-oxidative stress.

Material and Methods

The kits for detecting alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TCHO), triglycerides (TG), total protein (TP), albumin (ALB), and globulin (GLB) in the serum were purchased from Beijian Xinchuanyuan Biotechnology Cooperation (Beijing, China). The kits for detecting interleukin 2 (IL-2), interleukin 4 (IL-4), and interleukin 8 (IL-8) in the serum were offered by R&D Systems Inc. The kits for detecting immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) were provided by Beijing Jinhai Keyu Biotechnology Development Cooperation (Beijing, China). The kits for detecting malondialdehyde (MDA), total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), and CAT (catalase) were supplied by Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

This experiment mainly investigated the cold adaptation ability of Rongchang piglets during the latter half of cold stress. The thirty healthy, Rongchang piglets (2–3 days) provided from a Rongchang district farm (Chongqing, China) were randomly divided into a cold stress group (n = 15) and a control group (n = 15). The piglets in the control group were held in a constant temperature incubator (temperature = 22.80–25.70 °C, humidity = 46–66 %) and the piglets in cold stress group were kept in a piglet incubator (temperature = 13.20–16.20 °C, humidity = 77–93%; Qingdao Pairu Environmental Technology Co., Ltd, Shandong, China). All piglets were free to eat and drink under the same humidity, feed, light, personnel, feeding time, and other environmental conditions, except temperature. According to the experimental design, 3–5 ml blood was collected from the anterior vena cava on the 15th and 30th day.

The levels of ALT, AST, TCHO, TG, TP, ALB, and GLB were determined using a fully automated blood biochemistry analyzer (Toshiba FR120 fully automated biochemistry instrument, Japan) (Ding *et al*., 2022; Liu *et al*., 2022). After they were naturally stratified for 2 h, the blood samples were centrifuged at 3500 rpm for 10 min at room temperature and the upper serum was collected. The levels of IL-2, IL-4, IL-8, IgA, IgG, and IgM were measured using enzyme-linked immunosorbent assay (ELISA) and the optical density of each sample was finally measured at 450 nm using an enzyme-labelled instrument (MK3 enzyme marker, THERMO).

The levels of MDA, T-AOC, GSH-PX, and CAT were measured using the colorimetric method. The assay was performed using instructions, and the optical density of each sample was finally measured at 450 nm using an enzyme-labelled instrument (MK3 enzyme marker, THERMO).

All experimental results were obtained in three replicates and presented as mean ± standard deviation (SD). Data were analysed using one-way ANOVA in Graphpad Prism 8. Differences with P <0.05 were considered significant.

Results

The liver was analysed as it is an important organ for energy metabolism. On the 15th and 30th days, the average content of AST and ALT in the cold stress group were 1.5 times and 2.1 times higher, respectively, than that of control group (Figure 1A, B; P <0.01), and there was no increasing trend later (Figure 1A, B; blue line), whereas the contents of total cholesterol (TCHO) and triglycerides (TG) in the cold stress group were lower than that of control group (Figure 1C, D). In the cold stress group, total cholesterol showed no increasing trend in the second half of the experiment (Figure 1C, D), whereas the TG increased markedly and approached the normal by 30 d. These results indicate that Rongchang piglets had abnormal liver function after mild cold stress, but the degree of damage did not continue to increase, suggesting they had cold adaptation.

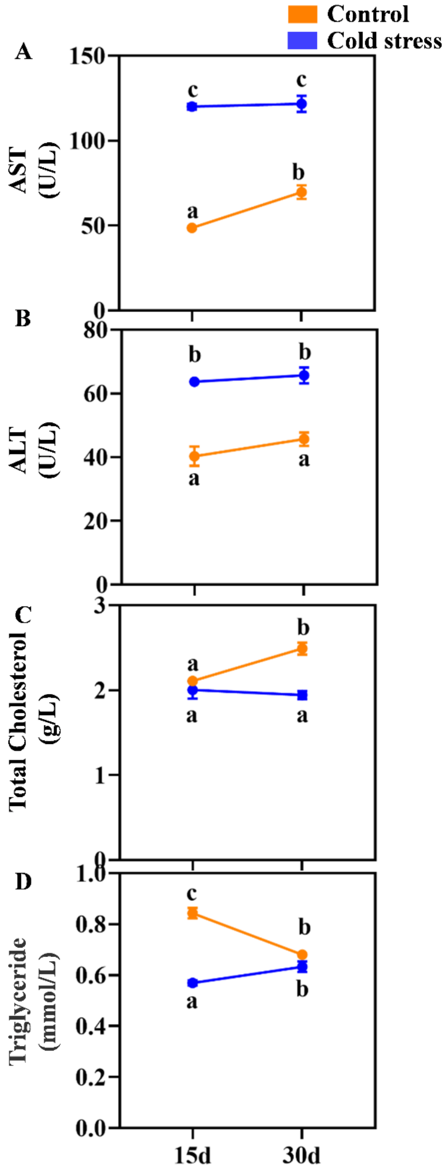

Figure 1 Effect of mild cold stress on liver energy metabolism of Rongchang piglets
Effects of mild cold stress on aspartate aminotransferase (A), alanine aminotransferase (B), total cholesterol (C) and triglyceride (D)

Body immunity was analysed because cold stress will impact immunity. The total protein (TP) content and ratio of GLB to ALB in the cold stress group were lower than that in control group (Figure 2A, B), resulting from the low levels of GLB (Figure 2C), not ALB (Figure 2D), after being analysed.

Overall, the interleukins and immunoglobulins of the cold stress group were lower than that of the control group. However, the disparity in IL-2, IgG, and IgM between the cold-stressed group and the control group was reduced (Figure 2 E, G, M); only IgA in the cold-stressed group increased by 19% (*P* < 0.05, Figure 2H). IL-4 and IL-8 tended to decrease in the cold stress group (Figure 2F and G). Therefore, we can preliminarily speculate that cold stress caused damage to the immune function of the Rongchang piglets, but with time, IgA, as a primary immune factor, participated in the immune process such that the body could recover.

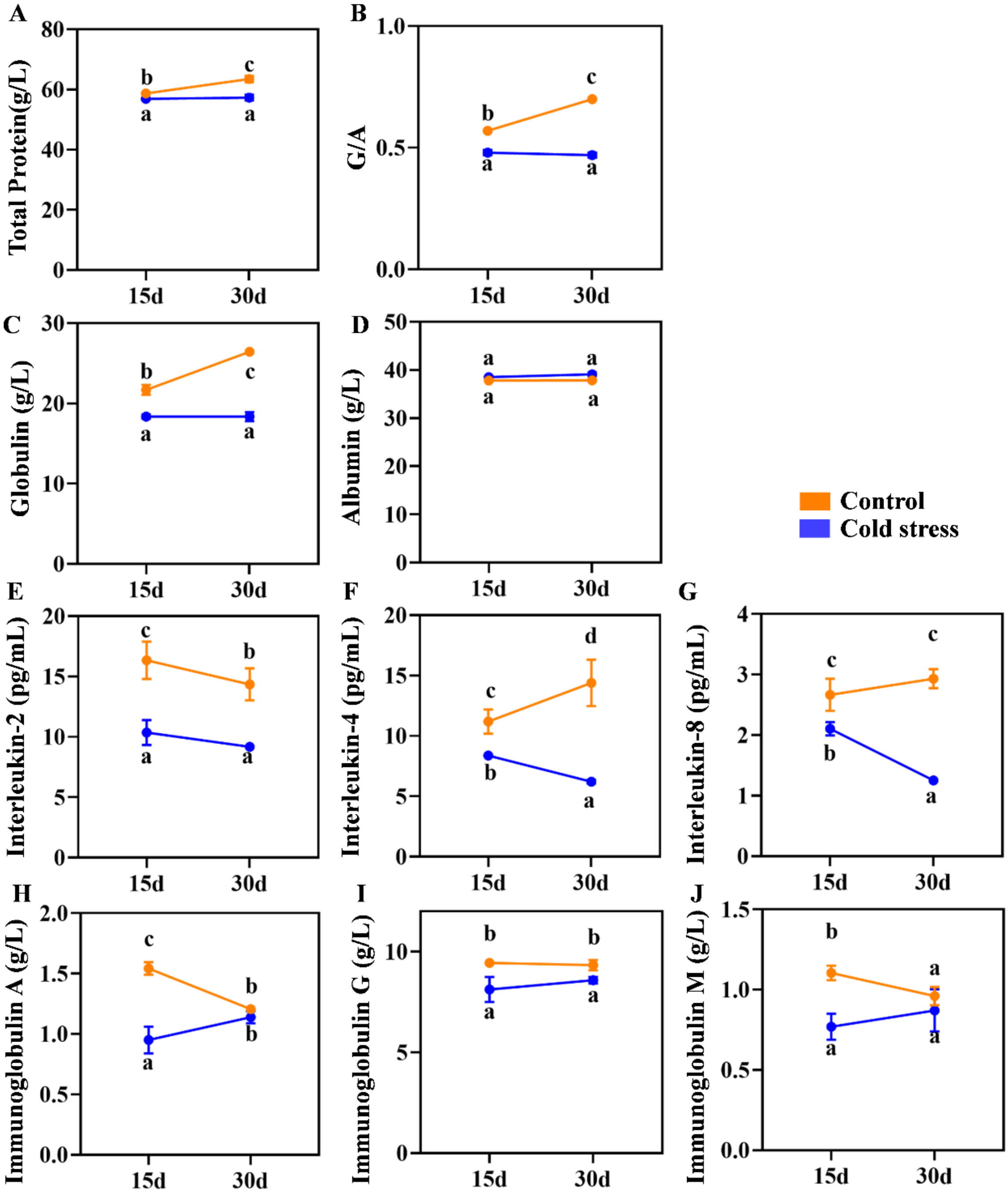

Figure 2 Effect of mild cold stress on immune function of Rongchang piglets
Effects of mild cold stress on total protein (A), globulin (B), globulin (C), albumin (D), inflammatory factor 2 (E), inflammatory factor 4 (F), inflammatory factor 8 (G), immunoglobulin A (H), immunoglobulin M (I), immunoglobulin G (J)

Oxidative stress needs to be elucidated due to the immature immune system of Rongchang piglets. Malondialdehyde (MDA), the oxidized product of reactive oxygen species (ROS), and lipid peroxidation can be used to measure the degree of oxidative damage. The experimental results suggest that, compared with the control group, the level of MDA was markedly higher at 15 d, gradually decreased from 15 to 30 d, and showed no marked difference on the 30th day (Figure 3A).

The total antioxidation capability (T-AOC) level in the cold stress group was lower than that in the control group but was enhanced with increasing treatment time (Figure 3B). Analysis of the antioxidant system indicated that glutathione peroxidase (GPH-Px) and catalase (CAT) played a crucial role and increased substantially by 12% and 17% from 15 to 30 d (Figure 3C, D), whereas superoxide dismutase (SOD) showed no remarkable change (Figure 3E). Therefore, we can surmise that the oxidative damage occurred in Rongchang piglets in the early stage of cold treatment and that GPH-Px and CAT were the main factors in antioxidant stress.

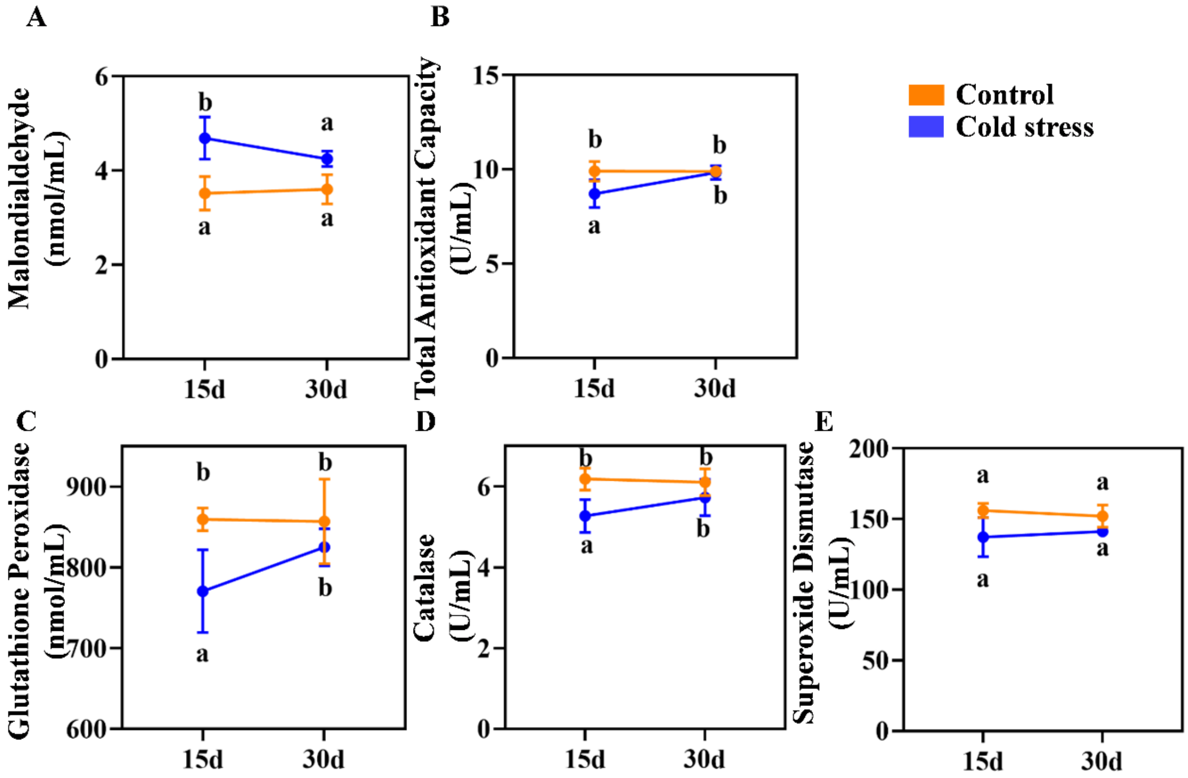

Figure 3 Effect of mild cold stress on oxidative stress of Rongchang piglets
Effects of mild cold stress on malondialdehyde (A), total antioxidant capacity (B), glutathione peroxidase (C), hydrogen peroxide (D) enzyme, superoxide dismutase (E)

Discussion

This study indicated that low temperature induced liver damage in Rongchang piglets via levels of AST and ALT, with oxidative damage caused by MDA. However, with time, the liver gradually recovered from damage, and the antioxidant stress system was enhanced. During the latter stages of the experiment, IgA increased, whereas the antioxidant stress system represented by GSH-Px and CAT tended to recover. GPH-Px and CAT were the main factors in antioxidant stress and played an important role.

The purpose of the study was to investigate the mechanism of cold acclimatization of Rongchang piglets. The liver is the main organ of energy metabolism and can produce ATP by breaking down liver glycogen and fatty acids (Zhang *et al*., 2021). Under normal conditions, AST and ALT exist in the mitochondria of the hepatocytes and are released into the blood when the liver is damaged (Ji *et al*., 2020; Loomba *et al*., 2021). It was found that the AST and ALT in the cold stress group were substantially higher than those in the control group, which is consistent with the report of Ali & Rifaai (2019). However, there was no marked fluctuation in AST and ALT in the cold stress group in this experiment, suggesting that liver injury was not exacerbated, providing the possibility of recovery and adaptation of the organism.

Confronted with liver injury and inflammation, the immune system was analysed. However, the interleukin and immunoglobulin levels in the cold stress group were lower than those in the control group with the immature immune function of piglets (Zhang *et al*., 2020; Chen *et al*., 2022). However, only the IgA appeared to increase after cold treatment, probably because it is involved in mucosal immunity. This is consistent with the results of Lai (2019), where the diarrhoea rate reduced during the late phase of cold stress (Lai *et al*., 2019).

Due to the close relationship between the immune system and oxidative stress, the oxidative stress and anti-oxidative stress systems were analysed in this study. The MDA content (indicative of the degree of oxidative damage) suggested that cold stress led to oxidative stress, i.e., oxidative damage (Chen and Zhong, 2014). However, the level of the antioxidant stress system (represented by GPH-Px and CAT) gradually increased with time, which was probably due to the activation of the Nrf2 signalling pathway by oxidative stress (Ma, 2013). GPH-Px, located downstream, catalysed reduced glutathione to the oxidized form, whereas CAT reduced hydrogen peroxide to water and oxygen (Figure 4). In addition, SOD showed no marked changes, indicated that cold stress did not affect the superoxide anion (O_2^-). GPH-Px requires selenium for its protein structure, and this needs to be obtained extrinsically. Therefore, we speculate that the oxidative stress caused by cold stress in this experiment was induced by superoxide and selenium supplementation may have the effect of promoting cold adaptation in Rongchang piglets.

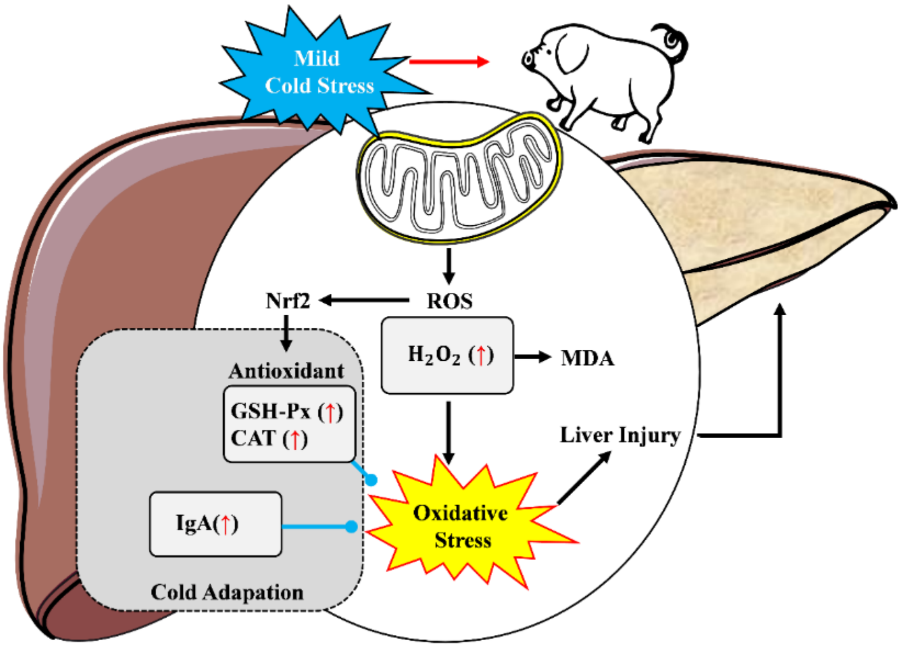

Figure 4 Mechanism of cold adaptation in Rongchang piglets

Conclusions

These results demonstrate that mild cold stress leads to liver damage, decreased immunity, and oxidative stress in Rongchang piglets. However, cold adaptation is achieved through the synergistic effect of immune regulation involving IgA and the involvement of GPH-Px and CAT in hydrogen peroxide catabolism. It provides a theoretical and experimental basis for exploring cold adaptation in Rongchang piglets.

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Author contributions

JC, ZY: Data Curation (equal); Formal analysis (lead); Visualization equal (equal); Writing—Original Draft Preparation (equal). XL， ZT, TZ: Data Curation (equal); Visualization equal (equal); Writing—Original Draft Preparation (equal). XW, JY, ZG: Conceptualization (equal); Writing—Review & Editing (equal). PW, LC: Conceptualization (equal); Funding Acquisition (lead); Project Administration (equal); Resources (lead); Supervision (equal); Writing—Review & Editing (equal). All authors have read and agreed to the published version of the manuscript.

Conflict of interest declaration

The authors declare no conflict of interests.

Institutional review board statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by Chongqing Academy of Animal Science's Institutional Animal Care and Use Committee.

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