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# N-acetylcysteine and intestinal health: a focus on mechanisms of its actions

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## 1. ABSTRACT

The integrity of the intestinal epithelium ensures its normal physiological function. Consequently, damage to the mucosal epithelium can impair the absorption of nutrients, thereby reducing the growth performance and compromising the health of animals. N-acetylcysteine (NAC) is pharmaceutically available either intravenously, orally, or by inhalation for reducing endothelial dysfunction, inflammation, fibrosis, invasion, cartilage erosion, acetaminophen detoxification, and transplant prolongation. NAC is rapidly metabolized by the small intestine to produce glutathione and can not be detected in animals without supplementation. The physiologic functions and therapeutic effects of NAC are largely associated with maintaining intracellular concentrations of reduced glutathione. Results from recent studies indicate that NAC reduces inflammation, alleviates oxidative stress, improves energy status, and ameliorates tissue damage in the intestine of lipopolysaccharide-challenged piglets. Moreover, dietary supplementation with NAC ameliorates acetic acid-induced colitis in a porcine model. The effects of NAC are associated with some intestinal cell signaling pathways, such as EGFR, TLR4, apoptosis and tight junction signaling. The current review focuses on the protective effects of NAC on intestinal health and the molecular mechanisms of its action.

## 2. INTRODUCTION

The intestinal integrity plays an essential role in nutrition (1-2), metabolism (3-4), and whole-body homeostasis (5-7). The gastrointestinal tract is important as the first defense of the body against bacteria-derived endogenous and exogenous harmful agents. Neonates are prone to various stresses, such as early-weaning, infection, and inflammatory bowel disease, resulting in intestinal mucosal injury and absorptive dysfunction (1, 8-9). Intestinal dysfunction severely impairs the health and growth of both humans and animals (particularly piglets).

N-acetylcysteine (NAC) is a thiol, a mucolytic agent, and an acetylated precursor of L-cysteine and reduced glutathione (GSH) (10). NAC is pharmaceutically available either intravenously, orally, or by inhalation (11). NAC is a source of sulfhydryl groups in cells and a scavenger of free

**Table 1.** Effects of N-acetylcysteine supplementation on the growth performance of weanling piglets after LPS challenge (10-20 days)

| Items | +LPS | | -LPS | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | -NAC | +NAC | -NAC | +NAC | | LPS | NAC | NAC×LPS |
| N | 6 | 6 | 6 | 6 | | | | |
| Initial BW, kg | 15.7 | 15.4 | 15.7 | 16.0 | 0.78 | 0.58 | 0.76 | 0.87 |
| Final BW, kg | 18.3 | 18.6 | 20.9 | 21.0 | 0.57 | 0.08 | 0.75 | 0.97 |
| ADG, g | 262 | 317 | 520 | 490 | 24 | 0.001 | 0.30 | 0.04 |
| ADFI, g/d | 656 | 666 | 950 | 913 | 67 | 0.009 | 0.72 | 0.83 |
| F/G | 2.52 | 2.11 | 1.84 | 1.86 | 0.22 | 0.04 | 0.82 | 0.34 |

Values are means with pooled SEM, n = 6. Adapted from Hu *et al.* (16). BW, body weight; F/G: feed/gain ratio; SEM, standard error of the mean.

**Table 2.** Effects of dietary NAC supplementation on the growth performance of piglets after LPS challenge

| Items | Control | LPS | LPS + NAC |
|---|---|---|---|
| Body weight at day 10 of the trial (kg) | 15.4 ± 0.55 | 15.7 ± 0.54 | 16.0 ± 0.63 |
| Average daily feed intake between days 10 and 20 of the trial (g/day) | 657 ± 60.3 | 664 ± 58.6 | 667 ± 61.2 |
| Average daily weight gain between days 10 and 20 of the trial (g/day) | 310 ± 12.3$^{a}$ | 269 ± 11.8$^{b}$ | 289 ± 13.0$^{a}$ |

Data are means ± SD, n = 6. Control (non-challenged control) = piglets fed the basal diet and injected with sterile saline; LPS (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; LPS + NAC (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg and challenged with LPS. a-b: Values within a row with different letters differ ($P$<0.05). Adapted from Hou *et al.* (17).

radicals as it interacts with reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radical (12). Therefore, NAC plays an important role in protecting cells against oxidative stress (13). Orally administered NAC is very rapidly utilized by the intestine to produce glutathione (14-15). There is growing evidence that dietary supplementation with NAC could improve intestinal morphology and function in animals, including piglets. The current review focuses on recent developments in the studies regarding protective effects of NAC on intestinal functions of mammals (particularly pigs) and the underlying molecular mechanisms.

## 3. GROWTH PERFORMANCE OF ANIMALS

Growth performance is a major criterion used to evaluate outcomes of animal production. Dietary supplementation with 0.05% NAC increased daily body weight gains of lipopolysaccharide (LPS)-challenged pigs (Tables 1 and 2) (16-17). This means that NAC could relieve growth depression in weaned piglets chronically challenged by the endotoxin. In contrast, in the absence of stress, administration of NAC may not affect the growth of young pigs in a short-term, as reported for piglets receiving 0.05% NAC as a supplement in a typical corn- and soybean meal-based diet (16).

## 4. NAC AND INTESTINAL ACTIONS

### 4.1. Intestinal morphology

The indicators of the small-intestinal morphology are villus height, crypt depth, the ratio of villus height to crypt depth, and villous surface area. Usually, an increase in villus height, villous surface area, or villus/crypt ratio corresponds to improvement in intestinal absorption capacity and health (17-19). There is evidence that dietary supplementation with NAC could improve intestinal histological morphology. For example, NAC supplementation has the following effects: (1) increased villus height; (2) decreased crypt depth; (3) an increased ratio of villus height to crypt depth; and (4) increased villous surface area in the small intestinal mucosa of LPS-challenged piglets (Table 3) (17). Notably, dietary supplementation with

**Table 3.** Effects of NAC supplementation on the intestinal mucosal morphology of pigs after LPS challenge

| Items | Control | LPS | LPS+NAC |
|---|---|---|---|
| Villus height, μm | | | |
| Duodenum | 388.17 ± 14.64 | 376.83 ± 9.39 | 369.75 ± 17.26 |
| Jejunum | 421.15 ± 11.23$^{a}$ | 399.53 ± 12.78$^{b}$ | 406.11 ± 8.24$^{b}$ |
| Ileum | 375.43 ± 17.23$^{a}$ | 349.34 ± 13.80$^{b}$ | 373.68 ± 12.68$^{a}$ |
| Crypt depth, μm | | | |
| Duodenum | 102.98 ± 5.23$^{b}$ | 118.81 ± 7.08$^{a}$ | 113.28 ± 7.87$^{a}$ |
| Jejunum | 111.70 ± 6.86$^{b}$ | 122.81 ± 7.12$^{a}$ | 113.18 ± 7.41$^{b}$ |
| Ileum | 114.34 ± 10.16 | 117.10 ± 9.96 | 112.59 ± 5.03 |
| Villus height/crypt depth | | | |
| Duodenum | 3.78 ± 0.21$^{a}$ | 3.18 ± 0.13$^{b}$ | 3.28 ± 0.25$^{b}$ |
| Jejunum | 3.78 ± 0.17$^{a}$ | 3.26 ± 0.21$^{b}$ | 3.61 ± 0.29$^{a}$ |
| Ileum | 3.31 ± 0.40 | 3.01 ± 0.32 | 3.32 ± 0.13 |
| Villous surface area, cm$^{2}$ | | | |
| Duodenum | 5.08 ± 0.28 | 4.81 ± 0.47 | 5.03 ± 0.27 |
| Jejunum | 5.61 ± 0.39$^{a}$ | 4.69 ± 0.25$^{c}$ | 5.14 ± 0.29$^{b}$ |
| Ileum | 5.28 ± 0.35$^{a}$ | 4.67 ± 0.32$^{b}$ | 4.96 ± 0.39$^{ab}$ |

Data are means ± SD, n = 6. Control (non-challenged control) = piglets fed the basal diet and injected with saline; LPS (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; LPS+NAC (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg and challenged with LPS. Villous surface area = 2πrh (r = villus width/2, h = Villus height). a-c; Values within a row with different letters differ ($P$<0.05). Adapted from Hou *et al.* (17).

500 mg/kg NAC could alleviate gross mucosal injury caused by acetic acid administration (8). In the piglet model of ulcerative colitis induced by intrarectal administration with acetic acid, NAC reduced the elevated histopathology score in the colon of acetic acid-challenged piglets and ameliorated acetic acid-induced histological abnormalities (8). Dietary supplementation with NAC increased the numbers of goblet cells and cell density, while decreasing the number of intraepithelial lymphocytes (IEL) and the lymphocytic density of acetic acid-challenged piglets (Table 4) (8). These findings support the notion that NAC could maintain the normal morphology of the intestine and favorably alleviate the LPS-induced damage in the small intestine and acetic acid-induced damage in the colon.

## 4.2. Absorptive and barrier function

Absorption of D-xylose from the intestinal lumen into plasma is a useful marker of *in vivo* intestinal function in animals (20-21). Generally, one hour blood D-xylose test is used to measure intestinal absorption capacity and mucosal integrity (17). Dietary supplementation with 500 mg/kg NAC augmented the entry of orally administered D-xylose into the systemic circulation in LPS-challenged piglets (Table 5) (17). In healthy piglets, D-xylose is readily absorbed by the small intestines. However, under LPS challenge or malabsorption, the entry of D-xylose from the intestinal lumen to the portal vein is impaired, thereby reducing D-xylose concentrations in both blood and urine (17-18, 21). In addition, dietary supplementation of 500 mg/kg NAC alleviated a decrease of diamine oxidase (DAO) activity in the small-intestinal mucosa and increase of DAO activity in plasma in response to LPS administration (Table 5) (17). DAO is present in the mammalian intestinal mucosa or the small intestine villi, and this enzyme is particularly abundant in rapidly dividing cells (20, 22). The activity of the mucosal DAO can serve as a useful marker of intestinal mucosal maturation and integrity, and of mucosal injury and recovery. In addition, plasma DAO is an indicator of

**Table 4.** Effects of NAC supplementation on the colonic mucosal morphology of acetic acid-treated piglets

| Items | Control | AA | NAC |
|---|---|---|---|
| Score | 6.3 ± 1.5[a] | 12.5 ± 2.6[b] | 8.3 ± 1.8[a] |
| Crypt Depth[1], μm | 190 ± 2.2 | 200 ± 3.5 | 231 ± 3.8 |
| Goblet cells/100 enterocytes | 10.3 ± 2.2[b] | 7.4 ± 0.7[a] | 9.8 ± 1.6[b] |
| IEL[2]/100 enterocytes | 1.6 ± 0.5[a] | 2.7 ± 0.8[b] | 1.8 ± 0.9[a] |
| Cell density[3] | 1.3 ± 0.4 | 0.9 ± 0.3 | 1.0 ± 0.1 |
| Lymphocytic density[3] | 1.6 ± 0.2[a] | 2.4 ± 0.7[b] | 1.7 ± 0.3[a] |

Data are means ± SD, n = 6. Control = piglets fed the basal diet and received intrarectal administration of sterile saline; AA = piglets fed the basal diet and received intrarectal administration of acetic acid; NAC = piglets fed the basal diet supplemented with 500 mg/kg NAC and received intrarectal administration of acetic acid. a-b: Values within a row with different letters differ ($P$<0.05). [1]Crypt depth: The distance from the crypt mouth to the base. The same crypt columns were used to determine the number of IEL, goblet cells expressed per 100 enterocytes. [2]IEL = Intraepithelial lymphocytes. [3]Intravillus lamina propria cell and lymphocytic density expressed as number of total cells or number of lymphocytes per 1,000 μm$^2$. Adapted from Wang *et al*. (8).

**Table 5.** Effects of dietary NAC supplementation on plasma D-xylose, the activity of DAO in plasma and intestinal mucosa of piglets after LPS challenge

| Items | Control | LPS | LPS+NAC |
|---|---|---|---|
| D-xylose in plasma (μg/mL) | 0.590 ± 0.100[a] | 0.409 ± 0.029[b] | 0.510 ± 0.049[a] |
| DAO in plasma (U/mL) | 6.974 ± 0.682[b] | 8.684 ± 0.933[a] | 7.584 ± 0.598[b] |
| DAO in duodenum (U/mg pro) | 0.278 ± 0.060[a] | 0.190 ± 0.064[b] | 0.172 ± 0.047[b] |
| DAO in jejunum (U/mg pro) | 0.199 ± 0.033[a] | 0.120 ± 0.027[b] | 0.165 ± 0.021[a] |
| DAO in ileum (U/mg pro) | 0.061 ± 0.023 | 0.042 ± 0.022 | 0.054 ± 0.012 |

Data are means ± SD, n = 6. Control (non-challenged control) = piglets fed the basal diet and injected with saline; LPS (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; LPS + NAC (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg and challenged with LPS. a-b: Values within a row with different letters differ ($P$<0.05). Adapted from Hou *et al*. (17).

the severity of mucosal injury (17, 20, 22). Under certain circumstances, intestinal mucosal cells undergo necrosis and slough off into the intestinal lumen, and DAO is released into the intestinal lymphatic and vascular space, leading to a decrease in intestinal mucosal DAO and an increase in circulating levels of DAO (17). This accompanies by the increase of intestinal permeability in response to LPS and inflammatory cytokines (20, 23). Taken together, these data support the notion that NAC can enhance the ability of the small intestine to absorb nutrients and improve mucosal barrier function particularly under inflammatory conditions.

### 4.3. Antioxidative capacity

NAC may be beneficial against lipid peroxidation-mediated damage. The adverse effects of LPS on intestinal oxidative stress can be attenuated by dietary NAC supplementation (7).

Reactive oxygen species (ROS) oxidize lipids, proteins and DNA, thereby causing cellular damage and subsequent cell death (11). Thus, ROS has been implicated in the pathophysiology of many disorders including neurodegenerative diseases (11). In addition to the possible effects of hypoperfusion on endotoxin-induced gut injury, LPS can result in tissue ischemia and hypoxia, changes in cell oxidative metabolism of the microsomal enzyme system, and production of a large number of free radicals (20, 24). NAC enhances the intestinal antioxidative capacity by partly increasing the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px). This action

**Table 6.** Effects of NAC on redox status in the jejunal mucosa

| Items | Control group | LPS group | NAC group |
|---|---|---|---|
| SOD, U/mg protein | 85.5 ± 2.5[a] | 71.8 ± 1.8[b] | 78.5 ± 2.2[a] |
| CAT, U/g protein | 52.4 ± 3.1[a] | 27.5 ± 0.70[c] | 37.3 ± 2.7[b] |
| GSH-Px, U/g protein | 85.4 ± 6.8[a] | 57.1 ± 7.2[b] | 70.7 ± 4.4[ab] |
| MDA, μmol/g protein | 0.37 ± 0.02[b] | 0.48 ± 0.01[a] | 0.36 ± 0.05[b] |
| $H_2O_2$, μmol/g protein | 0.66 ± 0.01[b] | 0.77 ± 0.03[a] | 0.68 ± 0.03[b] |
| $O_2^{\bullet -}$, μmol/g protein | 2.52 ± 0.08[c] | 4.76 ± 0.43[a] | 3.22 ± 0.10[b] |
| GSSG/GSH | 0.10 ± 0.01[b] | 0.23 ± 0.03[a] | 0.11 ± 0.01[b] |

Data are means ± SEM, n = 6. Control group (non-challenged control) = piglets fed the basal diet and injected with saline; LPS group (LPS challenged control)=piglets fed the basal diet and challenged with *Escherichia coli* LPS; NAC group (LPS + 500 mg/kg NAC)=piglets fed the basal diet supplemented with 500 mg/kg and challenged with LPS. [a, b, c]Values within a row with different letters differ ($P$<0.05). CAT, catalase; GSSG, oxidized glutathione; GSH, reduced glutathione; GSH-Px = glutathione peroxidase; $H_2O_2$ = hydrogen peroxide; MDA = malondialdehyde; $O_2^{\bullet -}$ = superoxide anion; SOD = superoxide dismutase. Adapted from Hou *et al.* (7).

of NAC is consistent with the decreased content of malondialdehyde (MDA), hydrogen peroxide ($H_2O_2$), superoxide anion ($O_2^{\bullet -}$) content, and GSSG/GSH ratio in the jejunal mucosa of LPS- challenged piglets (Table 6) (7). ROS, such as superoxide radical, hydrogen peroxide, and hydroxyl radical, are produced primarily by the mitochondria of cells as by-products of normal metabolism during conversion of molecular oxygen to water (7, 25). Whole-body homeostasis is maintained in the oxidative stress-antioxidant balance (20). The anti-oxidative enzymes include SOD, CAT, and GSH-Px (26). SOD functions to convert superoxide anion ($O_2^{\bullet -}$) into $H_2O_2$ and $O_2$, and is part of free radical scavenging systems (7, 10, 20). $H_2O_2$ is degraded to water by CAT (7). GSH-Px protects intracellular organelles from the damaging effects of hydroperoxides. This enzyme catalyses the reduction of $H_2O_2$ to water, with the simultaneous conversion of reduced glutathione to oxidized glutathione (13). The circulating level of MDA, which is an important indicator to reflect the extent in the accumulation of free radicals in the body caused by oxidative damage, serves as a useful bio-marker of *in vivo* oxidative stress (25). Moreover, NAC prevented LPS-induced increases in abundances of intestinal heat shock protein 70 (HSP70) protein expression of LPS-challenged piglets (Figure 1) (7). High concentrations of HSP70 are indicative of oxidative stress (7, 18, 27). In response to stress, HSP70 is expressed at elevated levels to promote refolding and prevent aggregation of partially-denatured proteins, thereby protecting cells from injury (20, 27). These findings suggest that NAC substantially ameliorated oxidative stress in the intestinal mucosa of piglets. One of the antioxidant defenses in cells is the endogenous thiols such as glutathione and thioredoxin (10, 28). NAC is a thiol, a source of sulfhydryl groups in cells and a scavenger of free radicals as it interacts with ROS (10), thereby protecting cells against oxidative damage (29). As stated above, the antioxidant effects of NAC may be directly related to its chemical structure or to the secondary induction of glutathione production. Among the direct effects of NAC are reactions with hydroxyl radicals, resulting in their inactivation (30). In this process, NAC is converted into NAC thiol radical intermediates, and finally into NAC disulfide (7, 10). A secondary antioxidant effect of NAC is exerted indirectly via increasing glutathione synthesis, which is essential for cellular defense against oxidative damage (7, 30).

Indeed, NAC supplementation decreased myeloperoxidase (MPO) activity in the plasma, as well as MDA concentrations in the plasma and colon of acetic acid-induced colitis in piglets (Table 7) (8). MPO is an enzyme found predominantly in neutrophils and has been used as a valid quantitative indicator of inflammation due to a positive correlation between MPO activities and neutrophil infiltration in the colon (8, 31-32). These findings suggest that NAC could alleviate acetic acid-induced oxidative injury in the colonic mucosa of piglets.

### 4.4. Anti-inflammatory effects

LPS challenge can stimulate macrophages to synthesize and secrete proinflammatory cytokines and enhances the production of inflammatory cytokines by multiple organs, including the small intestine (7, 33-34). Hou *et al*. (2013) recently reported

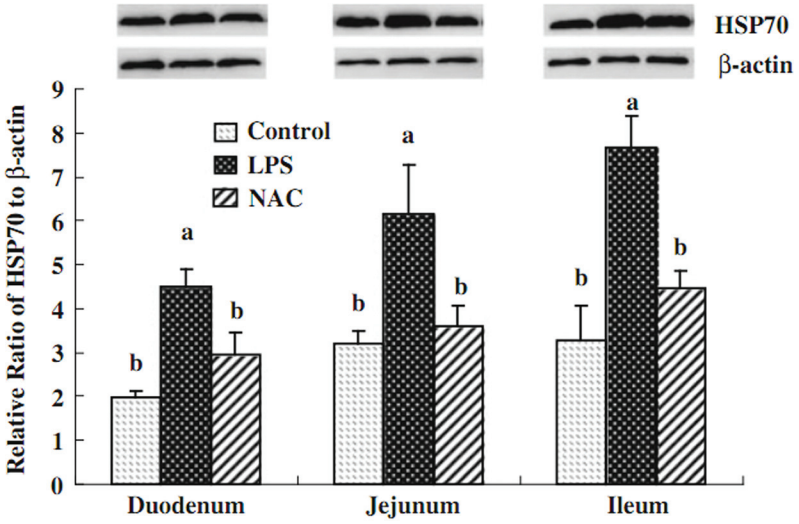


**Figure 1.** Relative levels of heat shock protein 70 (HSP70) expressed in the small-intestinal mucosa of piglets. Mucosal extracts (50 μg protein/sample) were separated by 10% SDS-polyacrylamide gel electrophoresis for determination of HSP70 and β-actin. Values for relative HSP70 were normalized for β-actin. Data are means ± SEM, n = 6. Control (non-challenged control) = piglets fed the basal diet and injected with sterile saline; LPS (LPS challenged control) = piglets fed the same control diet and challenged with *Escherichia coli* LPS; NAC (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg NAC and challenged with LPS. [a, b] Within same intestinal segment, means with different superscripts differ ($P$<0.05). Adapted from Hou *et al.* with permission (7).

**Table 7.** Effects of NAC on redox status in the plasma and colonic mucosa of acetic acid-induced piglets

| Items | Control | AA | NAC |
|---|---|---|---|
| Plasma | | | |
| MPO, U/L | 136 ± 12[a] | 172 ± 24[b] | 150 ± 24[a] |
| SOD, U/mL | 87.3 ± 30.2 | 82.0 ± 13.6 | 77.2 ± 11.8 |
| CAT, U/mL | 4.58 ± 1.29 | 3.64 ± 1.03 | 6.50 ± 3.19 |
| MDA, nmol/mg protein | 5.12 ± 0.51[a] | 6.97 ± 1.24[b] | 5.41 ± 1.02[a] |
| Colonic mucosa | | | |
| MPO, U/g wet mucosa | 0.071 ± 0.003[a] | 0.095 ± 0.018[b] | 0.063 ± 0.016[a] |
| SOD, U/mg protein | 20.9 ± 1.4 | 18.5 ± 4.2 | 20.5 ± 6.0 |
| CAT, U/mg protein | 1.16 ± 0.12[b] | 0.99 ± 0.17[a] | 0.87 ± 0.16[a] |
| MDA, nmol/mg protein | 0.33 ± 0.04[a] | 0.58 ± 0.16[b] | 0.41 ± 0.15[a] |

Data are means ± SD, n = 6. Control=piglets fed the basal diet and received intrarectal administration of sterile saline; AA = piglets fed the basal diet and received intrarectal administration of acetic acid; NAC = piglets fed the basal diet supplemented with 500 mg/kg NAC and received intrarectal administration of acetic acid. a-b: Values within a row with different letters differ ($P$ < 0.05). MPO = Myeloperoxidase; SOD = superoxide dismutase, CAT = catalase; MDA = malondialdehyde. Adapted from Wang *et al.* (8).

that LPS administration increased the concentrations of tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, cortisol, and prostaglandin $E_2$ ($PGE_2$) in the plasma and small intestinal mucosa and that dietary supplementation with 500 mg/kg NAC reduced concentrations of the inflammatory mediators (Table 8). These results indicate an important role for NAC in reducing inflammation (7). Consistent with the above observations, pretreatment of mice with NAC prior to endotoxin injection resulted in reduced

**Table 8.** Effects of NAC on concentrations of proinflammatory mediators in plasma and intestinal mucosae and of EGF in plasma

| Item | Control group | LPS group | NAC group |
|---|---|---|---|
| Plasma (Day 10) | | | |
| TNF-α, ng/mL | 0.68 ± 0.19$^{c}$ | 3.48 ± 0.34$^{a}$ | 2.25 ± 0.24$^{b}$ |
| Cortisol, ng/mL | 22.0 ± 6.4$^{c}$ | 272 ± 29$^{a}$ | 201 ± 8$^{b}$ |
| IL-6, pg/mL | 109 ± 6.4$^{b}$ | 210 ± 19$^{a}$ | 136 ± 19$^{b}$ |
| $PGE_2$, pg/mL | 51.1 ± 3.3$^{c}$ | 71.1 ± 2.9$^{a}$ | 62.3 ± 1.9$^{b}$ |
| EGF, ng/mL | 1.03 ± 0.04$^{a}$ | 0.83 ± 0.05$^{b}$ | 1.05 ± 0.08$^{a}$ |
| Plasma (Day 20) | | | |
| TNF-α, ng/mL | 0.63 ± 0.01$^{b}$ | 1.14 ± 0.11$^{a}$ | 0.66 ± 0.14$^{b}$ |
| Cortisol, ng/mL | 22.0 ± 5.4$^{b}$ | 117 ± 23$^{a}$ | 66.5 ± 14.0$^{ab}$ |
| IL-6, pg/mL | 104 ± 9$^{b}$ | 156 ± 4$^{a}$ | 119 ± 19$^{b}$ |
| $PGE_2$, pg/mL | 50.3 ± 1.1$^{b}$ | 60.6 ± 2.6$^{a}$ | 52.4 ± 3.0$^{b}$ |
| EGF, ng/mL | 1.26 ± 0.11$^{a}$ | 0.82 ± 0.09$^{b}$ | 1.35 ± 0.17$^{a}$ |
| Duodenal mucosa | | | |
| TNF-α, ng/mL | 2.19 ± 0.34$^{b}$ | 4.46 ± 0.51$^{a}$ | 3.18 ± 0.30$^{b}$ |
| Cortisol, ng/mL | 2.30 ± 0.11$^{b}$ | 5.99 ± 0.76$^{a}$ | 4.77 ± 0.69$^{a}$ |
| IL-6, pg/mL | 174 ± 8$^{b}$ | 267 ± 22$^{a}$ | 206 ± 37$^{ab}$ |
| $PGE_2$, pg/mL | 81.7 ± 5.4 | 91.8 ± 2.7 | 84.5 ± 3.1 |
| Jejunal mucosa | | | |
| TNF-α, ng/mL | 2.92 ± 0.45$^{b}$ | 5.11 ± 0.18$^{a}$ | 3.16 ± 0.28$^{b}$ |
| Cortisol, ng/mL | 2.29 ± 0.23$^{b}$ | 5.45 ± 0.87$^{a}$ | 3.49 ± 0.49$^{b}$ |
| IL-6, pg/mL | 210 ± 19$^{b}$ | 366 ± 18$^{a}$ | 263 ± 20$^{b}$ |
| $PGE_2$, pg/mL | 80.1 ± 1.6$^{b}$ | 95.7 ± 4.5$^{a}$ | 82.4 ± 2.2$^{b}$ |
| Ileal mucosa | | | |
| TNF-α, ng/mL | 1.61 ± 0.26$^{b}$ | 3.47 ± 0.44$^{a}$ | 2.18 ± 0.32$^{b}$ |
| Cortisol, ng/mL | 2.73 ± 0.52$^{c}$ | 7.87 ± 0.88$^{a}$ | 4.81 ± 0.52$^{b}$ |
| IL-6, pg/mL | 142 ± 15$^{b}$ | 235 ± 23$^{a}$ | 144 ± 7$^{b}$ |
| $PGE_2$, pg/mL | 86.0 ± 3.1$^{b}$ | 104 ± 3$^{a}$ | 92.9 ± 2.5$^{b}$ |

Data are means ± SEM, n = 6. Control group (non-challenged control) = piglets fed the basal diet and injected with saline; LPS group (LPS challenged control) = piglets fed the basal diet and challenged with Escherichia coli LPS; NAC group (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg and challenged with LPS. $^{a, b, c}$Values within a row with different letters differ ($P < 0.05$). IL-6 = interleukin 6; PGE2 = prostaglandin E2. Adapted from Hou *et al.* (7).

nuclear factor κB (NF-κB) activation and neutrophilic alveolitis (35). Furthermore, NAC was shown to inhibit collagen-induced arthritis in mice by inhibiting inflammatory cytokines and NF-κB activity (36-37). Through suppressing NF-κB activation, NAC also suppressed the production of nitric oxide by the inducible form of nitric-oxide synthetase and the generation of IL-6 by cells of the immune system (38). In line with these reports, NAC prevented the LPS-induced increase in NF-κB expression in the small-intestinal mucosa (Figure 2) (7). Similarly, NAC supplementation decreased TNF-α concentrations in the plasma of acetic acid-treated piglets (Table 9) (8).

NF-κB is normally bound to IκB protein in the cytoplasm, but is released from the bound complex to enter the nucleus in response to infection, oxidative stress or inflammation. The release of NF-κB can increase transcription of genes coding for TNF-α, IL-1 and IL-6, which ultimately results in a positive feedback loop (39). These findings suggest that NAC could alleviate LPS or acetic acid-induced oxidative injury in piglets and may have positive effects on reducing the severity of intestinal inflammation.

Notably, NAC reduced the elevated mRNA levels for toll-like receptor 4 (TLR4) in the jejunal and ileal mucosa of LPS-challenged piglets (Table 10) (7). TLR4 activation leads to an increase in enterocyte apoptosis and a loss of mucosal barrier integrity (40-41). In addition to activating pathways that lead to cytokine release from tissues, TLR4 may directly contribute to perturbations in mucosal healing and further exacerbate the inflammatory response within the intestinal mucosa (42). NAC reduced the elevated concentrations of the TNF-α in the plasma and small intestinal mucosa of LPS-challenged piglets, and prevented LPS-induced increases in the abundance of intestinal NF-κB p65 proteins. Taken together, these results provide evidence that NAC prevented the activation of the TLR4 signaling, and alleviated inflammatory response and the mucosal damage in the small intestine (7).

### 4.5. Improved energy status in the intestinal mucosa

Mitochondria are key cellular organelles that regulate biochemical reactions related to ATP production and apoptosis (43). Most of the ATP is generated by the proton gradient that develops across the inner mitochondrial membrane. ATP is a complex *nanomachine* that serves as the primary energy currency of the cell. AMP is a good indicator of cellular

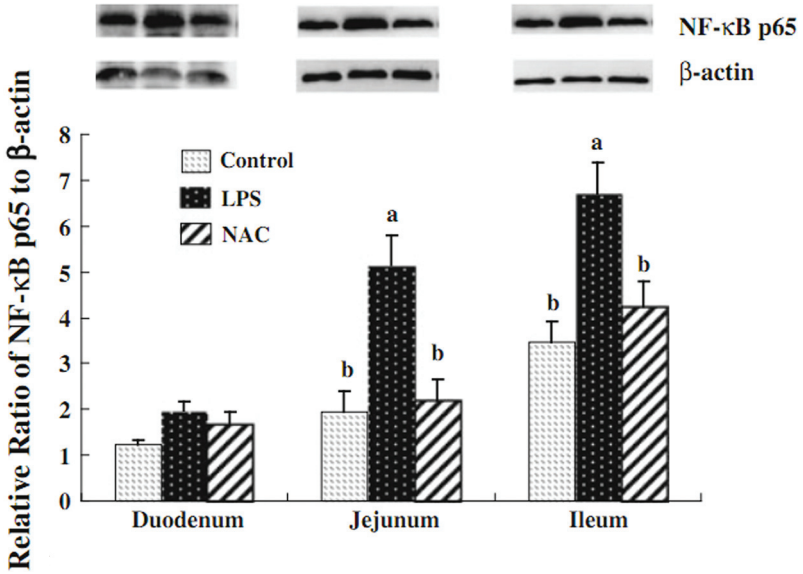


**Figure 2.** Relative levels of nuclear factor κB (NF-κB p65) expressed in the small-intestinal mucosa of piglets. Mucosal extracts (60 μg protein/sample) were separated by 10% SDS-polyacrylamide gel electrophoresis for determination of NF-κB p65 and β-actin. Values for relative NF-κB p65 were normalized for β-actin. Data are means ± SEM, n = 6. Control (non-challenged control) = piglets fed the bsasl diet and injected with sterile saline; LPS (LPS challenged control) = piglets fed the same control diet and challenged with *Escherichia coli* LPS; NAC (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg NAC and challenged with LPS. [a,b]Within same intestinal segment, means with different superscripts differ ($P$<0.05). Adapted from Hou *et al.* with permission (7).

**Table 9.** Effects of NAC on proinflammatory mediators and growth modulator in the plasma and colonic mucosa of acetic acid-treated pigs

| Item | Control | AA | NAC |
|---|---|---|---|
| Plasma | | | |
| TNF-α, ng/mL | 0.61 ± 0.17[a] | 0.84 ± 0.11[b] | 0.49 ± 0.14[a] |
| IL-6, pg/mL | 106.4 ± 23.6 | 115.2 ± 34.2 | 113.6 ± 18.3 |
| $PGE_2$, pg/mL | 57.9 ± 11.5 | 55.1 ± 13.1 | 51.9 ± 11.8 |
| EGF, ng/mL | 0.65 ± 0.08[ab] | 0.60 ± 0.07[a] | 0.76 ± 0.10[b] |
| Colonic mucosa | | | |
| IL-6, pg/mL | 134.3 ± 12.7 | 133.6 ± 17.2 | 130.8 ± 11.3 |
| $PGE_2$, pg/mL | 74.5 ± 3.9[a] | 96.0 ± 14.5[b] | 90.5 ± 15.0[b] |
| TGF-α, pg/mL | 3.50 ± 0.83[a] | 4.28 ± 0.33[b] | 2.56 ± 0.54[c] |

Data are means ± SD, n = 6. Control = piglets fed the basal diet and received intrarectal administration of sterile saline; AA = piglets fed the basal diet and received intrarectal administration of acetic acid; NAC = piglets fed the basal diet supplemented with 500 mg/kg NAC and received intrarectal administration of acetic acid. [a, b, c] Values within a row with different letters differ ($P$ < 0.05). TNF-α = tumor necrosis factor-alpha; IL-6 = interleukin 6; PGE2 = prostaglandin $E_2$; EGF = epidermal growth factor; TGF-α = transforming growth factor-α. Adapted from Wang *et al.* (8).

stress because an increased rate of ATP hydrolysis leads to a rapid accumulation of AMP in the cell (44). The adenylate energy charge (AEC) of the adenylate pool is a better measure of the energy status in a tissue than the level of a single nucleotide. The hydrolysis of ATP first increases cellular ADP concentrations, and ADP is then converted by the adenylate kinase reaction (2 ADP↔ATP+AMP) to ATP and AMP (45-46).

**Table 10.** Effects of NAC on EGFR, TLR4 and AQP8 mRNA levels in jejunal and ileal mucosae of LPS-challenged pigs

| Items | Control group | LPS group | NAC group |
|---|---|---|---|
| EGFR mRNA | | | |
| Jejunum | 1.00 ± 0.06[a] | 0.64 ± 0.07[b] | 0.76 ± 0.08[ab] |
| Ileum | 1.00 ± 0.05[a] | 0.72 ± 0.05[b] | 0.99 ± 0.07[a] |
| TLR4 mRNA | | | |
| Jejunum | 1.00 ± 0.24[b] | 2.07 ± 0.42[a] | 1.09 ± 0.16[b] |
| Ileum | 1.00 ± 0.19[b] | 1.89 ± 0.28[a] | 1.38 ± 0.17[ab] |
| AQP8 mRNA | | | |
| Jejunum | 1.00 ± 0.08[a] | 0.42 ± 0.05[b] | 0.52 ± 0.09[b] |
| Ileum | 1.00 ± 0.16 | 1.11 ± 0.32 | 1.13 ± 0.45 |

Data are means ± SEM, n = 6. Control group (non-challenged control) = piglets fed the basal diet and injected with saline; LPS group (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; NAC group (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg and challenged with LPS.
[a, b] Values within a row with different letters differ ($P < 0.05$).
Adapted from Hou *et al.* (7).

Therefore, during increased consumption of ATP, AMP accumulates well before any changes in cellular ATP or ADP concentrations occur (44). LPS could induce alterations in gastrointestinal oxygen metabolism and result in mitochondrial injury in the ileum (47-48). LPS treatment reduced ATP concentrations and AEC, therefore altering the cellular energy status in the piglet intestinal mucosa (45, 49). Notably, NAC supplementation resulted in: 1) increased ATP concentrations; 2) reduced AMP/ATP ratio; and 3) increased AEC in the small intestine of LPS-challenged piglets (Table 11) (49). These lines of evidence indicate that NAC could modulate the adenine nucleotide pool and support the notion that NAC beneficially alleviates the LPS-induced damage of the intestinal energy metabolism. The results also reveal a hitherto unrecognized role for NAC in improving energy status in the small intestine.

The activity of the AMPK in endothelial cells can be regulated by stimuli that affect cellular ATP levels (50). Moreover, hypoxia, which occurs in the small intestine of LPS-treated animals, leads to activation of AMPK via an increase in the AMP/ATP ratio (51-53). When activated, AMPK switches on catabolic pathways for ATP regeneration, such as glucose uptake, fatty acid β-oxidation, while switching off ATP-requiring pathways, such as the synthesis of protein, fatty acids, and triglyceride (51, 54). LPS challenge resulted in an increase in the ratio of phosphorylated AMPK-α to AMPK-α (p-AMPK/AMPK) in jejunal mucosa, whereas NAC supplementation decreased the p-AMPK/AMPK ratio in the jejunum (Figure 3) (49). Similarly, α-ketoglutarate (AKG), as a precursor of glutamine, prevented the LPS-induced increase in phosphorylated AMPK expression in the intestinal mucosa (45). Thus, NAC relieves the LPS-induced energy depletion in the intestinal mucosa possibly via the AMPK pathway, thereby playing a protective effect on the small intestinal mucosa (49). It is unknown whether NAC directly or indirectly phosphorylates AMPK in cells.

### 4.6. Promotion of cell survival and anti-apoptotic activities

Oxidative stress causes programmed cell death or apoptosis in several pathological processes (10, 55). High concentrations of ROS initiate necrotic or apoptotic cell death (56). Based on studies of various cell types, it is increasingly clear that NAC has growth-promoting activities (10). Consistent with this view, we found dietary supplementation with 500 mg/kg NAC attenuated the decreases in DNA concentrations, as well as rations of RNA/DNA and protein/DNA, in the jejunal mucosa of LPS-challenged piglets (Table 12) (7). In addition, NAC prevented the acetic acid-induced decrease in the protein/DNA ratio in the colon of piglets (8). Thus, intestinal biochemical indices, such as DNA concentrations, as well as RNA/DNA and protein/DNA ratios, can be used to assess intestinal growth and development (7, 57-58). DNA concentration reflects the rate of mitosis to produce new columnar epithelial cells, RNA/DNA ratio indicates cellular efficiency, and protein/DNA ratio implicates the efficiency of protein synthesis in cells (7, 57). The results of these studies clearly reveal that NAC supplementation can stimulate the growth of the intestinal mucosa in response to endotoxin or acetic acid treatment.

One of the putative mechanisms for NAC's action may involve the expression of caspase-3. Caspase-3 is one of the key components of the apoptotic pathway in the small intestine (17, 40, 59). Apoptosis is typically accompanied by the activation of a class of "death" proteases (caspases) (8, 60). Caspase-3 is commonly activated by numerous "death" signals to cleave a variety of important cellular proteins (61). This protein is either partially

**Table 11.** Effects of NAC on adenine nucleotide concentrations in the intestinal mucosa of piglets after LPS challenge

| Item | Control group | LPS group | NAC group |
|---|---|---|---|
| Duodenal mucosa | | | |
| ATP, μg/g wet wt | 87.81 ± 13.58$^{a}$ | 56.18 ± 3.95$^{b}$ | 65.63 ± 4.07$^{ab}$ |
| ADP, μg/g wet wt | 105.49 ± 4.63 | 98.65 ± 10.41 | 110.15 ± 2.99 |
| AMP, μg/g wet wt | 343.06 ± 20.68$^{b}$ | 439.05 ± 31.32$^{a}$ | 336.13 ± 11.30$^{b}$ |
| AMP/ATP | 4.20 ± 0.76$^{a}$ | 7.81 ± 0.50$^{b}$ | 5.32 ± 0.45$^{b}$ |
| TAN | 524.46 ± 12.09 | 516.10 ± 32.60 | 551.34 ± 19.05 |
| AEC | 0.27 ± 0.028$^{a}$ | 0.19 ± 0.012$^{b}$ | 0.23 ± 0.098$^{ab}$ |
| Jejunal mucosa | | | |
| ATP, μg/g wet wt | 78.61 ± 6.67$^{a}$ | 53.07 ± 4.72$^{b}$ | 61.69 ± 5.10$^{ab}$ |
| ADP, μg/g wet wt | 104.04 ± 5.83 | 95.72 ± 6.23 | 98.76 ± 13.77 |
| AMP, μg/g wet wt | 432.08 ± 25.62$^{b}$ | 548.55 ± 23.83$^{a}$ | 453.73 ± 26.73$^{b}$ |
| AMP/ATP | 6.12 ± 0.54$^{b}$ | 9.75 ± 1.29$^{a}$ | 6.88 ± 0.85$^{b}$ |
| TAN | 575.84 ± 34.34 | 671.60 ± 45.88 | 660.48 ± 55.46 |
| AEC | 0.22 ± 0.0087$^{a}$ | 0.16 ± 0.010$^{c}$ | 0.19 ± 0.0046$^{b}$ |
| Ileal mucosa | | | |
| ATP, μg/g wet wt | 60.05 ± 5.67$^{a}$ | 44.38 ± 3.21$^{b}$ | 54.22 ± 3.02$^{ab}$ |
| ADP, μg/g wet wt | 66.34 ± 3.82 | 73.18 ± 4.42 | 70.15 ± 6.23 |
| AMP, μg/g wet wt | 216.86 ± 10.26$^{b}$ | 323.69 ± 16.31$^{a}$ | 240.90 ± 8.37$^{b}$ |
| AMP/ATP | 3.73 ± 0.36$^{b}$ | 6.75 ± 1.20$^{a}$ | 3.95 ± 0.21$^{b}$ |
| TAN | 348.40 ± 9.49 | 397.41 ± 57.49 | 383.12 ± 19.79 |
| AEC | 0.26 ± 0.017$^{a}$ | 0.20 ± 0.016$^{b}$ | 0.25 ± 0.0083$^{ab}$ |

Data are means ± SEM, n = 6. Control group (non-challenged control) = piglets fed the basal diet and injected with saline; LPS group (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; NAC group (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg and challenged with LPS. $^{a, b}$ Values within a row with different letters differ ($P < 0.05$). TAN = ATP + ADP + AMP; AEC (adenylate energy charge) = (ATP + 0.5ADP)/(ATP + ADP + AMP); $^{a, b, c}$ Means in the same row with different superscripts differ significantly ($P < 0.05$). Adapted from Yang (49).

or totally responsible for the proteolytic cleavage of many key "death" proteins (17, 59). Much evidence shows that LPS or acetic acid induces cell death through the activation of caspase-3 (7, 17, 59).

We recently reported that dietary supplementation with 500 mg/kg NAC attenuated caspase-3 protein expression in the small intestine of LPS-challenged pigs (Figure 4) (17). Moreover, NAC prevented the acetic acid-induced increase in the abundance of caspase-3 protein in the colon of pigs (Figure 5) (8). Thus, the positive effect of NAC on LPS-induced enterocyte death is in line with its positive effect on acetic acid-induced apoptosis and its promoting effects on cell growth and survival.

On the other hand, NAC supplementation increased the circulating levels of epidermal growth factor (EGF) and relieved growth depression in weanling piglets under LPS challenge (16). In addition, NAC enhanced EGF receptor (EGFR) mRNA levels in the small intestinal mucosa of LPS-challenged piglets (Table 10) (7). Moreover, NAC increased mRNA levels for EGF in the colonic mucosa of acetic acid-challenged piglets (Table 13) (8). Epidermal growth factor (EGF) and its receptor (EGFR) play important roles in the repair of the intestinal mucosa following damage (62-64). The signal transduction mediated by EGFR is characterized by a plethora of beneficial responses, including the enhancement of cell proliferation, repair and migration, and the stabilization of internal environment (7, 63-64). This is consistent with the observations that NAC supplementation mitigated cell apoptosis (indicated by decreased expression of the caspase-3 protein) in the small intestine of LPS-challenged pigs and the colonic mucosa of acetic acid-challenged piglets. Additionally, mRNA levels for the amphiregulin gene was markedly elevated in the colon of NAC-supplemented piglets (Table 13) (8). Amphiregulin (AR) is a bi-functional growth modulator: it can promote the growth of normal epithelial cells but can also inhibit the growth of certain aggressive carcinoma cell lines (8, 65). Therefore, it is possible that NAC alleviates intestinal injury partly via its growth-promoting effects, particularly its anti-apoptotic actions and regulatory roles in the EGFR signaling pathway.

### 4.7. Regulation of tight junction protein expression (claudin-1 and occludin)

Intestinal epithelial integrity is maintained by cohesive interactions between cells via the formation of tight junctions (66). We found that

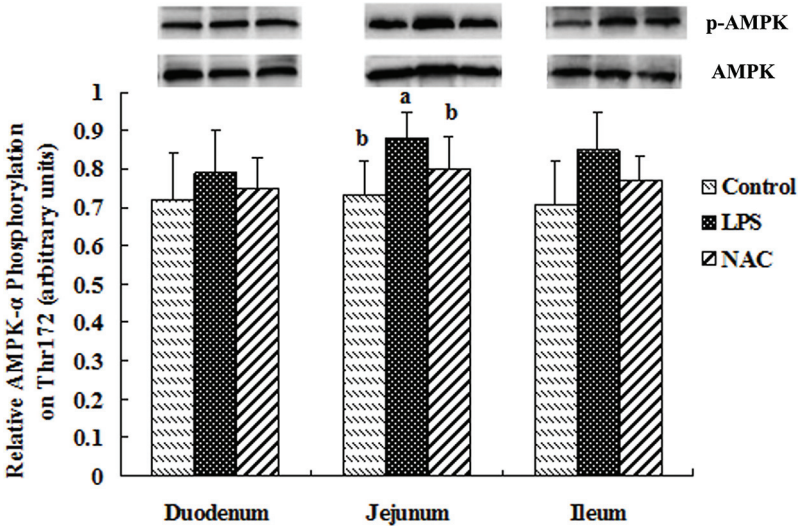


**Figure 3.** The phosphorylation state of AMPK in the small-intestinal mucosa of piglets. Mucosal extracts (150 μg protein/sample) from the small intestine were separated by 10% SDS-polyacrylamide gel electrophoresis for determination of phosphorylated AMPK-α (p-AMPK) and total AMPK (AMPK). Values for phosphorylated AMPK were normalized for total AMPK. Data are means ± SEM, n = 6. Control (non-challenged control) = piglets fed the bsasl diet and injected with sterile saline; LPS (LPS challenged control) = piglets fed the same control diet and challenged with *Escherichia coli* LPS; NAC (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg NAC and challenged with LPS. [a, b] Within same intestinal segment, means with different superscripts differ ($P$<0.05). Adapted from Yang (49).

**Table 12.** Effects of NAC supplementation on intestinal mucosal growth of piglets challenged with LPS

| Items | Control | LPS | LPS+NAC |
|---|---|---|---|
| DNA, mg/g | | | |
| Duodenum | 0.373 ± 0.028 | 0.354 ± 0.039 | 0.363 ± 0.015 |
| Jejunum | 0.317 ± 0.254[a] | 0.217 ± 0.026[b] | 0.283 ± 0.030[a] |
| Ileum | 0.540 ± 0.028[a] | 0.408 ± 0.025[b] | 0.430 ± 0.018[b] |
| RNA/DNA | | | |
| Duodenum | 5.365 ± 0.430[a] | 4.280 ± 0.364[b] | 4.579 ± 0.571[ab] |
| Jejunum | 7.124 ± 1.768[a] | 4.835 ± 0.382[c] | 6.743 ± 1.634[b] |
| Ileum | 4.544 ± 0.654[a] | 2.644 ± 0.223[b] | 3.960 ± 0.449[a] |
| Total protein/DNA | | | |
| Duodenum | 165.97 ± 22.76 | 141.46 ± 23.35 | 155.17 ± 26.05 |
| Jejunum | 333.22 ± 44.71[a] | 199.46 ± 27.87[c] | 268.16 ± 35.28[b] |
| Ileum | 175.39 ± 15.18[a] | 115.56 ± 8.65[b] | 158.53 ± 12.87[a] |

Data are means ± SD, n = 6. Control (non-challenged control) = piglets fed the basal diet and injected with saline; LPS (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; LPS + NAC (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg and challenged with LPS. a-b: Values within a row with different letters differ ($P$ < 0.05). Adapted from Hou *et al.* (17).

NAC-induced enhancement of epithelial barrier function was associated with increased expression of claudin-1 and occludin (7). The members of the claudin-family proteins play a critical role in

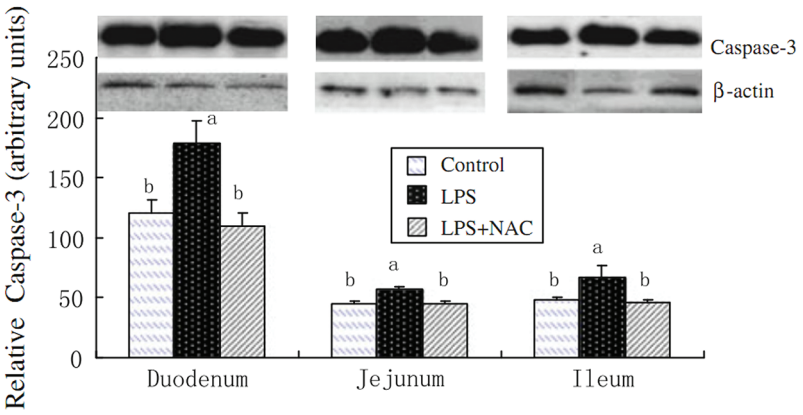


**Figure 4.** Relative levels of the caspase-3 protein in the small-intestinal mucosa of piglets. Mucosal extracts (150 μg protein/sample) from the small intestine were separated by 10% SDS-polyacrylamide gel electrophoresis for determination of caspase-3 and β-actin. Values for the caspase-3 protein were normalized for β-actin. Data are means ± SD, n = 6. Control (non-challenged control) = piglets fed the basal diet and injected with sterile saline; LPS (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; LPS+NAC (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg and challenged with LPS. [a, b] Within same intestinal segment, means with different superscripts differ ($P$<0.05). Adapted from Hou *et al.* with permission (17).

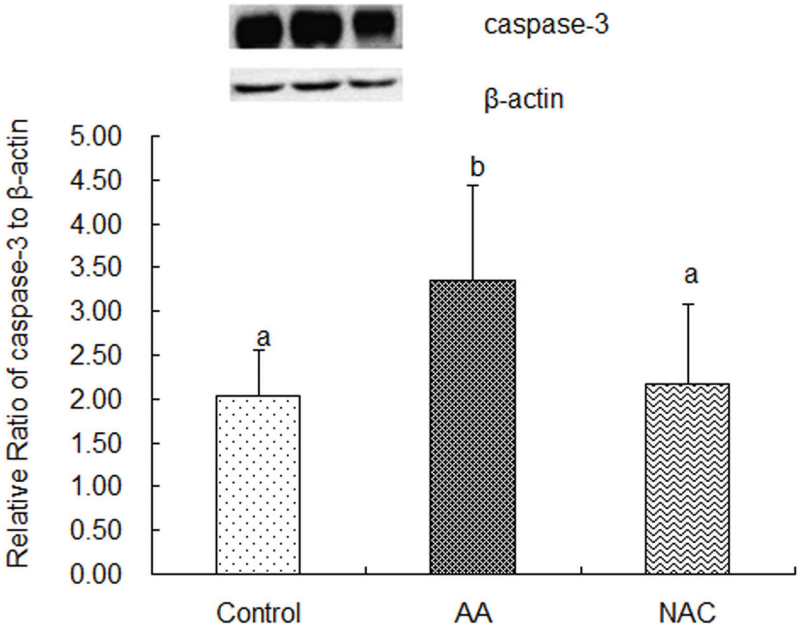


**Figure 5.** Relative levels of caspase-3 expressed in the colonic mucosa of piglets. Mucosal extracts (39 μg protein/sample) from the colon were separated by 10% SDS-polyacrylamide gel electrophoresis for determination of caspase-3 and β-actin. Values for relative caspase-3 abundance were normalized for β-actin. Data are means ± SD, n = 6. Control = piglets fed the basal diet and received intrarectal administration of sterile saline; AA = piglets fed the same control diet and received intrarectal administration of AA; NAC (AA + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg NAC and received intrarectal administration of AA. [a, b] Within the same intestinal segment, means with different superscripts differ ($P$<0.05). Adapted from Wang *et al.* (8).

tight junction formation and affect permeability characteristics in the gut (17, 59, 67-68). Claudin-1 and occludin integrate such diverse processes as gene transcription, tumor suppression, and cell proliferation to modulate intestinal-mucosal structure and function (69-70). LPS disrupts

**Table 13.** Effects of NAC on EGFR, AR, TNF-α and TLR4 mRNA levels in the colonic mucosa of acetic acid-treated pigs

| Items | Control | AA | NAC |
|---|---|---|---|
| EGFR | 1.00 ± 0.29$^{b}$ | 0.82 ± 0.19$^{ab}$ | 0.61 ± 0.12$^{a}$ |
| AR | 1.00 ± 0.17$^{a}$ | 1.28 ± 0.20$^{a}$ | 1.58 ± 0.17$^{b}$ |
| TNF-α | 1.00 ± 0.16$^{b}$ | 0.61 ± 0.16$^{a}$ | 0.60 ± 0.11$^{a}$ |
| TLR4 | 1.00 ± 0.04 | 0.86 ± 0.34 | 0.71 ± 0.10 |

Data are means ± SD, n = 6. Control = piglets fed the basal diet and received intrarectal administration of sterile saline; AA = piglets fed the basal diet and received intrarectal administration of acetic acid; NAC = piglets fed the basal diet supplemented with 500 mg/kg NAC and received intrarectal administration of acetic acid. a-b: Values within a row with different letters differ ($P < 0.05$). EGFR = epidermal growth factor receptor; AR = amphiregulin; TNF-α = tumor necrosis factor-alpha; TLR4 = toll-like receptor 4. Adapted from Wang *et al.* (8).

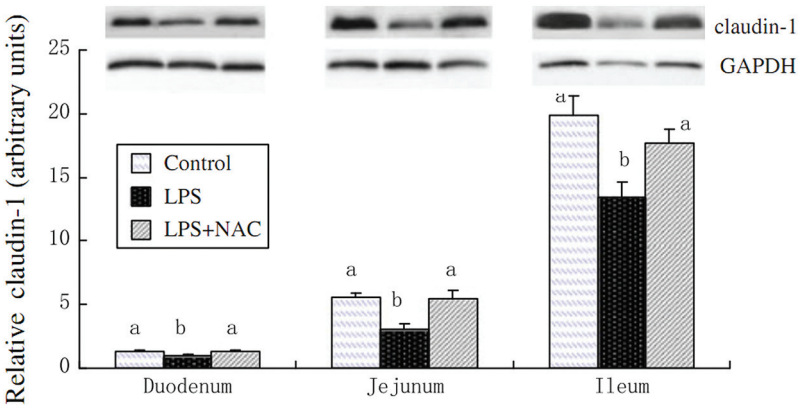


**Figure 6.** Relative levels of the claudin-1 protein in the small-intestinal mucosa of piglets. Mucosal extracts (60 μg protein/sample) from the small intestine were separated by 12% SDS-polyacrylamide gel electrophoresis for determination of claudin-1 and GAPDH. Values for relative claudin-1 were normalized for GAPDH. Data are means ± SD, n = 6. Control (non-challenged control) = piglets fed the basal diet and injected with saline; LPS (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; LPS+NAC (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg and challenged with LPS. $^{a, b}$ Within same intestinal segment, means with different superscripts differ ($P<0.05$). Adapted from Hou *et al.* with permission (17).

the gut morphology and increases paracellular permeability, while inducing a redistribution of tight junction proteins (e.g. occludin, claudin-1, claudin-4, and zonula occludens (ZO)-1) from intercellular junctions and reducing the expression of ZO-1 (71). This is consistent with our observations that LPS reduced the abundance of claudin-1 and occludin in the small intestinal mucosa of piglets (Figures 6 and 7) (7). Notably, dietary supplementation with NAC prevented the LPS-induced decreases of claudin-1 and occludin expression in the small intestinal mucosa (Figures 6 and 7), indicating that NAC can effectively protect the small intestine from oxidative injury and is a safe agent for clinical management of neonates with intestinal inflammation (7). Furthermore, NAC substantially stimulated expression of claudin-1 protein in the colonic mucosa of acetic acid-challenged piglets, suggesting that NAC may improve the colonic epithelial barrier function and alleviate the acetic acid induced mucosal damage in the animals (Figure 8) (8). Overall, through regulating tight junction protein expression, NAC exerts beneficial effects on epithelial barrier and protects the mucosa against severe insult by LPS or acetic acid. Similarly, preservation of epithelial barrier integrity by TGF-β (transforming growth factor-β) in the face of EHEC (enterohemorrhagic *Escherichia coli* O157:H7) infection was accompanied by maintenance of the levels and distribution of claudin-2, occludin, and ZO-1 (67). Collectively, these data support the view that NAC affects expression of key proteins involved in anti-inflammatory responses via the tight junction signaling (17).

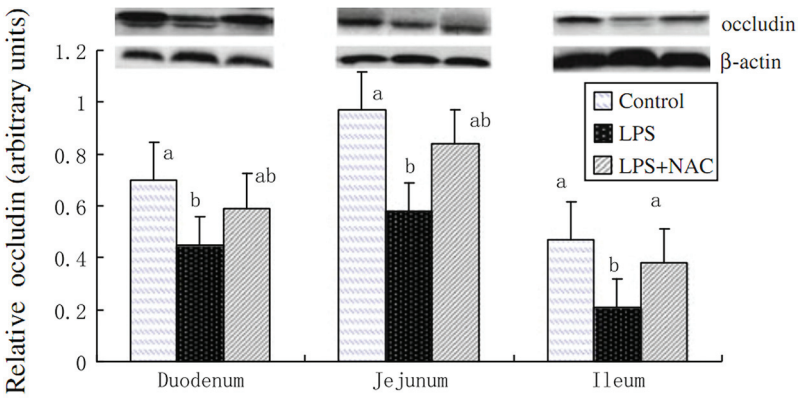


**Figure 7.** Relative levels of the occludin protein in the small-intestinal mucosa of piglets. Mucosal extracts (120 μg protein/sample) from the small intestine were separated by 10% SDS-polyacrylamide gel electrophoresis for determination of occludin and β-actin. Values for relative occludin were normalized for β-actin. Data are means ± SD, n = 6. Control (non-challenged control) = piglets fed the basal diet and injected with saline; LPS (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; LPS+NAC (LPS + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg and challenged with LPS. [a, b] Within same intestinal segment, means with different superscripts differ ($P$<0.05). Adapted from Hou *et al.* (17).

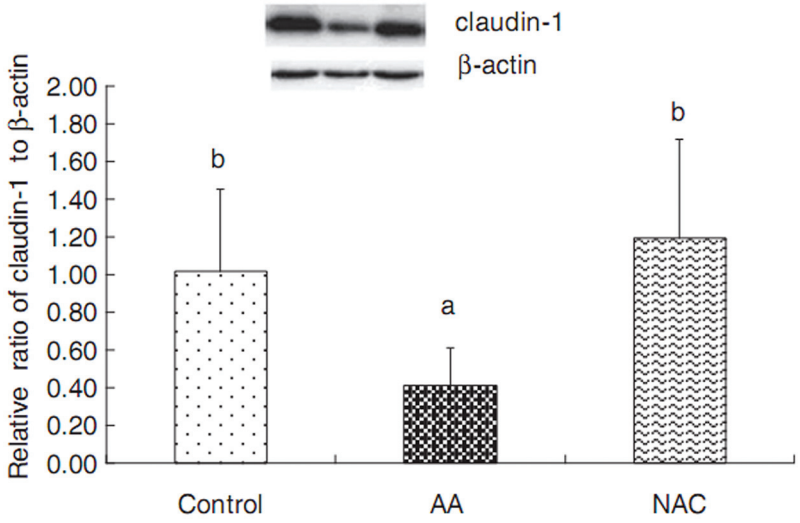


**Figure 8.** Relative levels of claudin-1 expressed in the colonic mucosa of piglets. Mucosal extracts (52 μg protein/sample) from the colon were separated by 12% SDS-polyacrylamide gel electrophoresis for determination of claudin-1 and β-actin. Values for relative claudin-1 abundance were normalized for β-actin. Data are means ± SD, n = 6. Control = piglets fed the basal diet and received intrarectal administration of sterile saline; AA = piglets fed the same control diet and received intrarectal administration of AA; NAC (AA + 500 mg/kg NAC) = piglets fed the basal diet supplemented with 500 mg/kg NAC and received intrarectal administration of AA. [a, b] Within the same intestinal segment, means with different superscripts differ ($P$<0.05). Adapted from Wang *et al.* with permission (8).

## 5. CONCLUSION AND PERSPECTIVES

Enterocytes, colonocytes, and other intestinal cells readily transport NAC and convert it into L-cysteine, which has recently been classified as a nutritionally essential amino acid in animal and human nutrition to maintain the integrity, growth, and function of the intestinal mucosa and other cell types (72, 73). Along with glutamate and glycine in the lumen of the gut (74-77), cysteine is utilized for synthesis of glutathione in the small intestine. Based on its regulatory function in cell nutrition, metabolism and signaling, cysteine is a functional amino acid in animals (78). Thus, humans, livestock (including pigs), poultry, and fish have dietary requirements for cysteine (79). Plant (e.g., corn and soybean meal)-based diets usually

do not contain sufficient cysteine to support maximal growth of mammals, birds or fish (80-82). In support of this notion, dietary supplementation with NAC alleviates intestinal injury and dysfunction in LPS-challenged piglets. The beneficial effects of NAC are associated with the following actions: 1) reduced oxidative stress (indicated by increased activities of anti-oxidant enzymes, decreased production of reactive oxygen species, and reduced expression of the HSP70 protein); 2) reduced inflammation (indicated by reduced concentrations of inflammatory mediators) via the TLR4 signaling (indicated by decreased expression of the NF-κB p65 protein and TLR4 mRNA); 3) improved energy status of the intestinal mucosa (indicated by increased ATP and AEC, reduced AMP/ATP ratio and phosphorylated AMPK expression); 4) reduced cell apoptosis and enhanced recovery of the injured intestine (indicated by decreased expression of the caspase-3 protein in the intestinal mucosa and improved mucosal repair via the EGF signaling); and 5) regulated tight junction protein expression (indicated by increased expression of claudin-1 and occludin proteins in the small-intestinal mucosa). These findings not only aid in understanding the mode of NAC's actions in the gut but also have important implications for development of new interventions to ameliorate intestinal injury and dysfunction in animals and humans under inflammatory conditions. Finally, NAC has anti-cancer effects, including counteractions against carcinogens and prevention of tumor progression (83). Thus, in addition to its use as a food additive in animal and human nutrition, NAC may hold great promise in preventing and treating a wide array of cancers, including brain, colon, hepatic, kidney, lung, and pancreatic cancers.

## 6. ACKNOWLEDGMENTS

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## 7. REFERENCES

1. Bergen WG, Wu G: Intestinal nitrogen recycling and utilization in health and disease. J Nutr 139, 821-825 (2009) DOI: 10.3945/jn.109.104497
2. Dai ZL, Li XL, Xi PB, Zhang J, Wu G, Zhu WY: L-Glutamine regulates amino acid utilization by intestinal bacteria. Amino Acids 45, 501-512 (2013) DOI: 10.1007/s00726-012-1264-4
3. Brosnan JT, Brosnan ME: Glutamate: a truly functional amino acid. Amino Acids 45, 413-418 (2013) DOI: 10.1007/s00726-012-1280-4
4. Liu YL, Huang JJ, Hou YQ, Zhu HL, Zhao SJ, Ding BY, Yin YL, Yi GF, Shi JY, Fan W: Dietary arginine supplementation alleviates intestinal mucosal disruption induced by Escherichia coli lipopolysaccharide in weaned pigs. Br J Nutr 100, 552-560 (2008) DOI: 10.1017/S0007114508911612
5. Dai ZL, Wu G, Zhu WY: Amino acid metabolism in intestinal bacteria: links between gut ecology and host health. Front Biosci 16, 1768-1786 (2011) DOI: 10.2741/3820
6. Xi P, Jiang Z, Zheng C, Lin Y, Wu G: Regulation of protein metabolism by glutamine: implications for nutrition and health. Front Biosci 16, 578-597 (2011) DOI: 10.2741/3707
7. Hou YQ, Wang L, Yi D, Ding BY, Yang ZG, Li J, Chen X, Qiu YS, Wu GY: N-acetylcysteine reduces inflammation in the small intestine by regulating redox, EGF and TLR4 signaling. Amino Acids 45, 513-522 (2013) DOI: 10.1007/s00726-012-1295-x
8. Wang QJ, Hou YQ, Yi D, Wang L, Ding BY, Chen X, Long MH, YL Liu, Wu GY: Protective effects of N-acetylcysteine on acetic acid-induced colitis in a porcine model. BMC Gastroenterol 13, 133 (2013) DOI: 10.1186/1471-230X-13-133
9. Blikslager AT, Moeser AJ, Gookin JL, Jones SL, Odle J: Restoration of barrier

function in injured intestinal mucosa. Physiol Rev 87, 545-564 (2007)
DOI: 10.1152/physrev.00012.2006

10. Zafarullah M, Li WQ, Sylvester J, Ahmad M: Molecular mechanisms of N-acetylcysteine actions. Cell Mol Life Sci 60, 6-20 (2003)
DOI: 10.1007/s000180300001

11. Samuni Y, Goldstein S, Dean OM, Berk M: The chemistry and biological activities of N-acetylcysteine. Biochim Biophys Acta 1830, 4117-4129 (2013)
DOI: 10.1016/j.bbagen.2013.04.016

12. Aruoma OI, Halliwell B, Hoey BM, Butler J: The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic Biol Med 6, 593-597 (1989)
DOI: 10.1016/0891-5849(89)90066-X

13. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND: Glutathione metabolism and its implications for health. J Nutr 134, 489-492 (2004)

14. Hagen TM, Wierzbicka GT, Sillau AH, Bowman BB, Jones DP: Bioavailability of dietary glutathione: Effect on plasma concentration. Am J Physiol 259, G524-G529 (1990)

15. Vincenzini M T, Favilli F, Iantomasi T: Glutathione-mediated transport across intestinal brush-bordermembranes. Biochim Biophys Acta 942,107-114 (1988)
DOI: 10.1016/0005-2736(88)90279-9

16. Hu Y, Zhang LL, Hou YQ, Ding BY, Zhu HL, Liu YL, Yang ZG, Zhang W: Effects of N-acetylcysteine on growth performance and plasma biochemical indices of lipopolysaccharide challenged piglets. Chinese Journal of Animal Nutrition 22, 1007-1011 (2010)

17. Hou YQ, Wang L, Zhang W, Yang ZG, Ding BY, Zhu HL, Liu YL, Qiu YS,Yin YL, Wu GY: Protective effects of N-acetylcysteine on intestinal functions of piglets challenged with lipopolysaccharide. Amino Acids 43, 1233-1242 (2012)
DOI: 10.1007/s00726-011-1191-9

18. Hou YQ, Wang L, Ding BY, Liu YL, Zhu HL, Liu J, Li YT, Wu X, Yin YL, Wu GY: Dietary α-ketoglutarate supplementation ameliorates intestinal injury in lipopolysaccharide-challenged piglets. Amino acids 29, 555-564 (2010)
DOI: 10.1007/s00726-010-0473-y

19. Montagne L, Pluske JR, Hampson DJ: A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim Feed Sci Tech 108, 95-117 (2003)
DOI: 10.1016/S0377-8401(03)00163-9

20. Hou YQ, Wang L, Ding BY, Liu YL, Zhu HL, Liu J, Li YT, Kang P, Yin YL, Wu G: Alpha-ketoglutarate and intestinal function. Front Biosci 16, 1186-1196 (2011)
DOI: 10.2741/3783

21. Mansoori B, Nodeh H, Modirsanei M, Rahbari S, Aparnak P: D-Xylose absorption test: A tool for the assessment of the effect of anticoccidials on the intestinal absorptive capacity of broilers during experimental coccidiosis. Anim Feed Sci Tech 148, 301-308 (2009)
DOI: 10.1016/j.anifeedsci.2008.04.009

22. Luk GD, Bayless TM, Baylin SB: Plasm a postheparin diamine oxidase: Sensitive provocative test for quantitating length of acute intestinal mucosa injury in the rat. J Clin Invest 71, 1308-1315 (l983)

23. Ropelle ER, Pauli JR, Fernandes MFA, Rocco SA, Marin RM, Morari J, Souza KK, Dias MM, Gomes-Marcondes MC, Gontijo JAR, Franchini KG, Velloso LA, Saad MJA, José BC: Carvalheira. A central role for neuronal amp-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) in high-protein diet–induced weight loss. Diabetes 57, 594-605 (2008)
DOI: 10.2337/db07-0573

24. Nishi K, Oda T, Takabuchi S, Oda S, Fukuda K, Adachi T, Semenza GL, Shingu K, Hirota K: LPS induces hypoxia-Inducible factor 1 activation in macrophage-differentiated cells in

a reactive oxygen species-dependent manner. Antioxid Redox Signal 10, 983-995 (2008)
DOI: 10.1089/ars.2007.1825

25. Fang YZ, Yang S, Wu G: Free radicals, antioxidants, and nutrition. Nutrition 18, 872-879 (2002)
DOI: 10.1016/S0899-9007(02)00916-4

26. Yu BP: Cellular defenses against damage from reactive oxygen species. Physiol Rev 74, 139-162 (1994)

27. Sepponen K, Pösö AR: The inducible form of heat shock protein 70 in the serum, colon and small intestine of the pig: comparison to conventional stress markers. Vet J 171, 519-524 (2006)
DOI: 10.1016/j.tvjl.2005.01.005

28. Davis W Jr, Ronai Z, Tew KD: Cellular thiols and reactive oxygen species in drug-induced apoptosis. J Pharmacol Exp Ther 296,1-6 (2001)

29. Knight KR, MacPhadyen K, Lepore DA, Kuwata N, Eadie PA, O'Brien BM: Enhancement of ischaemic rabbit skin flap survival with the antioxidant and free-radical scavenger N-acetylcysteine. Clin Sci (Lond) 81, 31-36 (1991)

30. Fishbane S, Durham JH, Marzo K, Rudnick M: N-Acetylcysteine in the prevention of radiocontrast-induced nephropathy. J Am Soc Nephrol 15, 251-260 (2004)
DOI: 10.1097/01.ASN.0000107562.68920.92

31. Cetinkaya A, Bulbuloglu E, Kurutas EB, Ciralik H, Kantarceken B, Buyukbese MA: Beneficial effects of N-acetylcysteine on acetic acid-induced colitis in rats. Tohoku J Exp Med 206, 131-139 (2005)
DOI: 10.1620/tjem.206.131

32. Krawisz JE, Sharon P, Stenson WF: Quantitative assay for acute intestinal inflammation based on myeloper-oxidase activity. Gastroenterology 1984, 87, 1344-1350 (1984)

33. Liu YL, Li DF, Gong LM, Yi GF, Gaines AM, Carroll JA: Effects of fish oil supplementation on performance as well as immunological, adrenal, and somatotropic responses of weaned pigs after Escherichia coli lipopolysaccharide challenge. J Anim Sci 81, 2758-2765 (2003)

34. Webel DM, Finck BN, Baker DH, Johnson RW: Time course of increased plasma cytokines, cortisol and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. J Anim Sci 75,1514-1520 (1997)

35. Blackwell TS, Blackwell TR, Holden EP, Christman BW, Christman JW: In vivo antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation. J Immunol 157, 1630-1637 (1996)

36. Kroger H, Miesel R, Dietrich A, Ohde M, Altrichter S, Braun C, Ockenfels H: Suppression of type II collagen-induced arthritis by N-acetyl-L-cysteine in mice. Gen Pharmacol 29, 671-674 (1997)
DOI: 10.1016/S0306-3623(96)00570-8

37. Tsuji F, Miyake Y, Aono H, Kawashima Y, Mita S: Effects of bucillamine and N-acetyl-L-cysteine on cytokine production and collagen-induced arthritis (CIA). Clin Exp Immunol 115, 26-31 (1999)
DOI: 10.1046/j.1365-2249.1999.00749.x

38. Li P, Yin YL, Li D, Kim SW, Wu G: Amino acids and immune function. Br J Nutr 98, 237-252 (2007)
DOI: 10.1017/S000711450769936X

39. Lehmann GL, Carreras FI, Soria LR, Gradilone SA, Marinelli RA: LPS induces the TNF-alpha-mediated downregulation of rat liver aquaporin-8: role in sepsis-associated cholestasis. Am J Physiol Gastrointest Liver Physiol 294, G567-575 (2008)
DOI: 10.1152/ajpgi.00232.2007

40. Tan B, Yin Y, Kong X, Li P, Li X, Gao H, Li X, Huang R, Wu G: L-Arginine stimulates proliferation and prevents endotoxin-induced death of intestinal cells. Amino Acids 38, 1227-1235 (2010)
DOI: 10.1007/s00726-009-0334-8

41. Leaphart CL, Cavallo J, Gribar SC, Cetin S, Li J, Branca MF, Dubowski TD, Sodhi CP,

Hackam DJ: A critical role for TLR4 in the pathogenesis of necrotizing enterocolitis by modulating intestinal injury and repair. J Immunol 179, 4808-4820 (2007) DOI: 10.4049/jimmunol.179.7.4808

42. Gribar SC, Anand RJ, Sodhi CP, Hackam DJ: The role of epithelial Toll-like receptor signaling in the pathogenesis of intestinal inflammation. J Leukoc Biol 83, 493-498 (2008) DOI: 10.1189/jlb.0607358

43. Psarra AM, Solakidi S, Sekeris CE: The mitochondrion as a primary site of action of steroid and thyroid hormones: presence and action of steroid and thyroid hormone receptors in mitochondria of animal cells. Mol Cell Endocrinol 246 (1-2), 21-33 (2006) DOI: 10.1016/j.mce.2005.11.025

44. Frederich M, O'Rourke MR, Furey NB, Jost JA: AMP-activated protein kinase (AMPK) in the rock crab, Cancer irroratus: an earlyindicator of temperature stress. J Exp Biol 212, 722-730 (2009) DOI: 10.1242/jeb.021998

45. Hou YQ, Yao K, Wang L, Ding BY, Fu DB, Liu YL, Zhu HL, Liu J, Li YT, Kang P, Yin YL, Wu GY: Effects of α-ketoglutarate on energy status in the intestinal mucosa of weaned piglets chronically challenged with lipopolysaccharide. Br J Nutr 106, 357-363 (2011) DOI: 10.1017/S0007114511000249

46. Hardie DG, Scott JW, Pan DA, Hudson ER: Management of cellular energy by the AMP-activated protein kinase system. FEBS Lett 546, 113-120 (2003) DOI: 10.1016/S0014-5793(03)00560-X

47. Crouser ED, Julian MW, Weinstein DM, Fahy RJ, Bauer JA: Endotoxin-induced ileal mucosal injury and nitric oxide dysregulation are temporally dissociated. Am J Respir Crit Care Med 161, 1705-1712 (2000) DOI: 10.1164/ajrccm.161.5.9907043

48. Lobo SM, De Backer D, Sun Q, Tu Z, Dimopoulos G, Preiser JC, Nagy N, Vray B, Vercruy V, Terzi RG, Vincent JL: Gut mucosal damage during endotoxic shock is due to mechanisms other than gut ischemia. J Appl Physiol 95, 2047-2054 (2003)

49. Yang Z: The effects of N-acetylcysteine on immunological stress and energy status of piglets challenged with lipopolysaccharide. DM.S. Thesis, Wuhan Polytechnic University, Wuhan, China (2011)

50. Fisslthaler B, Fleming I: Activation and signaling by the amp-activated protein kinase in endothelial cells. Circ Res 105,114-127 (2009) DOI: 10.1161/CIRCRESAHA.109.201590

51. Winder WW, Hardie DG: The AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am J Physiol 277, E1-E10 (1999)

52. Hardie DG, Hawley SA: AMP-activated protein kinase: the energy charge hypothesis revisited. Bioessays 23:1112-1119 (2001) DOI: 10.1002/bies.10009

53. Evans AM, Mustard KJW, Wyatt CN, Peers C, Dipp M, Kumar P, Kinnear NP, Hardie DG: Does AMP-activate protein kinase couple inhibition of mitochondrial oxidative phosphorylation by hypoxia to calcium signaling in O2-sensing cells? J Biol Chem 280, 41504–41511 (2005) DOI: 10.1074/jbc.M510040200

54. Hardie DG, Carling D, Carlson M: The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? Annu Rev Biochem 67, 821-55 (1998) DOI: 10.1146/annurev.biochem.67.1.821

55. Chandra J, Samali A, Orrenius S: Triggering and modulation of apoptosis by oxidative stress. Free Radic Biol Med 29: 323-333 (2000) DOI: 10.1016/S0891-5849(00)00302-6

56. Novitsky VV, Ryazantseva NV, Chasovskih NY, Starikova EG, Kaygorodova EV, Starikov YV, Jukova OB: Modulation of apoptosis of mononuclear cells under

conditions of oxidative stress. Bull Exp Biol Med 145, 283-286 (2008)
DOI: 10.1007/s10517-008-0071-0

57. Fasina YO, Moran ET, Ashwell CM, Conner DE, Leslie M, Mckee SR: Effect of dietary gelatin supplementation on the expression of selected enterocyte genes, intestinal development and early chick performance. Int J Poultry Sci 6, 944-951 (2007)
DOI: 10.3923/ijps.2007.944.951

58. Iji PA, Saki A, Tivey DR: Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. Anim Feed Sci Technol 89, 175-188 (2001)
DOI: 10.1016/S0377-8401(00)00223-6

59. Hou YQ, Wang L, Yi D, Ding BY, Chen X, Wang QJ, Zhu HL, Liu YL, Yin YL, Gong J, Wu G: Dietary supplementation with tributyrin alleviates intestinal injury in piglets challenged with intrarectal administration of acetic acid. Br J Nutr 111, 1748-1758 (2014)
DOI: 10.1017/S0007114514000038

60. Nicholson DW, Thornberry NA: Caspases: killer proteases. Trends Biochem Sci 22, 299-306 (1997)
DOI: 10.1016/S0968-0004(97)01085-2

61. Jänicke RU, Sprengart ML, Wati MR, Porter AG (1998) Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J Biol Chem 273:9357-9360
DOI: 10.1074/jbc.273.16.9357

62. Liu F, Yin J, Du M, Yan P, Xu J, Zhu X, Yu J: Heat-stress-induced damage to porcine small intestinal epithelium associated with downregulation of epithelial growth factor signaling. J Anim Sci 87, 1941-1949 (2009)
DOI: 10.2527/jas.2008-1624

63. Helmrath MA, Shin CE, Erwin CR: Intestinal adaptation is enhanced by epidermal growth factor independent of increased ileal epidermal growth factor receptor expression. J Pediatr Surg 33, 980-985 (1998)
DOI: 10.1016/S0022-3468(98)90518-8

64. Nair RR, Warner BB, Warner BW: Role of epidermal growth factor and other growth factors in the prevention of necrotizing enterocolitis. Semin Perinatol 32, 107-113 (2008)
DOI: 10.1053/j.semperi.2008.01.007

65. Plowman GD, Green JM, McDonald VL, Neubauer MG, Disteche CM, Todaro GJ, Shoyab M: The amphiregulin gene encodes a novel epidermal growth factor-related protein with tumor-inhibitory activity. Mol Cell Biol 10, 1969-1981 (1990)

66. Boudreau F, Lussier CR, Mongrain S, Darsigny M, Drouin JL, Doyon G, Suh ER, Beaulieu JF, Rivard N, Perreault N: Loss of cathepsin L activity promotes claudin-1 overexpression and intestinal neoplasia. FASEB J 21, 3853-3865 (2007)
DOI: 10.1096/fj.07-8113com

67. Howe KL, Reardon C, Wang A, Nazli A, McKay DM: Transforming growth factor-β regulation of epithelial tight junction proteins enhances barrier function and blocks enterohemorrhagic escherichia coli O157:h7-induced increased permeability. Am J Pathol 167, 1587-1597 (2005)
DOI: 10.1016/S0002-9440(10)61243-6

68. Tsukita S, Furuse M: The structure and function of claudins, cell adhesion molecules at tight junctions. Ann NY Acad Sci 915, 129-135 (2000)
DOI: 10.1111/j.1749-6632.2000.tb05235.x

69. Rhoads JM, Wu G: Glutamine, arginine, and leucine signaling in the intestine. Amino Acids 37, 111-122 (2009)
DOI: 10.1007/s00726-008-0225-4

70. Schneeberger EE, Lynch RD: The tight junction: a multifunctional complex. Am J Physiol Cell Physiol 286, C1213-C1228 (2004)
DOI: 10.1152/ajpcell.00558.2003

71. Sheth P, Delos Santos N, Seth A, LaRusso NF, Rao RK: Lipopolysaccharide disrupts tight junctions in cholangiocyte monolayers by a c-Src-, TLR4-, and LBP-dependent mechanism. Am J

Physiol Gastrointest Liver Physiol 293, G308-G318 (2007)
DOI: 10.1152/ajpgi.00582.2006

72. Wu G, Wu ZL, Dai ZL, Yang Y, Wang WW, Liu C, Wang B, Wang JJ, Yin YL: Dietary requirements of "nutritionally nonessential amino acids" by animals and humans. Amino Acids 44, 1107-1113 (2013)
DOI: 10.1007/s00726-012-1444-2

73. Wu G: Dietary requirements of synthesizable amino acids by animals: A paradigm shift in protein nutrition. J Anim Sci Biotechnol 5, 34 (2014)
DOI: 10.1186/2049-1891-5-34

74. Wang WW, Wu ZL, Dai ZL, Yang Y, Wang JJ, Wu G: Glycine metabolism in animals and humans: implications for nutrition and health. Amino Acids 45, 463-477 (2013)
DOI: 10.1007/s00726-013-1493-1

75. Rezaei R, Knabe DA, Tekwe CD, Dahanayaka S, Ficken MD, Fielder SE, Eide SJ, Lovering SL, Wu G: Dietary supplementation with monosodium glutamate is safe and improves growth performance in postweaning pigs. Amino Acids 44, 911-923 (2013)
DOI: 10.1007/s00726-012-1420-x

76. Rezaei R, Wang WW, Wu ZL, Dai ZL, Wang JJ, Wu G: Biochemical and physiological bases for utilization of dietary amino acids by young pigs. J Anim Sci Biotechnol 4, 7 (2013)
DOI: 10.1186/2049-1891-4-7

77. Wang WW, Wu ZL, Lin G, Hu SD, Wang B, Dai ZL, Wu G: Glycine stimulates protein synthesis and inhibits oxidative stress in pig small-intestinal epithelial cells. J Nutr (2014).
DOI: 10.3945/jn.114.194001

78. Wu G: Functional amino acids in nutrition and health. Amino Acids 45, 407-411 (2013)
DOI: 10.1007/s00726-013-1500-6

79. Wu G, Bazer FW, Dai ZL, Li DF, Wang JJ, Wu ZL: Amino acid nutrition in animals: protein synthesis and beyond. Annu Rev Anim Biosci 2, 387-417 (2014)
DOI: 10.1146/annurev-animal-022513-114113

80. Li P, Mai KS, Trushenski J, Wu G: New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino Acids 37, 43-53 (2009)
DOI: 10.1007/s00726-008-0171-1

81. Li XL, Rezaei R, Li P, Wu G: Composition of amino acids in feed ingredients for animal diets. Amino Acids 40, 1159-1168 (2011)
DOI: 10.1007/s00726-010-0740-y

82. Dai ZL, Wu ZL, Jia SC, Wu G: Analysis of amino acid composition in proteins of animal tissues and foods as pre-column o-phthaldialdehyde derivatives by HPLC with fluorescence detection. J Chromatogr B 964, 116-127 (2014)
DOI: 10.1016/j.jchromb.2014.03.025

83. Mates JM, Segura JA, Alonso FJ, Marquez J: Sulphur-containing non enzymatic antioxidants: therapeutic tools against cancer. Front Biosci (Schol Ed) 4, 722-748 (2012)
DOI: 10.2741/S296

**Abbreviations:** AEC, adenylate energy charge; AR, amphiregulin; DAO, diamine oxidase; EGF, EGFR, epidermal growth factor receptor; HSP 70, heat shock protein 70; LPS, lipopolysaccharide; NAC, N-acetylcysteine; NF-κB, nuclear factor κB; SEM, standard error of the mean; SD, standard deviation of the mean; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-alpha

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