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Original article

Chlorogenic acid supplementation regulates redox status, hepatic inflammation, and mitochondrial function in weaned piglets with intrauterine growth retardation

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Abstract

This study was designed to investigate the effect of chlorogenic acid (CGA) on redox status, hepatic inflammation, and mitochondrial function in weaned piglets with intrauterine growth retardation (IUGR). In total, 24 IUGR male piglets (1.59±0.11 kg) and 24 same-sex normal birth weight (NBW) littermates (0.94±0.06 kg) were selected at birth. After weaning at 26 days of age, NBW and IUGR piglets were fed either a basal diet or a CGA-supplemented diet for 21 days. Thus, all piglets were distributed into groups of 4 treatments × 6 replicates × 2 piglets per replicate. The IUGR piglets showed 18.05%, 28.59%, 23.78%, 27.17%, and 18.61% lower hepatic absolute weight, total superoxide dismutase (T-SOD) activity, GSH, ATP, and complex I levels, and 103.77% and 61.32% higher interleukin 1 beta (IL1β) content and IL18 mRNA expression, respectively, than the NBW piglets. Serum MDA concentration was 34.76% higher and glutathione peroxidase (GPX) activity was 24.18% lower in the IUGR piglets than in the NBW piglets. CGA supplementation decreased serum alanine aminotransferase activity by 29.44%, increased hepatic T-SOD activity by 24.43%, and upregulated complex I concentration by 21.76%. CGA supplementation reversed the IUGR-induced decreases in the activities of serum T-SOD and GPX and hepatic ATP level, increased hepatic IL1β level and IL18 mRNA expression in piglets. A diet supplemented with CGA reduced hepatic MDA content in the IUGR piglets. The results indicate that CGA may improve redox status, inflammation, and mitochondrial function, ultimately alleviating hepatic injury in IUGR piglets.

Keywords: chlorogenic acid, intrauterine growth retardation, liver injury, redox status, piglets

Introduction

A series of problems caused by intrauterine growth retardation (IUGR) have been investigated in the context of swine production and human health. IUGR refers to the failure of a fetus to achieve the expected in utero growth potential due to various genetic and environmental insults (Sankaran et al. 2009), with a higher incidence rate up to nearly 20% in neonatal piglets (Zhang et al. 2019b). The liver is the most important organ for nutrient metabolism and immune defense (Tang et al. 2023). IUGR would alter the normal maturation trajectory of the liver and subsequently disrupt its structure and physiological function from the fetal period to adulthood (Zhang et al. 2019a, Cheng et al. 2021), which is a critical contributor to higher perinatal mortality and morbidity, as well as impaired growth, metabolism, and carcass quality. Evidence shows that the liver from IUGR individuals exhibited compromised antioxidant capacity, immune imbalance, glycolipid metabolism disorder, endoplasmic reticulum stress, mitochondrial dysfunction and increased cell apoptosis, and ultimately tissue damage (Liu et al. 2018, Cheng et al. 2020, Cheng et al. 2021, Zhang et al. 2021). Therefore, there is an urgent need to develop effective treatment strategies for reducing liver damage and the associated diseases in IUGR offspring.

Chlorogenic acid (CGA), a phenolic compound generally formed by the condensation of quinic acid and caffeic acid, is abundant in honeysuckle, *Eucommia ulmoides* leaves, coffee beans, and tea (Upadhyay et al. 2013). Multiple in vitro and in vivo studies have demonstrated that CGA possesses a series of biological activities, including antioxidant, anti-inflammatory, antimicrobial and immunomodulatory effects (Hu et al. 2006, Miao et al. 2020, Cheng et al. 2023). An increasing body of experimental data indicates that CGA has excellent hepatoprotective effects against various liver diseases such as drug-induced liver injury (Hu et al. 2020), alcoholic liver disease (Kim et al. 2018), metabolic dysfunction-associated fatty liver disease (Shi et al. 2021), cholestatic liver disease (Tan et al. 2016), liver fibrosis (Wang et al. 2017) and liver cancer (Yan et al. 2017), which can be partially attributed to the activation of erythroid 2-related factor 2 (Nrf2) and the inhibition of toll-like receptor 4 (TLR4)/nuclear factor-κB (NF-κB) signaling pathways (Xue et al. 2023). Additionally, CGA has been reported to ameliorate endotoxin-induced liver injury by improving mitochondrial oxidative phosphorylation, as evidenced by the decreased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, the increased ATP content and activities of enzymes involved in oxidative phosphorylation (Zhou et al. 2016). However, it remains unclear whether CGA supplementation alleviates IUGR-induced hepatic damage in neonates. Therefore, this study aimed to elucidate the effects of CGA on the redox status, hepatic inflammation and mitochondrial function in IUGR piglets with the intention of providing an effective strategy to improve hepatic disorder in neonates induced by IUGR.

Materials and Methods

Experimental animals and design

The study was approved by the Henan University of Technology Institutional Animal Care and Use Committee (No. HAUT20230301). The selection criteria for NBW and IUGR (Duroc × [Landrace × Yorkshire]) piglets in the same litter were performed according to a previous study (Cheng et al. 2021); briefly, piglets with a birth weight (BW) near the population mean (<0.5 standard deviations) were labeled as normal BW (NBW) piglets, and those with 2 standard deviations lower than the population mean were labeled as IUGR piglets. 24 male NBW piglets (1.59±0.11 kg) and 24 same-sex IUGR piglets (0.94±0.06 kg) were selected at birth. After weaning at 26 days of age, they were assigned to the NC group (NBW piglets received a basal diet), the NG group (NBW piglets received a basal diet containing 1000 mg/kg CGA), the IC group (IUGR piglets received a basal diet) and the IG group (IUGR piglets received a basal diet containing 1000 mg/kg CGA). CGA was administered by mixing it into the basal diet. Each group had six replicates/pens and each replicate had two piglets. CGA was purchased from Hunan E.K. Herb Co. Ltd (Changsha, China) with a purity of 98%. The piglets were fed a diet based on corn, flour, and soybean meal (18.30% crude protein, 14.42 MJ/kg digestible energy, 0.78% calcium, 0.38% available phosphorus). The composition and nutrient levels of the basal diet were described in a previous study (Cheng et al. 2025), which was based on the NRC requirements (2012). Piglets had free access to feed and water. Initial body weight (IBW), final body weight (FBW), and the daily feed intake of piglets were weighed per pen, and the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) over the entire experimental period were then calculated.

Sample collection

On d 21 of the experiment, one piglet per replicate/pen was selected for sample collection and weighed after a 12-h fasting. Blood sample was collected from the anterior vena cava and centrifuged at 2000 × g for

Table 1. List of primers used in RT-qPCR.

Items[1]	Forward primer sequence (5' -> 3')	Reverse primer sequence (5' -> 3')
IL18	GCTGCTGAACCGGAAGACAA	CCGATTCCAGGTCTTCATCGT
p65	TGCCAGACACAGATGACCG	ATGGCGTAAAGGGATAGGGC
TNF	GCCCTTCCACCAACGTTTTC	CAAGGGCTCTTGATGGCAGA
GADPH	CCAAGGAGTAAGAGCCCCTG	AAGTCAGGAGATGCTCGGTG

[1] IL18, Interleukin 18; TNF, tumor necrosis factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

20 min at 4°C. The serum sample was stored at -80°C until analysis. All piglets were euthanized after anesthesia. The administration of the anesthetic agent (50 mg sodium pentobarbital/kg body weight, intramuscular injection) showed no influence on the physiological parameters assessed in the study. Subsequently, the entire liver was immediately collected and weighed to calculate the organ index. A fraction of chopped liver sample from the same left lobe was quickly snap frozen in liquid nitrogen, and stored at -80°C for subsequent analysis.

Serum parameters assay

Serum ALT (catalog No. C009-1-1), AST (catalog No. C010-1-1), total superoxide dismutase (T-SOD, catalog No. A001-1-2) and glutathione peroxidase (GPX, catalog No. A005-1-2) activities, malondialdehyde (MDA, catalog No. A003-1), total glutathione (T-GSH, catalog No. A061-2-1), reduced glutathione (GSH, catalog No. A061-2-1) and oxidized glutathione (GSSG, catalog No. A061-2-1) contents were measured using a microplate reader following the protocols from the manufacturer (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China).

Hepatic redox status assay

Each liver sample was homogenized with an iced saline solution at a weight-to-volume ratio of 1: 9 and then centrifuged at 4000 × *g* for 15 min at 4°C. The supernatant was collected for further analysis. Protein (catalog No. A045-3-2), MDA (catalog No. A003-1) and GSH (catalog No. A006-1-1) levels, T-SOD (catalog No. A001-1-2) and GPX (catalog No. A005-1-2) activities in the supernatant were determined with a microplate reader using commercial kits purchased from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China).

Hepatic cytokine analysis

Lnterleukin 1 beta (IL1β, catalog No. E78620) level in the liver was detected using a microplate reader and the commercial enzyme-linked immunosorbent assay (ELISA) kits purchased from Hongsheng Biotechnology Co. Ltd. (Nanjing, China).

Hepatic ATP and mitochondrial respiratory chain complex analysis

Hepatic ATP (catalog No. MM−32675O1), mitochondrial respiratory chain complex I (catalog No. MM−7758101), complex III (catalog No. MM−7757101) and complex V (catalog No. MM−7757301) contents were determined with a microplate reader using ELISA kits according to the manufacturer's protocol (Jiangsu Meimian Industrial Co. Ltd., Yancheng, China).

Hepatic gene expression measurement

Liver samples were used to extract total RNA using the Total RNA Isolation Reagent (catalog No. R401-01, Vazyme, Nanjing, China). The RNA concentration, reverse transcription and RT-qPCR procedure were performed, as previously described (Cheng et al. 2023). Expression of the target gene was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak et al. 2001) and individually normalized to *GAPDH* used as control. The primers used in this study are shown in Table 1.

Statistical analysis

SPSS statistical software (version 27.0; SPSS, Inc., Chicago, IL) was used for statistical analysis. Data were analyzed by the general linear model using a two-way analysis of variance. The classification variables were birth weight (NC + NG × IC + IG), diet (NC + IC × NG + IG), and the interaction between birth weight and diet (NC × NG × IC × IG). Tukey's post hoc test was used to explore all significant differences. The statistical significance level for all analyses was set at $p \leq 0.05$ and $0.05 < p < 0.10$ was considered as a trend. Data are presented as the mean and their pooled standard error (SEM).

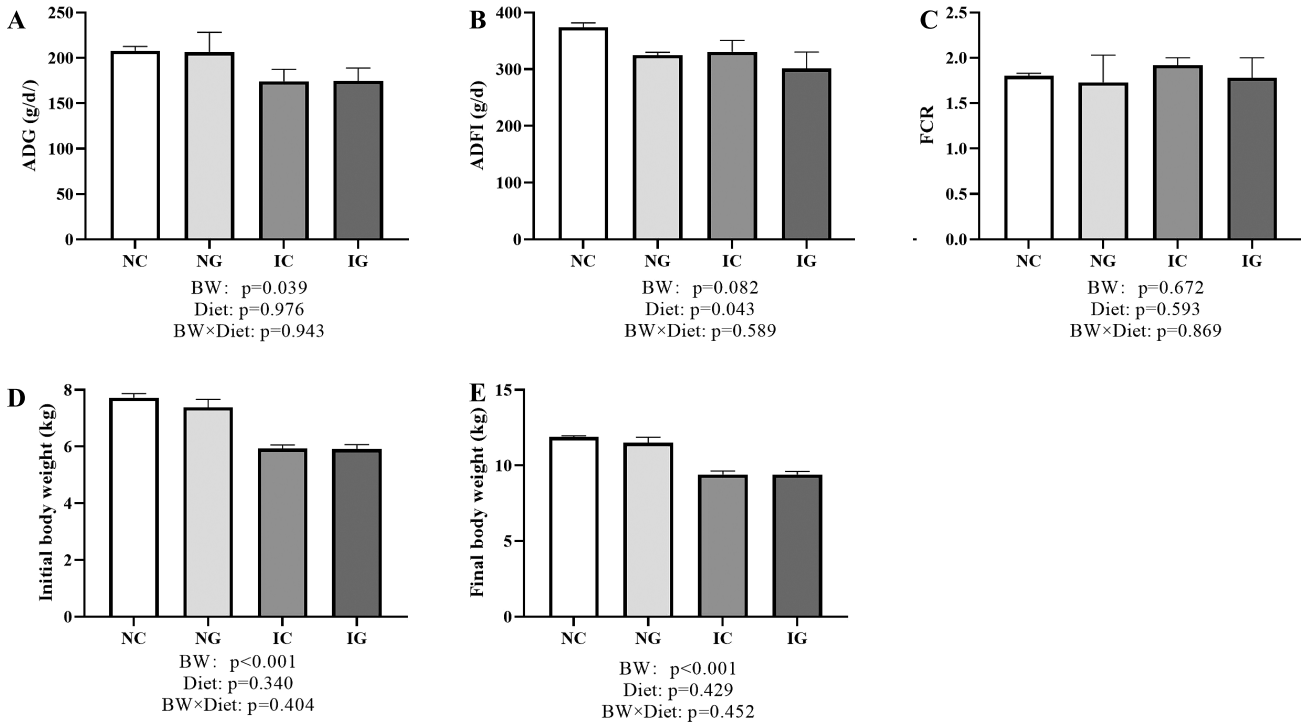

Fig. 1. Growth performance of weaned piglets with different birth weight receiving different dietary treatments. (A) ADG, average daily gain; (B) ADFI, average daily feed intake; (C) FCR, feed conversion ratio; (D) initial body weight; (E) final body weight. NC, normal birth weight piglets received a basal diet; NG, normal birth weight piglets received a chlorogenic acid-supplemented diet; IC, intrauterine growth retardation piglets received a basal diet; IG, intrauterine growth retardation piglets received a chlorogenic acid-supplemented diet. Results are expressed as means and standard errors.

Results

Growth performance

Compared with the NBW piglets, ADG (Fig. 1A, p=0.039), ADFI (Fig. 1B, p=0.082), IBW (Fig. 1D, p<0.001), and FBW (Fig. 1E, p<0.001) were lower in the IUGR piglets. Dietary CGA supplementation decreased ADFI in weaned piglets regardless of BW when compared with the basal diet (p=0.043). Treatments did not affect the FCR in weaned piglets (Fig. 3C, p>0.05). There was no interaction between BW and diet on the growth performance in weaned piglets (p>0.05).

Hepatic damage

When compared with the NBW piglets, IUGR decreased absolute weight of the liver (Fig. 2A, p=0.005) in weaned piglets. Serum ALT activity (Fig. 2C, p=0.024) was lower in piglets receiving the basal diet supplemented with CGA than that in piglets receiving the basal diet. A significant interaction of BW and diet was observed for serum AST activity in piglets (Fig. 2D, p=0.037); the increment in serum AST activity in the IUGR piglets was not reversed by CGA supplementation (p>0.05). However, relative weight of the liver (Fig. 2B, p>0.05) in piglets was not affected by these treatments.

Redox status in serum and liver

Table 2 shows that the IUGR piglets had a higher level of MDA (p=0.012) and lower activities of T-SOD (p=0.007) and GPX (p<0.001) in serum than the NBW piglets. CGA supplementation reversed the IUGR-induced decrease in the activities of T-SOD (p=0.014) and GPX (p=0.021) in piglets. Neither BW or diet had any effect on levels of T-GSH, GSSG, GSH, and GSH/GSSG in the serum of piglets (p>0.05).

Hepatic SOD activity (Fig 3B, p=0.001) and GSH level (Fig. 3D, p=0.003) in the IUGR piglets were lower than that in the NBW piglets. The diet supplemented with CGA enhanced hepatic T-SOD activity (p=0.012) in piglets (p<0.05), and reduced hepatic MDA content (Fig. 3A) in the IUGR piglets (p<0.05). No difference was observed in the activity of hepatic GPX among the groups (Fig. 3C, p>0.05).

Hepatic inflammation

Hepatic levels of IL1β (Fig. 4A, p<0.001) and *IL18* mRNA (Fig. 4B, p=0.013) were significantly higher in the IUGR piglets than in the NBW piglets.

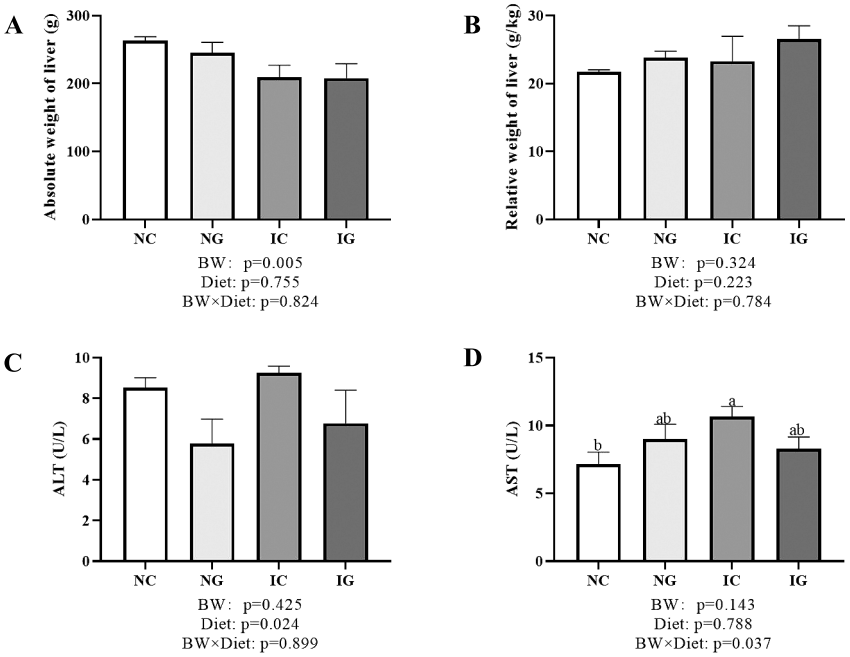

Fig. 2. Hepatic weight and serum parameters in weaned piglets with different birth weight receiving different dietary treatments. (A) absolute weight of liver; (B) relative weight of liver; (C) ALT, serum alanine aminotransferase activity; (D) AST, serum aspartate aminotransferase activity. NC, normal birth weight piglets received a basal diet; NG, normal birth weight piglets received a chlorogenic acid-supplemented diet; IC, intrauterine growth retardation piglets received a basal diet; IG, intrauterine growth retardation piglets received a chlorogenic acid-supplemented diet. Different letters on the error bars indicate significant differences ($p<0.05$). Results are expressed as means and standard errors.

Table 2. Serum redox status of weaned piglets with different birth weight receiving different dietary treatments.

Items	NC	NG	IC	IG	SEM	p_{BW}	p_{Diet}	$p_{BW\times Diet}$
MDA (nmol/mL)	4.67	3.99	5.54	6.13	0.31	0.012	0.930	0.246
T-SOD (U/mL)	101.37^{a}	89.98^{a}	43.74^{b}	86.79^{a}	6.77	0.007	0.129	0.014
GPX (U/mL)	89.59^{a}	92.98^{a}	56.40^{b}	82.02^{a}	3.85	<0.001	0.004	0.021
T-GSH (μmol/L)	2.74	2.95	3.11	3.15	0.18	0.459	0.745	0.833
GSSG (μmol/L)	0.60	0.57	0.78	0.60	0.04	0.187	0.178	0.325
GSH (μmol/L)	1.54	1.80	1.56	1.95	0.20	0.845	0.456	0.878
GSH/GSSG	2.95	3.26	2.10	3.79	0.46	0.869	0.311	0.476

MDA, malondialdehyde; T-SOD, total superoxide dismutase; GPX, glutathione peroxidase; T-GSH, total glutathione; GSSG, oxidized glutathione; GSH, reduced form of glutathione. NC, normal birth weight piglets received a basal diet; NG, normal birth weight piglets received a chlorogenic acid-supplemented diet; IC, intrauterine growth retardation piglets received a basal diet; IG, intrauterine growth retardation piglets received a chlorogenic acid-supplemented diet. Means within a row with different superscripts are different at $p<0.05$. Results are expressed as means and SEM.

The increased IL1β ($p<0.001$) and IL18 ($p=0.011$) mRNA levels in the liver induced by IUGR were inhibited due to CGA administration. No differences were observed in the mRNA expression of hepatic TNF (Fig. 4C) and p65 (Fig. 4D) among the groups ($p>0.05$).

Hepatic ATP and mitochondrial respiratory chain complex

As shown in Table 3, the IUGR piglets showed a decrease in levels of ATP ($p<0.001$) and complex I ($p=0.014$) in the liver compared with the NBW piglets. Dietary CGA supplementation increased the concentrations of hepatic ATP ($p<0.001$) and complex I ($p=0.017$) in piglets ($p<0.05$). A significant interaction between BW and diet was observed for ATP content in the liver of piglets; the decrease in hepatic ATP content in the IUGR piglets was not observed when IUGR piglets were supplemented with CGA ($p<0.001$). No differences were found in the hepatic complex III and complex V contents of piglets among these groups ($p>0.05$).

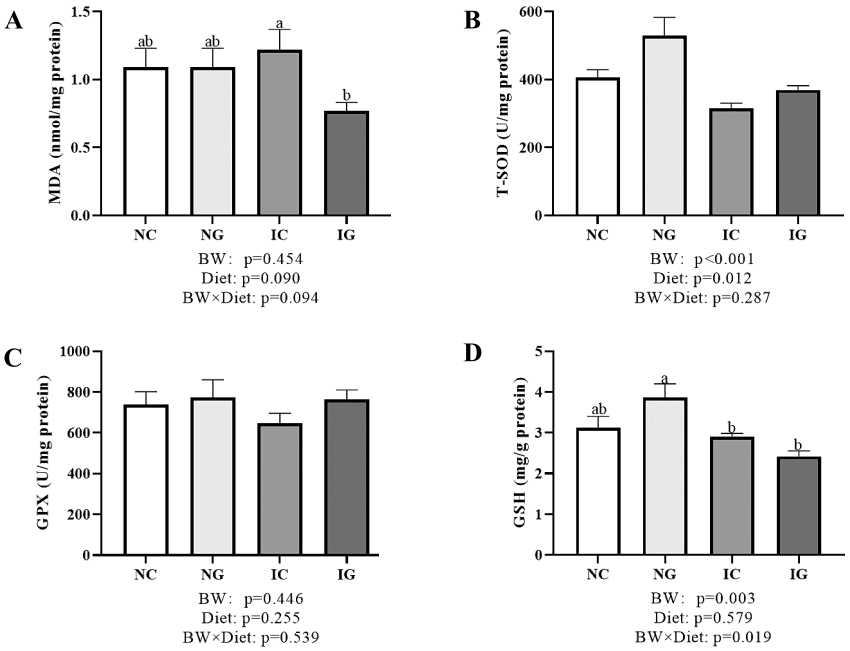

Fig. 3. Hepatic redox status in weaned piglets with different birth weight receiving different dietary treatments. (A) MDA, malondialdehyde; (B) T-SOD, total superoxide dismutase; (C) GPX, glutathione peroxidase; (D) GSH, reduced form of glutathione. NC, normal birth weight piglets received a basal diet; NG, normal birth weight piglets received a chlorogenic acid-supplemented diet; IC, intrauterine growth retardation piglets received a basal diet; IG, intrauterine growth retardation piglets received a chlorogenic acid-supplemented diet. Different letters on the error bars indicate significant differences ($p<0.05$). Results are expressed as means and standard errors.

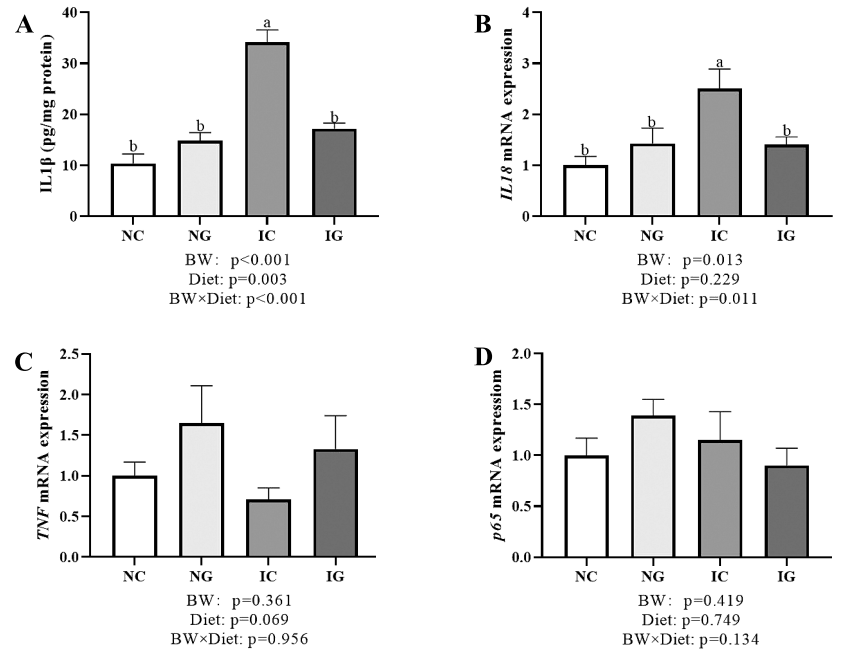

Fig. 4. Hepatic inflammation in weaned piglets with different birth weight receiving different dietary treatments. (A) IL1β, interleukin 1 beta concentration; (B) *IL18*, interleukin 18 mRNA expression; (C) *TNF*, tumor necrosis factor mRNA expression; (D) *p65* mRNA expression. NC, normal birth weight piglets received a basal diet; NG, normal birth weight piglets received a chlorogenic acid-supplemented diet; IC, intrauterine growth retardation piglets received a basal diet; IG, intrauterine growth retardation piglets received a chlorogenic acid-supplemented diet. Different letters on the error bars indicate significant differences ($p<0.05$). Results are expressed as means and standard errors.

Table 3. Hepatic mitochondrial function in weaned piglets with different birth weight receiving different dietary treatments.

Items	NC	NG	IC	IG	SEM	p_{BW}	p_{Diet}	$p_{BW\times Diet}$
ATP, nmol/g protein	254.51[a]	250.86[a]	101.17[b]	266.89[a]	16.52	<0.001	<0.001	<0.001
Complex I, ng/mg protein	13.55	16.28	10.85	13.43	0.64	0.014	0.017	0.938
Complex III, ng/mg protein	12.18	11.38	11.95	11.28	0.39	0.842	0.383	0.938
Complex V, ng/mg protein	6.21	5.25	5.28	5.16	0.25	0.330	0.307	0.418

ATP, adenosine triphosphate; NC, normal birth weight piglets received a basal diet; NG, normal birth weight piglets received a chlorogenic acid-supplemented diet; IC, intrauterine growth retardation piglets received a basal diet; IG, intrauterine growth retardation piglets received a chlorogenic acid-supplemented diet. Means within a row with different superscripts are different at $p<0.05$. Results are expressed as means and SEM

Discussion

Similar to previous studies (Zhang et al. 2021, Tang et al. 2023), the present study also showed that IUGR induced liver damage in weaned piglets, as evidenced by increased serum AST activity and lower absolute weight of liver. AST, an intracellular enzyme, is released into the blood circulation when the liver is damaged, and its activity can reflect the severity of liver injury. In addition, accumulation of serum MDA, decreased liver T-SOD activity, inhibited serum T-SOD and GPX activities, increased hepatic IL1β and IL18 mRNA expression were observed in the IUGR weaned piglets, which suggested that oxidative stress and inflammation occurred in the liver. These changes in the IUGR piglets may promote the initiation and development of liver injury. Considering the important roles that the liver plays in nutrient metabolism and immune response, hepatic damage induced by IUGR partly contributed to the inferior growth performance in the IUGR weaned piglets. As expected, in the present study, CGA alleviated the IUGR-induced hepatic injury by enhancing antioxidant defense capacity, inhibiting inflammation and improving mitochondrial oxidative phosphorylation.

In the present study, CGA stimulated hepatic T-SOD activity and serum GPX activity in piglets. As an important part of the endogenous antioxidant defense system, SOD catalyses the production of harmless hydrogen peroxide from the highly reactive, unstable superoxide anion, and plays a role in preventing and alleviating oxidative stress (Islam et al. 2022). Moreover, treatment with CGA reversed the decreased activities of serum T-SOD and GPX as well as hepatic MDA content in the IUGR piglets, suggesting that CGA alleviated oxidative stress in the IUGR piglets. Previous studies conducted on rodents have consistently reported that CGA can reverse the increase in various toxic substance-induced hepatic oxidative damage by stimulating antioxidant defense enzyme activity through activation of the Nrf2 signaling pathway (Hu et al. 2020, Hussein et al. 2021, Cheng et al. 2023). A similar effect of CGA on hepatic redox status was observed in broilers challenged with diquat (Zha et al. 2023), and hydrogen peroxide (Song et al. 2024). In pigs, supplementation with CGA has also been shown to improve serum, intestinal and muscular antioxidant capacity evidenced by increased GPX, T-SOD, and catalase activities (Chen et al. 2018, Xie et al. 2023). In addition, the antioxidant activity of CGA is at least partly due to its special molecular structure. It contains five active hydroxyl groups, which easily react with free radicals and can form hydrogen radicals to eliminate hydroxyl radicals and superoxide anions (Miao et al. 2020). Thus, in this study, the improvement of hepatic redox status by CGA may be due to the enhanced activity of T-SOD and its molecular structure, which plays a beneficial role in the alleviation of hepatic injury induced by IUGR in piglets.

The anti-inflammatory activity of CGA is another key factor promoting the recovery of hepatic inflammation and damage in IUGR piglets. As expected, CGA supplementation restored the IUGR-induced increases in hepatic IL1β level and IL18 mRNA abundance. Huang et al. (2022) reported that CGA suppressed the contents of IL1β and IL18 in lipopolysaccharide-induced Human gingival fibroblasts (Huang et al. 2022). The available literature indicates that CGA treatment attenuated hepatic inflammation in experimental animals caused by ethanol (Buko et al. 2021), CCL4 (Shi et al. 2016), cadmium (Ding et al. 2021), and high fructose (Alqarni et al. 2019), the mechanism of which was partially recognized as the regulation of TLR4/NFκB signaling cascade. However, in this study, hepatic NF-κBp65 mRNA expression in the IUGR piglets was not altered by CGA supplementation, suggesting that the possible mechanism of CGA against hepatic inflammation in the IUGR piglets was associated with the post-translational modification of NFκBp65 rather than the transcriptional level. The detailed mechanism of CGA in suppressing hepatic inflammation in the IUGR piglets needs to be investigated in the future. As expect-

ed, dietary CGA administration inhibited the phosphorylation of NFκB in the jejunum and ileum of weaned piglets, and reduced the secretion of cytokines including IL1β and IL22 (Zhang et al. 2024). The CGA-induced improvement in hepatic inflammation has also been found by Zha et al. (2023) in broilers exposed to diquat.

Mitochondria are highly enriched in liver tissue and are especially crucial to the liver due to its high energy demands. Five enzymatic complexes (i.e., complexes I, II, III, IV, and V) of the mitochondrial respiratory chain drive the production of ATP, which is used as a primary energy carrier in virtually all cellular processes (Vercellino et al. 2022). In recent years, the irreplaceable role of mitochondria in hepatic damage induced by IUGR has been gradually discovered. CGA increased the ATP and complex I levels in the liver of piglets, and also, CGA recovered the decreased generation of ATP in the liver of the IUGR piglets. These results indicate that the improvement in intracellular energy production resulting from CGA supplementation may be related to the enhanced complex I content. Previous studies have found that the hepatoprotective effect of CGA against endotoxin (Zhou et al. 2016), ischemia and reperfusion (Mu et al. 2015) may be associated with enhanced ATP production and stimulation of mitochondrial oxidative phosphorylation, and the mechanism was the activation of AMP-activated protein kinase or sirtuin 3 signaling pathways. Collectively, the beneficial effects of CGA on mitochondrial oxidative phosphorylation may aid in the recovery of the hepatic damage in the IUGR piglets. Furthermore, the results may provide a potential explanation for the antioxidant and anti-inflammatory action of CGA in the liver of IUGR piglets, since mitochondria play an important role in reactive oxygen species production and cytokine release (Begriche et al. 2006). However, additional studies will be needed to fill in more molecular details in this process.

In conclusion, the results of this study suggest that CGA supplementation could protect against hepatic damage in IUGR piglets, and the possible mechanism is associated with the improvement of redox status, inflammatory response, and mitochondrial oxidative phosphorylation. This study may help in the development of appropriate nutritional interventions to alleviate hepatic damage in the IUGR piglets.

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