FULL PAPER *Pharmacology*

Protective effect of quercetin on pig intestinal integrity after transport stress is associated with regulation oxidative status and inflammation

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ABSTRACT. This experiment was conducted to evaluate the effects of quercetin supplementation on intestinal integrity, intestinal reactive oxygen species (ROS) levels and intestinal inflammation in pigs under transport stress. A total of 170 finishing pigs were randomly assigned into two groups. Animals in the control group consumed a basal diet, while those in the treatment group consumed the same diet supplemented with 25 mg quercetin per kg feed. After a 4-week period, pigs were transported for 5 hr. The quercetin-supplemented pigs showed decreased serum levels of endotoxin ($P<0.05$), increased height of jejunum villi ($P<0.05$), and increased occludin and zonula occudens-1 (ZO-1) mRNA expression in the jejunum ($P<0.05$). These parameters are associated with intestinal health and were markedly improved by quercetin supplementation. Pigs consuming the quercetin-supplemented diet had lower intestinal levels of ROS and malondialdehyde (MDA) compared with the control group ($P<0.05$). This finding coincided with greater inhibition of the innate immune system ($P<0.05$), including mitogen-activated protein kinase (MAPK), protein kinase B (Akt) and nuclear factor κB (NF-κB) signaling pathways, as well as decreased expression of inflammatory cytokines in the jejunum. These results indicate that quercetin alleviates intestinal injury in pigs during transport, probably through modulation of intestinal oxidative status and inflammation.

KEY WORDS: inflammatory disorder, pig intestine, quercetin, reactive oxygen species, transport stress

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Pigs are often subjected to road transportation, which is complicated by various physical and psychological challenges including noise, motion, fasting, dehydration, crowding and changes in temperature [28, 39]. The stresses induced by animal transport have been associated with impairment of intestinal barrier function [33, 43, 45]. Effects of transport stress on the gastrointestinal tract are of great interest, because they may increase the risk of bacterial translocation, leading to carcass damage, poor meat quality and high morbidity [14, 15, 33, 34].

During transportation, the level of reactive oxygen species (ROS) can increase dramatically and result in oxidative stress [26, 47, 48]. ROS have been implicated in the pathogenesis of stress-induced gastrointestinal mucosal injury [3]. ROS interact with target cell membrane constituents to cause lipid peroxidation, membrane disintegration and endothelial cell damage [9]. ROS also lead to the activation of mitogen-activated protein kinase (MAPK) (e.g., p38, ERK1/2 and JNK), protein kinase B (Akt) and Nuclear factor κB (NF-κB) signaling pathways, and the release of proinflammatory substances [30, 37]. The accumulation of proinflammatory substances further contributes to ROS formation and intestinal tissue damage, resulting in bacterial translocation [16]. Therefore, the oxidative status of pigs at the time of slaughter is a critical factor in intestinal barrier integrity.

Several antioxidants are available to alleviate the negative effects of transport stress in pigs. Phytochemicals have been supplemented in the diets of pigs during transport, because of their reported benefits [27, 48]. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the most widely distributed bioflavonoids [10]. It is present in high concentrations in apples, onions, mulberries, potatoes, broccoli, tea, peanuts, soybeans and red wine. It has potent antioxidant effects, because of its metal chelation and free-radical scavenging activities [19]. There is evidence supporting the use of quercetin supplementation in the treatment of intestinal barrier defects in rats [18, 24]. However, to our knowledge, there have been no studies demonstrating beneficial effects of nutritional supplements on intestinal barrier function in transported pigs.

The objective of the present study was to evaluate the effects of dietary quercetin supplementation on intestinal integrity, intestinal oxidative status and intestinal immune system function in pigs undergoing transport. We hypothesized that dietary quercetin supplementation would be associated with regulation of the intestinal oxidative status and improved intestinal immune system function. The transport procedure used in the present study was known to induce stress in pigs [5, 48].

MATERIALS AND METHODS

All animal handling protocols were approved by the

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Huazhong Agricultural University Animal Care and Use Committee guidelines.

Animals, diets and treatments: A total of 170 finishing pigs (Large White × Landrace) with an initial body weight (BW) of 72 ± 4 kg were obtained from the same farm (Wuhan Chinapork Co., Ltd., Wuhan, China). The pigs were split into two groups according to diet. Each group of 85 pigs was further split into five replicate pens, each holding 17 pigs. The control group received feed without supplementation. The composition of the control diet is shown in Table 1. The treatment group received supplementation with 25 mg quercetin per kg feed (as-fed basis). Quercetin 98% extract from *Sophora japonica* L. was purchased from YuanCheng Biotechnology Co., Ltd., Wuhan, China. Pigs were allowed feed and water *ad libitum* over a period of 4 weeks.

Transport procedure: On the day of slaughter, 36 pigs per treatment (total of 72 pigs) with the final BW closest to the 100 kg were selected. The pigs were transported using the method described by Zhang *et al.* [48]. Selected pigs were transported in an open truck at an average ambient temperature between 20°C and 30°C. The trucks used to transport the pigs had 3 levels, each containing 4 special vehicle pens (length × width × height: 190 × 112.5 × 120 cm). Pigs were loaded into the special vehicle pens with stocking densities of 6 pigs per pen or 275 kg/m^2. Pigs in each of special vehicle pens were from the same diet treatment. In the present study, a total of 12 special vehicle pens of pigs were transported. The transport took place on ordinary roads, highways and bumpy roads at speeds between 60 and 90 km/hr.

Sample collection: After 5 hr transportation, one pigs per vehicle pen (total of 12 pigs) were selected to be harvested for intestinal integrity, intestinal oxidative status and intestinal immune system evaluation. Samples of the jejunum itself were removed from the middle jejunum segment and then rinsed with ice-cold physiological saline. One section was snap-frozen in liquid nitrogen and then stored at −80°C until further analysis. Other sections of ileum (3 cm) were kept in 4% neutral buffered formalin for gut morphological analysis. Blood samples were collected by beaker during exsanguination and then quickly separated into five tubes. A 10 m*l* sample was placed on ice immediately and subsequently centrifuged at 1,300 × g at 4°C for 15 min to obtain serum. The serum samples were stored at −80°C for subsequent analysis.

Gut morphological analysis: The digestive tract was removed, and the jejunum was cut and fixed in 10% phosphate-buffered formalin. The samples were sectioned at 5 μm thickness and stained with hematoxylin and eosin. Villous height and crypt depth were measured on the stained sections using a light microscope fitted with an image analyzer (Image Pro Plus 6.0; Media Cybernetics, Bethesda, MD, U.S.A.). The measurements of 20 villi and crypts were taken for each segment.

Measurement of serum endotoxin level: Serum endotoxin level was measured by a quantitative chromogenic endpoint tachypleus amebocyte lysate endotoxin detection kit following the manufacturer's instructions (Xiamen TAL Experimental Plant Co., Ltd., Xiamen, China). Briefly, serum samples were diluted to 1:10 with water/Tris–HCl buffer. After centrifuged at 1,270 × g for 10 min, the supernatant was removed and incubated with limulus amebocyte lysate at 37°C for 10 min, followed by incubation with the provided chromogenic substance for 6 min. The absorbance at 545 nm was measured after adding appropriate-reagents.

Table 1. Composition and analysis of the basal diet

Composition (g/kg)	Basal diet[a)]
Wheat	380.00
Corn, grains	464.10
Soybean meal (46%)	89.00
Monocalcium phosphate	14.00
Limestone	7.00
Mycetes adsorbent	1.50
Antimildew agent	0.50
Salt	3.50
Soybean oil	20.00
Ethoxyquin	0.25
Probiotics	0.20
Y402 premix[b)]	20.00
Analysis[c)]	
Dry matter – DM (%)	86.80
Metabolism energy (MJ/ kg)	13.20
Crude protein – CP (%)	13.90
Crude fiber (%)	2.80
Ash (%)	3.60
Fat (%)	4.30
Calcium (%)	0.60
Phosphorus (%)	0.60

a) Control group (C) was fed with the above basal diet, whereas the quercetin group consumed the basal diet supplemented with 25 mg kg^{-1} Quercetin, respectively. b) Premix contained per kg: 10.5 g Fe, 1.4 g Cu, 8.5 g Zn, 4 g Mn, 7.5 mg Se, 30 mg I,350 kIU of vitamin A, 40 kIU of vitamin D3, 1.5 kIU of vitamin E, 50 mg of vitamin K3, 50 mg of vitamin B1, 150 mg of vitamin B2, 100 mg of vitamin B6, 0.1 mg of vitamin B12, 86.4 g lysine, 17.5 g methionine, 25 g threonine, 4 g phytase and 15 g choline (kIU: 1,000 international units). c) Metabolism energy was calculated from data provided by Feed Database in China (1999).

Chemiluminescence measurement of ROS: Levels of ROS were measured in the jejunum of pig by chemiluminescence assay using luminol (5-amino-2, 3-dihydro-1, 4-phthalazinedione, Sigma, St. Louis, MO, U.S.A.) as probe. The measurements had minor changes according to the method of Kobayashi *et al.* [20]. Jejunum tissues homogenates (10%) were prepared in chilled normal saline. Luminescence of the tissue samples was recorded at room temperature using a LB 940 luminometer (Berthold Technologies, Bad Wildbad, Germany) in the presence of enhancers. Fifty microliters homogenates and 20 μl horseradish peroxidase (HRP) (1 U of HRP, typeVI 310 U/mg; Sigma) were added into 200 μl Krebs-HEPES buffer. 25 microliters of 5 mM luminol was added to mixture. Levels of ROS were determined by measuring chemiluminescence in the integrate mode until the chemiluminescence was down to the start value. All chemiluminometric counts were obtained at 0.05 sec intervals for 5 sec, and the results were expressed as areas under the curve

Table 2. Species and genus specific primers used for real time PCR

Gene	Primers (sense/antisense 5'-3')	Size (bp)	Annealing temperature (°C)
TNF-α	F: CACCACGCTCTTCTGCCTACTG R: TTGAGACGATGATCTGAGTCCTTGG	115	63
MCP-1	F: GTCCTTGCCCAGCCAGATG R: CGATGGTCTTGAAGATCACTGCT	148	60
IL1-β	F: AAAGGGGACTTGAAGAGAG R: CTGCTTGAGAGGTGCTGATGT	286	58
IL-6	F: AAGGTGATGCCACCTCAGAC R: TCTGCCAGTACCTCCTTGCT	151	60
ZO-1	F: GGCGCACGGCGAAGGTAATT R: CTATCAAACTCAGGAGGCGGCACT	405	60
Occludin	F: GGAGTGATTCGGATTCTGTCTATGCT R: CGCCTGGGCTGTTGGGTTGA	423	60
β-actin	F: CCAGGTCATCACCATCGG R: CCGTGTTGGCGTAGAGGT	158	60

(AUCs) of relative light units (RLU).

Assessment of lipid peroxidation: Jejunum tissues homogenates (10%) were prepared in chilled normal saline. The contents of malondialdehyde (MDA) in jejunum were assayed using colorimetric methods with a spectrophotometer (Biomate 5, Thermo Electron Corporation, Rochester, NY, U.S.A.). The assays were conducted with the assay kits purchased from Nanjing Jiancheng Insititute of Bioengineering (Nanjing, China) and the procedures accordingly

Protein immunoblot analysis: Phosphorylated c-Jun N-terminal protein kinase (p-JNK), phosphorylated extracellular signal-regulated kinases 1/2 (p-ERK1/2), phosphorylated p38 (p-p38), phosphorylated protein kinase B (p-Akt) and nuclear p65 proteins were analyzed by western blotting. Briefly, 100 mg of frozen tissue of jejunum was homogenized in 1 m*l* RIPA lysis buffer (with 1 mM PMSF). For nuclear p65 measurement, the nuclear fractions were isolated using the Nuclear/Cytosol Fractionation Kit (BestBio, Shanghai, China). Next, they were centrifuged at 12,000 g at 4°C for 10 min, and the supernatants were collected for assay. After the protein concentration was determined by a standard BCA protein assay, protein sample was loaded per lane and separated on SDS-PAGE. The target protein was then electrophoretically transferred to nitrocellulose membranes, which were blocked in TBST (5% nonfat milk, 10 mM Tris, 150 mM NaCl and 0.05% Tween-20) for 2 hr. Next, they were incubated with first antibodies, anti-Phospho-p38 MAPK (1:1,000; Cell Signaling Signaling, Billerica, MA, U.S.A.), anti-Phospho-JNK (1:1,000; Cell Signaling), anti-Phospho-ERK1/2 (1:1,000; Cell Signaling), anti-Phospho-Akt (1:1,000; Cell Signaling), anti-NF-κB p65 (1:1,000; Cell Signaling), anti-PCNA (1:5,000; BD Transduction Laboratories, San Diego, CA, U.S.A.) or anti-actin antibodies (1:1,000; Cell Signaling) at 4°C overnight. After three washes with Tris-buffered saline containing 0.1% Tween 20, blots were incubated with the HRP-conjugated secondary antibodies, anti-rabbit IgG (1:15,000; Jackson ImmunoResearch, West Grove, PA, U.S.A.) or anti-mouse IgG (1:15,000; Jackson ImmunoResearch) for 2 hr and were washed again. Chemiluminescence detection was performed using the ECL reagent (Thermo Scientific, Rockford, IL, U.S.A.) according to the manufacturer's instructions. Specific bands were detected and were analyzed and quantified by Image J Software (NIH, Bethesda, MD, U.S.A.).

Quantitative PCR. Total RNA was extracted from samples of jejunum using Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer's instructions. Primers (Table 2) used in this study were either synthesized according to our previous protocols or designed with Primer 5.0 according to pig gene sequences. Real-time PCR was performed according to our previous study [44]. The relative expression of genes in the treatment group was normalized based on the values of the control group.

Statistical analysis: Statistical analysis was performed using Prism software (Prism 5.0; GraphPad Software, La Jolla, CA, U.S.A.). Numbers (*n*) used for statistics are noted in the figures. All data were analyzed by *t* test procedures of SAS (v 8.2, SAS Inst., Inc., Cary, NC, U.S.A.). All the values were presented as means ± standard error of the mean (SEM), and those at $P<0.05$ were considered significant.

RESULTS

Gut morphology in the jejunum of pigs: The status of the gut and its microscopic structure are good indicators of the stress response of the intestinal tract [41]. As shown in Table 3, when pigs endured transportation, villus height was significantly increased in pigs fed the quercetin supplemented diet compared with those fed with the control diet ($P<0.05$). As shown in Fig. 1, the villi were scattered and seriously desquamated in the jejunum of the control group, while higher and intact villi were observed in the jejunum of quercetin treated pigs.

Endotoxin levels in the pigs serum: The endotoxin level is a useful biomarker for evaluating the integrity of the gastrointestinal tract [11]. The effects of treatment with quercetin on endotoxin levels in the serum of pigs are shown in Fig. 2. After slaughter, pigs fed the diet supplemented with quercetin had a significantly lower ($P<0.05$) concentration of endotoxin in the serum than pigs fed the control diet.

The mRNA levels of barrier tight junction protein in the jejunum of pigs: To determine the effects of quercetin

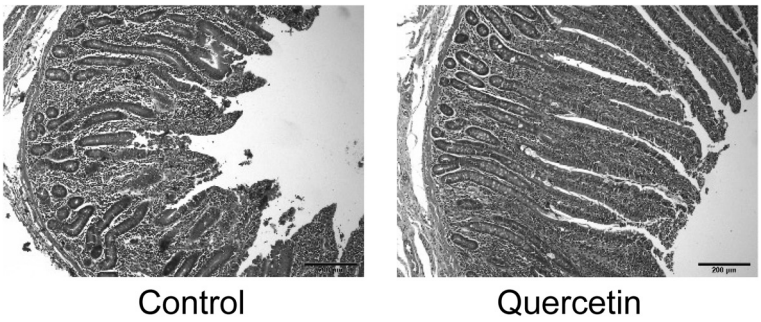

Fig. 1. Effect of quercetin on morphology in the jejunum of pig stimulated with transportation. The jejunum was cut off and fixed in 10% formaldehyde-phosphate buffer and then stained with hematoxylin and eosin. Hematoxylin and eosin staining with original magnification × 100. Bars represent 200 μm.

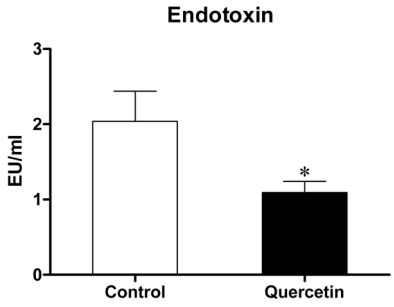

Fig. 2. Effect of quercetin on endotoxin levels in the serum of pig stimulated with transportation. Values are means ± SEMs, n=6. *Significant different (P<0.05) from the control group.

Table 3. Effect of quercetin on gut morphology in the jejunum of pig stimulated with transportation

Items	Control	Quercetin	SEM	*P* value
Villous height (μm)	320.41	442.33*	30.97	0.02
Villous width (μm)	123.45	128.20	7.13	0.77
Crypt depth (μm)	222.39	266.97	24.71	0.42
Villous height: crypt depth ratio	1.33	1.64	0.11	0.18

Values are means ± SEM, n=6. *Significant different (P<0.05) from the control group. **Significant different (P<0.01) from the control group.

supplementation on the intestinal mucosal tight junction, the expressions of *Occludin* and *zonula occudens-1* (*ZO-1)* were measured at mRNA levels. As shown in Fig. 3, the mRNA levels of *Occludin* and *ZO-1* were moderately higher in the quercetin group than in the control group (P<0.01).

ROS and MDA (malondialdehyde) levels in the jejunum: Levels of ROS and MDA are represented in Fig. 4. Pigs offered diets containing quercetin had lower levels of ROS and MDA in the jejunum compared with the control group (P<0.05).

MAPK, Akt and NF-κB pathways in the jejunum: MAPK, Akt and NF-κB pathways are induced by ROS and have been implicated in the induction of proinflammatory genes [30]. As shown in Fig. 5a, the treatment group showed inhibited activation of JNK and ERK1/2 due to a decrease in levels of phosphorylated JNK and ERK1/2 proteins compared with the control group (P<0.05). In contrast, quercetin supplementation had no effect on phosphorylated p38. Compared with the control group, dietary supplementation with quercetin reduced the amount of phosphorylated Akt protein in the jejunum (P<0.05). Similarly, dietary supplementation with quercetin decreased the amount of NF-κB p65 protein in jejunal tissues compared with the control group (Fig. 5b, P<0.05).

Pro-inflammatory cytokine mRNA levels in the jejunum: We examined gene expression levels of four major inflammatory cytokines involved in mucosal inflammation of jejunal tissues: Tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and Monocyte chemotactic protein-1 (MCP-1). The results are presented in Table 4. When pigs were given dietary supplementation with quercetin, the levels of *TNF-α*, *IL-1β*, *IL-6* and *MCP-1* were significantly decreased (P<0.01) compared with the control group.

DISCUSSION

The negative effect of environmental stressors on intestinal function has been increasingly recognized and studied [43, 45]. In previously published reports, intestinal permeability and height of villi appeared to decrease in pigs and rats after transport stress [36, 42, 43, 51]. Quercetin has been reported to have beneficial effects on the animal intestine. It is one of the most common flavonoids in the animal diet and exhibits considerable radical scavenging activity as a function of its phenolic chemical structure. Oral administration of quercetin to rats significantly reduced ischemia/reperfusion induced gastric mucosal injury [24]. The effect of quercetin on pig intestinal integrity under transport stress has not been reported previously. Therefore, in this study, we examined whether dietary supplementation with quercetin could have a protective effect on intestinal integrity in a transport stress model of the pig.

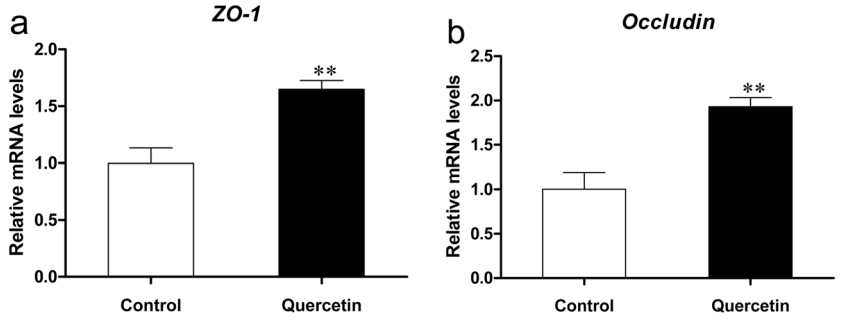

Fig. 3. Effect of quercetin on the *ZO-1* and *Occludin* mRNA levels in the jejunum of pig stimulated with transportation. (a) *ZO-1* mRNA levels, (b) *Occludin* mRNA levels. Values are means ± SEM, n=6. **Significant different (P<0.01) from the control group. ZO-1, Zonula occludens-1.

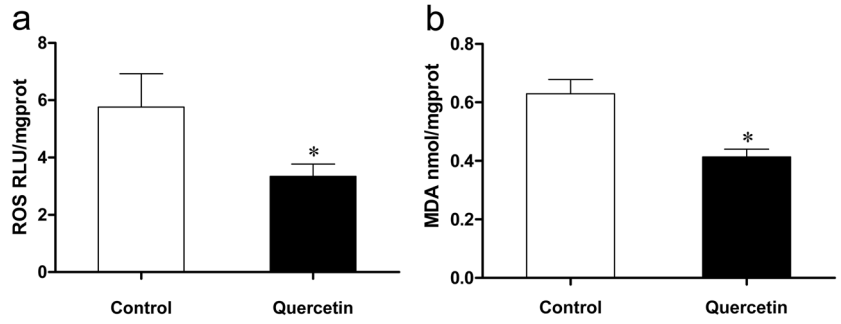

Fig. 4. Effect of quercetin on the ROS and MDA levels in the jejunum of pig stimulated with transportation. (a) All chemiluminometric counts were obtained at 0.05 sec intervals for 5 sec, and the results were expressed as areas under the curve (AUCs) of relative light units (RLU) for 5 sec per mgprot of tissue luminol. (b) MDA levels. Values are means ± SEM, n=6. *Significant different (P<0.05) from the control group. RLU, relative light units; MDA, malondialdehyde.

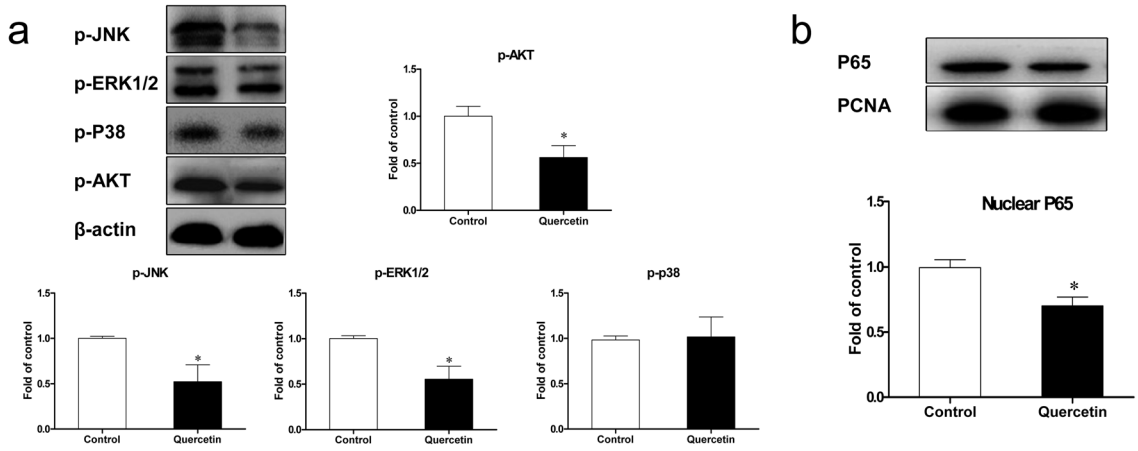

Fig. 5. Effect of quercetin on the p-Akt, p-ERK, p-p38, p-JNK and nuclear NF-κB p65 protein levels in the jejunum of pig stimulated with transportation. (a) p-Akt protein levels, p-ERK protein levels, p-p38 protein levels and p-JNK protein levels. Equal loading was assessed by β-actin immunoblotting. (b) Nuclear p65 protein levels. Equal loading was assessed by PCNA immunoblotting. Values are means ± SEM, n=6. *Significant different (P<0.05) from the control group. p-ERK1/2, phosphorylated extracellular signal-regulated kinases 1/2; p-JNK, phosphorylated c-Jun N-terminal protein kinase; p-P38, phosphorylated p38; p-AKT, phosphorylated protein kinase B; NF-κB, nuclear factor kappa B.

Table 4. Effect of quercetin on the pro-inflammatory cytokines mRNA levels in the jejunum of pig stimulated with transportation

Gene	Control	Quercetin	SEM	*P* value
IL-1β	1.00	0.29**	0.11	<0.01
IL-6	1.00	0.12**	0.18	<0.01
TNF-α	1.00	0.29**	0.13	<0.01
MCP-1	1.00	0.28**	0.13	<0.01

Values are means ± SEM, n=6. **Significant different (P<0.01) from the control group.

The jejunum epithelial surface consists of a simple columnar epithelium, the surface area of which is increased by the presence of villi. Intestinal mucosal permeability is directly related to the integrity of the intestinal barrier [22]. Intestinal barrier integrity is commonly assessed by indices, such as serum endotoxin levels, gut morphology and intestinal tight junction proteins. Intestinal injury results in increased levels of serum endotoxin, decreased villous height, and decreased occludin and ZO-1 mRNA expression in intestinal tissues [11, 38, 40]. In this study, the height of villi in the jejunum was improved in the treatment group, indicating that quercetin may protect the intestine against villous atrophy and epithelial cell necrosis. In parallel with these findings, serum endotoxin levels were also significantly decreased in the treatment group. In addition, we found that the expression of occludin and ZO-1, the two major proteins responsible for the organization and stability of intestinal tight junctions [38], was significantly increased in the group supplemented with quercetin. Based on these results, quercetin supplementation may be a promising approach for protection of the intestinal barrier in pigs under transport stress.

ROS play an important role in the pathogenesis of gastrointestinal injury and dysfunction in pigs under stress [50]. During transportation, ROS levels increase dramatically. Any imbalance between production of these molecules and their safe disposal may culminate in oxidative stress [26, 47]. The pre-slaughter procedure used in the present study is known to increase generation of free radicals and other ROS in pig serum and liver [48]. Excessive levels of ROS damage cellular proteins, including cytoskeletal proteins, and ultimately disrupt the gastrointestinal barrier, thereby increasing gut permeability [4]. Quercetin is known to have antioxidant activity. *In vitro*, quercetin inhibits hypoxanthine-xanthine oxidase activity and scavenges superoxide and hydroxyl radicals [13, 31]. After oral administration, quercetin ameliorated diabetes-induced oxidative stress in rats [23]. At the molecular and cellular levels, quercetin protects HepG2 cells against oxidative stress induced by *tert*-butyl hydroperoxide [1]. In the present study, we have shown that there were decreases in levels of intestinal ROS in the group supplemented with quercetin. MDA is the end product of lipoperoxidation and is considered an excellent indicator of oxidative stress [17]. Therefore, it was also observed that quercetin was beneficial for decreasing MDA levels in the intestinal of transported pigs. In a previous study, in pigs after 5 hr transport, though ROS level significantly increased in serum and liver, MDA was not affected in serum [48]. The discrepancy may be that gastrointestinal (GI) tract is a key source of ROS [4], and compared with other tissue, the gastrointestinal tract is particularly sensitive to stress [35].

The highest expression of pro-inflammatory cytokines occurred on pigs intestines, on arrival or soon thereafter. Inflammation is activated as a defense mechanism and is generally beneficial [21]. However, if inflammation is uncontrolled, migration of innate immune cells, such as neutrophils, macrophages and dendritic cells, into target mucosal tissues results in mucosal injury [29]. In the present study, although there are no data to indicate whether this inflammatory state was caused by the long-distance transport, previous literature shows various stressors can cause imbalance between pro- and anti-inflammatory responses by increasing production of pro-inflammatory cytokines locally and systemically [2, 6, 7], resulting in inflammation, and that inflammation can interfere with normal intestinal integrity in animals [8, 30, 46]. Therefore, the beneficial effect of quercetin on intestinal integrity in the present studies may be through its anti-inflammatory effects described by several studies [12, 49], resulting in the decreased expression of pro-inflammatory cytokines in intestines of pigs. In the present study, the quercetin-supplemented group showed down-regulation of *TNF-α*, *IL-1β*, *IL-6* and *MCP-1* mRNA expression, consistent with inactivation of MAPK, Akt and NF-κB signaling pathways. Recently, it has been recognized that the ROS induce inflammation by stimulating MAPKs, Akt and NF-κB [30, 37]. The activation of these pathways is associated with increased expression of TNF-α, IL-1β, IL-6 and MCP-1 [25]. Intestinal ROS levels and intestinal inflammation are tightly correlated. ROS are a potential etiological and/or triggering factor for intestinal inflammation, and the detrimental effects of ROS in the inflammatory process have been well established [32]. Our study indicates that dietary supplementation with quercetin blocks intestinal inflammation caused by transport stress through decreasing intestinal levels of ROS, both directly and indirectly.

In conclusion, this study provides the first evidence that quercetin may serve as an effective and beneficial dietary supplement for improving intestinal integrity in transport-stressed pigs. The present data indicate that dietary supplementation with quercetin can reduce the production of ROS and attenuate the degree of intestinal permeability in pigs. The protective effects of quercetin on the intestine are associated with decreasing levels of intestinal ROS and inactivation of ERK1/2, JNK, Akt and NF-κB signaling pathways.

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