



Effects of dietary oregano essential oil and vitamin E supplementation on meat quality, stress response and intestinal morphology in pigs following transport stress

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ABSTRACT. This study investigates the effects of dietary oregano essential oil (OEO) and vitamin E (Vit E) supplementation on meat quality, stress response and intestinal morphology in pigs following transport stress. A total of 288 finishing pigs were randomly assigned to three groups: a basal diet or a basal diet supplemented either with 200 mg/kg Vit E or 25 mg/kg OEO. After a 28-day feeding trial, total of 132 finishing pigs according diet and transport stress were assigned to one of four treatment groups: 1) control treatment without transport stress (Control group), 2) control treatment with 5-hr transport stress (Negative group), 3) Vit E treatment with 5-hr transport stress and 4) OEO treatment with 5-hr transport stress. Transport stress pigs had lower muscle 45 min pH (pHi) and higher drip loss than control pigs. Dietary OEO and Vit E supplementation significantly increased 45min pH under transport stress, and the OEO groups produced lower 24-hr drip loss values ($P<0.05$) than that of pigs from the negative group. The OEO-supplemented pigs showed decreased serum levels of creatine kinase (CK) and cortisol ($P<0.05$), and decreased *Hsp 27* (heat shock protein 27) and *Hsp 70* (heat shock protein 70) mRNA expression in the muscle ($P<0.05$). Additionally, histological analysis revealed intestinal epithelial damage in transport stress pigs that was reversed by dietary supplementation with OEO. In conclusion, supplementation with dietary OEO may be superior to supplementation with dietary Vit E in alleviating the meat quality, stress response and intestinal morphology of pigs after challenge due to transportation stress.

KEY WORDS: intestinal morphology, meat quality, oregano essential oil, pig, 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 [20, 28]. These stressors might induce structural changes in muscle tissues and the intestine, thereby leading to deterioration in the quality of the meat and injury of the intestine [22]. At endocrinal levels, the pituitary-adrenal axis is stimulated under transport stress, which increases levels of glucocorticoids. This further takes a toll on an animal's productivity by bringing about substantial changes in body metabolism [29]. At the cellular level, the ability to survive and adapt to transport stress involves gene expression; increased expression of heat shock proteins (HSP) is integral to the cellular response to stress [9].

A number of ways exist to alleviate the negative effects caused by transport stress on meat quality, intestine structure and the stress response in pigs. Vitamin E (Vit E) is a well-known chain-breaking antioxidant and has been shown to reduce transport stress and improve meat quality in finishing pigs [4, 19, 21]. Plant extracts have been studied as alternative feed additives in recent years [35, 36]. Among these plant extracts, oregano essential oil (OEO) has been found to possess significant effects in alleviating transport stress in pigs. Previous studies from our laboratory have found that dietary OEO supplementation improved pig meat quality under transport stress conditions [35]. However, differences between transport stress and non-transport stress have not been considered. Thus, knowledge concerning the effects of dietary supplementation with OEO for alleviation of stress of transported pigs is limited.

This study aimed to evaluate the effects of OEO and Vit E on meat quality, stress response and intestinal morphology in pigs

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following transportation. The effects of dietary OEO on growth performance were also determined.

MATERIALS AND METHODS

All animal handling protocols were approved by the Huazhong Agricultural University Animal Care and Use Committee (ref. SCXK20080004).

Animals, diets and treatments

A total of 288 finishing pigs (Large White×Landrace) were selected from the same farm (Wuhan Chinapork Co., Ltd., Wuhan, China), based on body weight (BW), structural soundness and the health status of the pigs. All pigs (77 ± 5 kg BW) were individually ear-tagged, weighed and randomly assigned to three dietary treatments from blocks designed to balance initial BW across treatments. Each treatment had six replicate pens of 16 pigs per pen. The treatments were as follows: Control group, basal diet; Vit E group, basal diet supplemented with 200 mg Vit E/kg; or OEO group, basal diet supplemented with 25 mg OEO/kg. Pigs had *ad libitum* access to feed and water throughout the 4-week feeding experiment. The pig pens were kept in an environmentally controlled building with a temperature between 27 and 33°C. The weights of feed allocated and left in the feeders were recorded daily. The BW of each pig was recorded at the beginning and end of the experiment. At the end of the 4-week feeding experiment, the average daily gains (ADG), average daily feed intakes (ADFI) and feed/gain ratios (F/G) were calculated for each pen. The composition of the control diet is shown in Table 1. Vit E was purchased from New Weipu Additive Co., Ltd. (Zhejiang, China). The OEO was in the form of a powder called Phytogen (Meritech Bioengineering Co., Ltd., Guangzhou, China). The composition and analysis are shown in the supplementary Table 1 and supplementary Fig. 1.

Transport and slaughter

On the day of slaughter, sixty pigs from control group, thirty-six pigs from Vit E group and thirty-six pigs from OEO group with the final BW closest to the 100 kg were selected. All pigs according diet and transport stress were assigned to one of four treatment groups: 1) control treatment without transport stress (Control group), 2) control treatment with 5-hr transport stress (Negative group), 3) Vit E treatment with 5-hr transport stress (TS-Vit E) and 4) OEO treatment with 5-hr transport stress (TS-OEO). Pigs were transported using the method previously described by Zou *et al.* [35]. The truck used to transport pigs usually has three layers comprising 30 small vehicle pens; each vehicle pen has a dimension of $190 \times 112.5 \times 120$ cm (length × width × height) and covers an area of approximately 2.138 m². Twenty-four pigs were selected from control group of pigs, according to low stocking density (4 pigs per vehicle pen or 187 kg/m²), were directly transported to the abattoir (1-hr journey) a day prior to slaughter and housed in resting pens for 24 hr (14:00 to next day 14:00) with water *ad libitum* before slaughter. On the other hand, negative group, TS-Vit E group and TS-OEO group were shipped according to high stocking density (6 pigs per vehicle pen or 280 kg/m²) and taken on a continuous 5-hr journey on a country road at a speed of 60–90 km/hr with no feed or water provided before slaughter. The weather was hot, with outdoor temperature ranging from 27 to 34°C during the 5-hr transportation period (from 9:00 to 14:00).

Sampling and processing

After arrival in the abattoir, twelve pigs from each group (two pigs per vehicle pen) were randomly selected from each treatment group (control, TS, TS-vit E or TS-OEO) with final BW closest to 100 kg and were selected for slaughter for meat quality evaluation. Pigs were slaughtered quickly by severance of the jugular veins after electrical stunning (75V, 1.5A, 3–4 sec) on a single day to avoid interference arising from lairage time and treatment during lairage. *Longissimus thoracis et lumborum* (LTL) samples with backfat layer (30 cm around the 13th rib) were removed from the left side of pig carcasses [15]. Each sample was sliced into two sections. One section was divided into five pieces (about 1 g), vacuum packed and snap frozen in liquid nitrogen and then stored at –80°C until analysis; the other (about 2 kg) was kept for meat quality studies. Samples of the jejunum itself were removed from the middle jejunum segment and then rinsed with ice-cold physiological saline. Jejuna (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 ml sample was placed on ice immediately and subsequently centrifuged at $1,300 \times g$ at 4°C for 15 min to obtain serum. The serum samples were stored at –80°C for subsequent analysis.

The pH value and meat color (MC)

The pH of the LTL was measured using a glass electrode connected to a pH meter (Matthäus, Pöttmes, Germany), with the electrode inserted into the muscles. Approximately 20 g of LTL specimen were minced by meat chopper (Shenan, Shanghai, China) prior to testing and sampled at 45 min (pH immediately, pH_i) and 24 hr (pH ultimately, pH_u) postmortem. The pH meter was standardized by a three-point method against buffer standards of pH 6.86, pH 4.01 and pH 9.18. The part of the muscle between 12th and 13th ribs was sliced across the fibers, left exposed to the air at room temperature for 45 min and stored at 4°C for 24 hr. The pH value was determined as the mean of readings taken at three sites on the muscle. MC 45 min and MC 24 hr were measured 3 times per sample on the cut surface using the OPTO-STAR meat color determinator (Matthäus).

Electrical conductivity (EC)

Approximately 20 g of LTL specimen, between 12 th and 13 th ribs were left exposed to the air at room temperature for 45 min and stored at 4°C for 24 hr. Meat EC 45 min and EC 24 hr were measured 3 times per sample with the electrode inserted

Table 1. Composition and analysis of the basal diet

Composition (g/kg)	Basal diet
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 ^{a)}	20.00
Analysis ^{b)}	
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) 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). b) Metabolism energy was calculated from data provided by Feed Database in China (1999).

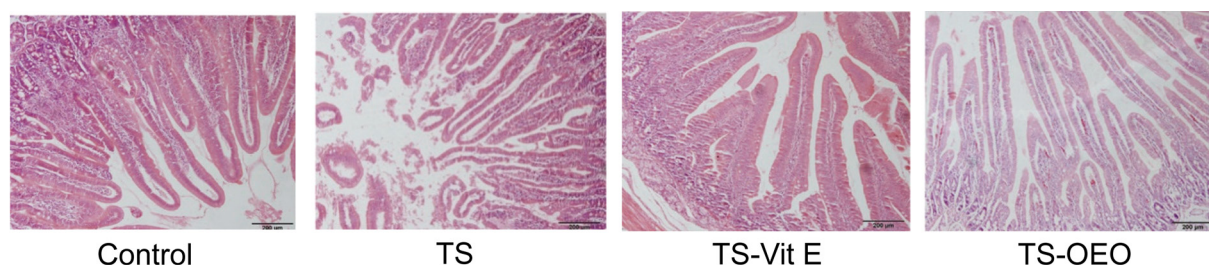


Fig. 1. Effect of dietary supplementation with OEO and Vit E on gut morphology in the jejunum in transport stress pigs. The jejunum was cut off and fixed in 10% formaldehyde-phosphate buffer and then stained with hematoxylin and eosin. Hematoxylin and eosin staining with original magnification $\times 100$. Bars represent 200 μ m. CT, control; TS, transport stress; OEO, oregano essential oil; Vit E, vitamin E.

into the muscles using the LF-STAR conductivity determinator (Matthäus). In order to minimize errors in the above mentioned characteristics, the location of the measurement was changed three times around the center point for each reading, and their average was used in the analysis.

Drip loss

In brief, three cores of 25 mm diameter and approximate 10 g each, were sampled from the ventral, middle and dorsal parts of a piece of LTL taken from the region of the 14th rib. Each sample was placed in a special EZ-DripLoss container (Taastrup, Denmark) and remained for storage at 4°C. All containers were tared before use. The meat samples were then removed from the EZ-DripLoss containers after 24 hr, and each container with exudated meat juice was weighed on the scale used for the taring procedure. All drip loss measurements were expressed as a percentage of the initial weight.

Intramuscular fat

Measurement of IMF was made according to the method first described by Folch *et al.* [10]. In brief, tissue samples were

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

Gene	Primers (sense/antisense 5'-3')	Size (bp)	Annealing temperature (°C)
<i>Hsp27</i>	F: CCGGTGTTTCACTCGAAAATACA R: GCTTTTCCGACTTTCCAGCTTCT	200	60
<i>Hsp70</i>	F: GCCCTGAATCCGCAGAATA R: TCCCCACGGTAGGAAACG	152	58
<i>Hsp90</i>	F: AATCGCCCAGTTGATGTCG R: TGTCCACTATCGTGAGGGTCC	206	60
<i>β-actin</i>	F: CCAGGTCATCACCATCGG R: CCGTGTGGCGTAGAGGT	158	60

Table 3. Effect of dietary OEO and Vit E supplementation on growth performance in finishing pigs^{1, 2)}

Parameters	Treatments			SEM	P-value
	Control	Vit E	OEO		
Pens ³⁾	6	6	6		
Initial BW (kg)	77.74	77.66	78.00	0.25	0.54
Final BW (kg)	102.08	102.75	104.95	0.42	0.06
ADG (kg/d)	0.88 ^{b)}	0.91 ^{a,b)}	0.97 ^{a)}	0.02	0.05
ADFI (kg/d)	2.44	2.39	2.47	0.03	0.55
FCR	2.78 ^{a)}	2.63 ^{a,b)}	2.54 ^{b)}	0.04	0.02

OEO, oregano essential oil; Vit E, vitamin E; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio. ¹Pigs were fed *ad libitum*; control group was fed with a pelleted diet, whereas the Vit E and OEO groups were fed the same diet, with the only difference that feed was uniformly supplemented with 200 mg/kg Vit E and 25 mg/kg OEO, respectively. ²Data were expressed as pooled standard error (SEM). ³sixteen pigs per pen, *n*=6. a, b) Letters means within a row that do not have a common superscript letter differ, *P*<0.05.

homogenized with 2:1 chloroform-methanol mixture to a final dilution 20-fold the volume of the tissue sample. The crude extract was mixed with 20% of its volume of water, and it was separated into two phases. The lower phase contained the tissue lipids.

Serum measurements

Serum cortisol level was measured using the double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) Kit (R&D, Minneapolis, MN, U.S.A.). Immunological detection of the cortisol was performed essentially according to the manufacturer's instruction. Serum creatine kinase (CK) level was assayed using colorimetric methods with a spectrophotometer (Thermo Electron Corporation, Rochester, NY, U.S.A.) and conducted with the assay kits purchased from Nanjing Jiancheng Insititute of Bioengineering (Nanjing, Jiangsu, China) and the procedures according to kit instructions.

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. Real-time PCR was performed according to our previous study [32]. The relative expression of genes in the treatment group was normalized based on the values of the control group. 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 sequence.

Determination of jejunal morphology

The jejunum was removed from the digestive tract, cut and fixed in 10% phosphate-buffered formalin. The samples were sectioned at 5 mm 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.

Statistics

Data were analyzed by ANOVA using SAS version 8.2 (SAS Inst., Inc., Cary, NC, U.S.A.). Performance was analyzed with pen as the experimental unit. Meat quality, stress response and jejunal morphology were analyzed with individual animal as the experimental unit. All data were tested for normality and homoscedasticity before analysis using the Shapiro–Wilk and Levene tests, respectively. Significant differences among treatment means were determined by Duncan's Multiple Range Test method (Duncan, 1955). Significance was accepted for *P*<0.05, and results were presented as mean and pooled standard error of the mean.

Table 4. Effect of dietary supplementation with OEO and Vit E on meat quality characteristics in transport stress pigs¹⁾

Parameters	CT	TS	TS-Vit E	TS-OEO	SEM	P-value
pHi (45 min)	6.63 ^{a)}	6.19 ^{c)}	6.42 ^{b)}	6.44 ^{b)}	0.04	<0.01
pHu (24 hr)	5.81	5.72	5.68	5.73	0.03	0.42
MC (45 min)	77.6	78.5	76.9	79.2	0.60	0.30
MC (24 hr)	53.7	49.8	52.7	51.0	0.68	0.25
EC (45 min) mS/cm	2.6	2.6	2.7	2.6	0.02	0.64
EC (24 hr) mS/cm	3.0	2.9	2.9	3.0	0.03	0.12
Drip loss (24 hr, %)	2.34 ^{b)}	3.50 ^{a)}	3.28 ^{a)}	1.77 ^{b)}	0.20	0.02
IMF (%)	2.20	2.31	2.13	2.30	0.07	0.82

CT, control; TS, transport stress; OEO, oregano essential oil; Vit E, vitamin E; pHi, pH immediately; pHu, pH ultimately; MC, meat color; EC, electrical conductivity; IMF, Intramuscular fat. ¹⁾Data were expressed as pooled standard error (SEM), $n=12$. a–c) Letters means within a row that do not have a common superscript letter differ, $P<0.05$.

Table 5. Effect of dietary supplementation with OEO and Vit E on gut morphology in the jejunum in transport stress pigs¹⁾

Parameters	CT	TS	TS-Vit E	TS-OEO	SEM	P-value
Villous height (μm)	393.99 ^{a)}	316.36 ^{b)}	346.16 ^{a,b)}	407.74 ^{a)}	11.08	0.02
Villous width (μm)	85.79	86.38	89.05	85.42	1.86	0.77
Crypt depth (μm)	130.62	132.86	130.84	120.09	4.86	0.42

CT, control; TS, transport stress; OEO, oregano essential oil; Vit E, vitamin E. ¹⁾Data were expressed as pooled standard error (SEM), $n=6$. a, b) Letters means within a row that do not have a common superscript letter differ, $P<0.05$.

RESULTS

Feed intake and growth performance

Although pigs from each group did not show much difference in their initial body weight and their feed intake was not significantly different (Table 3), the group with OEO supplementation increased their final BW ($P<0.05$) and ADG ($P<0.05$) and significantly decreased their FCR by 8.6 and 3.4%, respectively, compared with the control and Vit E groups.

Meat quality

The meat quality characteristics of pigs are described in Table 4. After 5 hr transportation, the pHi value of the *Longissimus thoracis et lumborum* (LTL) meat of transport stress pigs was significantly decreased compared with that of control pigs ($P<0.05$), while drip loss of the LTL was higher in transport stress pigs than in control pigs ($P<0.05$). Drip loss and pHi were significantly decreased after OEO supplementation compared with that of controls during transport stress ($P<0.05$). Vit E supplementation only significant effected pHi following transport stress.

Morphology of small intestine

As shown in Fig. 1, the jejunum morphology of pigs fed with control diet was obviously damaged after 5 hr transportation. Dietary OEO supplementation prevented the distortion of jejunal architecture induced by transport stress. Moreover, the intestinal morphological indices, including villous height (VH), crypt depth (CD) and villous width (VW), were calculated and shown in Table 5, and the results indicated that VH in transport stress pigs decreased significantly compared with that in control pigs ($P<0.05$). OEO supplementation increased VH in transport stress pigs ($P<0.05$).

Serum measurements

Serum cortisol and creatine kinase (CK) levels are shown in Fig. 2. After 5 hr transportation, serum cortisol and CK levels in transport stress pigs increased significantly compared with that of control pigs ($P<0.05$). When pigs endured transportation, serum cortisol was significantly increased in pigs fed the OEO supplemented diet compared with the negative group ($P<0.05$). Moreover, OEO and Vit E supplementation decreased serum CK levels of transport stress pigs in comparison with the group of control animals ($P<0.05$).

Muscle heat shock protein (Hsp) gene expression

Hsp 27, *Hsp 70* and *Hsp 90* mRNA expression levels in the muscle of control and transport stress pigs are shown in Fig. 3. *Hsp27* expression levels in transport stress pigs were significantly increased compared with those in control pigs ($P<0.05$). Pigs

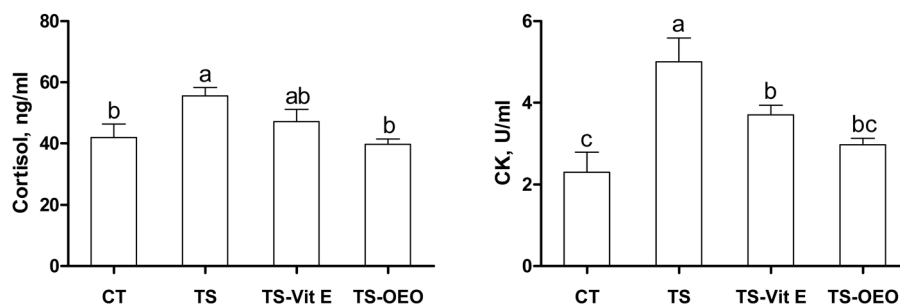


Fig. 2. Effect of dietary supplementation with OEO and Vit E on cortisol and CK in the serum in transport stress pigs. (a) Cortisol levels, (b) CK levels. Values are means \pm SEM, $n=6$. a, b, c Letters means within a row that do not have a common superscript letter differ, $P<0.05$. CT, control; TS, transport stress; OEO, oregano essential oil; Vit E, vitamin E; CK, creatine kinase.

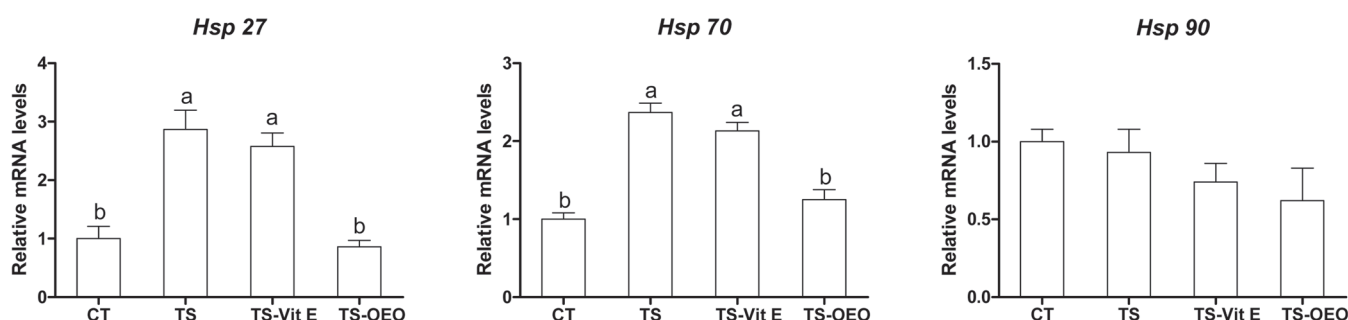


Fig. 3. Effect of dietary supplementation with OEO and Vit E on the *Hsps* mRNA levels in the jejunum of pig stimulated with transportation. (a) *Hsp 27* mRNA levels, (b) *Hsp 70* mRNA levels, (c) *Hsp 90* mRNA levels. Values are means \pm SEM, $n=6$. a, b, c Letters means within a row that do not have a common superscript letter differ, $P<0.05$. CT, control; TS, transport stress; OEO, oregano essential oil; Vit E, vitamin E.

receiving OEO supplementation showed a dramatic decrease in the stress-induced upregulation of *Hsp 27* in transport stress pigs. After 5 hr transportation, the relative expression levels of *Hsp 70* mRNA were dramatically increased in transport stress pigs compared with those in control pigs ($P<0.05$); dietary OEO supplementation significantly decreased *Hsp 70* expression ($P<0.05$). Furthermore, both *Hsp27* and *Hsp70* mRNA levels were not significantly different between pigs receiving Vit E supplementation and control pigs. Expression of *Hsp 90* did not change significantly after 5 hr transportation ($P>0.05$), and no significant differences in *Hsp 90* mRNA levels were observed amongst the dietary treatments ($P>0.05$).

DISCUSSION

Stress caused by transportation is one of the major factors influencing intestine and meat quality in pigs [28, 31, 33]. Deterioration of meat quality during the transport period is of particular economic importance in animal and meat production [25]. Effects of transport stress on the gastrointestinal tract are of great interest, because these effects may increase the risk of bacterial translocation, leading to carcass damage, poor meat quality and high morbidity [13, 14, 22, 23]. The status of the gut and its microscopic structure are good indicators of the stress response of the intestinal tract [30]. In the present study, transportation resulted in increased stress status accompanied by lower LTL pH values and high drip loss values. Additionally, the villi were scattered and seriously desquamated in the jejunum of the negative group, while lower villi were observed in the jejunum of transport stress pigs.

Blood parameters are considered to be the most effective marker of a physiological condition or disease [3]. Serum cortisol concentrations in serum are a hallmark of stress in pigs, especially transport stress [6]. Creatine kinase (CK) is released into the blood when there is muscle damage or when there is vigorous exercise [8]. It is clear that some kinds of damage that affect welfare result in CK release [6]. The present results are consistent with previous findings, in which transport stress increased the cortisol and CK levels in transported pigs. Cells in all organisms respond to environmental stressors by rapid gene transcription and subsequent mRNA translation, which generates a group of highly conserved proteins termed heat shock proteins (Hsps) [18]. These proteins protect cells from stress by restoring the function of damaged proteins, preventing protein aggregation and inhibiting denaturation [24]. These proteins are found in several protein families, including the *Hsp 27*, *Hsp 70* and *Hsp 90* families [27]. Research has shown that large Hsp proteins, such as *Hsp 90*, and small Hsp, such as *Hsp 27*, may be important in cell protection [7, 17]. Our investigation showed that the muscle levels of *Hsp 27* and *Hsp 70* mRNAs were enhanced after transportation

compared with control pigs, whereas the muscle levels of *Hsp 90* exhibited no obvious changes after transportation compared with control pigs. The reason for this finding may be that different HSPs differ in the capability to protect muscle in pigs responding to transport stress.

Feeding Vit E has a beneficial effect on meat quality by preserving the integrity of the muscle cell membranes [5]. Guo *et al.* [12] have reported that feeding pigs with 200 IU/kg Vit E during the growing and finishing periods significantly improves meat quality indicators, such as pH, conductivity, color and drip loss. The present results are consistent with previous findings, in which supplementing the diets of finishing pigs with vit E improved the 45-min pH values and the CK levels in transported pigs. Interestingly, it was also observed that OEO was beneficial for alleviating meat deterioration. The OEO used in the present study contains volatile, natural, complex compounds characterized by a strong odor, formed by *Origanum vulgare* L as secondary metabolites [1]. The main components of the OEO were thymol and carvacrol, while their biogenetic precursors, *p*-cymene and γ -terpinene were the most abundant monoterpenes (see Supplemental Materials). Compared with Vit E group, it was observed that a diet containing OEO was more effective in improving meat quality and reducing intestinal injury in transport stress pigs. These results are consistent with our previous laboratory finding that OEO could improve meat quality and reduce intestinal injury when pigs suffer transport stress [34, 35]. In the present study, serum cortisol and CK ratios were elevated following transport stress, but significantly reduced in pigs fed with OEO compared with the negative group. Similarly, the upregulated expression of stress markers, *Hsp 27* and *Hsp 70*, in transport stress pigs was suppressed by OEO supplementation. Hsps act as molecular chaperones, maintain the metabolic and structural integrity of the cell, and have protective effects on some tissues against stress [2, 16]. These parameters are associated with the stress response and were markedly improved by OEO supplementation. However, this suppressing effect on response was not obvious in the Vit E group. Poor meat quality and injury of the intestines are tightly correlated with transport stress. The effects of OEO on intestinal and meat quality may be caused by OEO administration attenuating stress responses. Our findings suggest that OEO is a potential alternative to Vit E for improving transport stress in pigs.

In addition, with regard to growth performance, our results showed that OEO supplementation had a larger effect than Vit E supplementation on improving ADG and FCR. These results are consistent with a previous study in which a diet containing 25 mg/kg OEO improved the ADG and FCR in growing pigs, but did not affect the ADFI [35]. Nevertheless, the results are different from previous reports on the use of OEO in pig diets [26], which showed no effect on growth performance, including final body weight, ADFI, ADG and FCR. Giannenas *et al.* [11] reported that the response to phytochemicals may be greater in a challenging environment and that stress could enhance the positive influence of these substances in poultry. Published differences in the effect of OEO on finishing pigs may be caused by different feeding conditions and individual differences.

In the present study, the dietary administration of OEO had positive effects on growth performance in the finishing period for pigs. Under the transportation procedures used in the study, OEO was superior to Vit E in decreasing the stress response, thereby reducing transportation-induced intestinal injury and improving meat quality. We found that OEO can act as an efficient dietary supplement to alleviate transport stress in finishing pigs.

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REFERENCES

1. Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. 2008. Biological effects of essential oils—a review. *Food Chem. Toxicol.* **46**: 446–475. [Medline] [CrossRef]
2. Bruce, J. L., Price, B. D., Coleman, C. N. and Calderwood, S. K. 1993. Oxidative injury rapidly activates the heat shock transcription factor but fails to increase levels of heat shock proteins. *Cancer Res.* **53**: 12–15. [Medline]
3. Buckham Sporer, K. R., Weber, P. S., Burton, J. L., Earley, B. and Crowe, M. A. 2008. Transportation of young beef bulls alters circulating physiological parameters that may be effective biomarkers of stress. *J. Anim. Sci.* **86**: 1325–1334. [Medline] [CrossRef]
4. Buckley, D. J., Morrissey, P. A. and Gray, J. I. 1995. Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *J. Anim. Sci.* **73**: 3122–3130. [Medline] [CrossRef]
5. Cannon, J. E., Morgan, J. B., Schmidt, G. R., Tatum, J. D., Sofos, J. N., Smith, G. C., Delmore, R. J. and Williams, S. N. 1996. Growth and fresh meat quality characteristics of pigs supplemented with vitamin E. *J. Anim. Sci.* **74**: 98–105. [Medline] [CrossRef]
6. Chai, J., Xiong, Q., Zhang, C., Miao, W., Li, F., Zheng, R., Peng, J. and Jiang, S. 2010. Effect of pre-slaughter transport plant on blood constituents and meat quality in halothane genotype of NN Large White×Landrace pigs. *Livest. Sci.* **127**: 211–217. [CrossRef]
7. Christians, E. S., Ishiwata, T. and Benjamin, I. J. 2012. Small heat shock proteins in redox metabolism: implications for cardiovascular diseases. *Int. J. Biochem. Cell Biol.* **44**: 1632–1645. [Medline] [CrossRef]
8. Clarkson, P. M. and Ebbeling, C. 1988. Investigation of serum creatine kinase variability after muscle-damaging exercise. *Clin. Sci.* **75**: 257–261. [Medline] [CrossRef]
9. Collier, R. J., Collier, J. L., Rhoads, R. P. and Baumgard, L. H. 2008. Invited review: genes involved in the bovine heat stress response. *J. Dairy Sci.* **91**: 445–454. [Medline] [CrossRef]
10. Folch, J., Lees, M. and Sloane Stanley, G. H. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**: 497–509. [Medline]
11. Giannenas, I., Bonos, E., Christaki, E. and Florou-Paneri, P. 2013. Essential oils and their applications in animal nutrition. *Med. Aromat. Plants* **2**: 1–12.

12. Guo, Q., Richert, B. T., Burgess, J. R., Webel, D. M., Orr, D. E., Blair, M., Fitzner, G. E., Hall, D. D., Grant, A. L. and Gerrard, D. E. 2006. Effects of dietary vitamin E and fat supplementation on pork quality. *J. Anim. Sci.* **84**: 3089–3099. [[Medline](#)] [[CrossRef](#)]
13. Hartung, J. 2003. Effects of transport on health of farm animals. *Vet. Res. Commun.* **27** Suppl 1: 525–527. [[Medline](#)] [[CrossRef](#)]
14. Hassoun, H. T., Kone, B. C., Mercer, D. W., Moody, F. G., Weisbrodt, N. W. and Moore, F. A. 2001. Post-injury multiple organ failure: the role of the gut. *Shock* **15**: 1–10. [[Medline](#)] [[CrossRef](#)]
15. Kauffman, R., Habel, R., Smulders, F., Bergstrom, P. and Hartman, W. 1990. Recommended terminology for the muscle commonly designated 'longissimus dorsi'. *Meat Sci.* **28**: 259–265. [[Medline](#)] [[CrossRef](#)]
16. Kawana, K., Miyamoto, Y., Tanonaka, K., Han-no, Y., Yoshida, H., Takahashi, M. and Takeo, S. 2000. Cytoprotective mechanism of heat shock protein 70 against hypoxia/reoxygenation injury. *J. Mol. Cell. Cardiol.* **32**: 2229–2237. [[Medline](#)] [[CrossRef](#)]
17. Lee, W. C., Lin, K. Y., Chiu, Y. T., Lin, J. H., Cheng, H. C., Huang, H. C., Yang, P. C., Liu, S. K. and Mao, S. J. 1996. Substantial decrease of heat shock protein 90 in ventricular tissues of two sudden-death pigs with hypertrophic cardiomyopathy. *FASEB J.* **10**: 1198–1204. [[Medline](#)]
18. Locke, M. and Noble, E. G. 1995. Stress proteins: the exercise response. *Can. J. Appl. Physiol.* **20**: 155–167. [[Medline](#)] [[CrossRef](#)]
19. Peeters, E., Driessen, B. and Geers, R. 2006. Influence of supplemental magnesium, tryptophan, vitamin C, vitamin E, and herbs on stress responses and pork quality. *J. Anim. Sci.* **84**: 1827–1838. [[Medline](#)] [[CrossRef](#)]
20. Peeters, E., Driessen, B., Steegmans, R., Henot, D. and Geers, R. 2004. Effect of supplemental tryptophan, vitamin E, and a herbal product on responses by pigs to vibration. *J. Anim. Sci.* **82**: 2410–2420. [[Medline](#)] [[CrossRef](#)]
21. Peeters, E., Neyt, A., Beckers, F., De Smet, S., Aubert, A. E. and Geers, R. 2005. Influence of supplemental magnesium, tryptophan, vitamin C, and vitamin E on stress responses of pigs to vibration. *J. Anim. Sci.* **83**: 1568–1580. [[Medline](#)] [[CrossRef](#)]
22. Rostagno, M. H. 2009. Can stress in farm animals increase food safety risk? *Foodborne Pathog. Dis.* **6**: 767–776. [[Medline](#)] [[CrossRef](#)]
23. Rotstein, O. D. 2000. Pathogenesis of multiple organ dysfunction syndrome: gut origin, protection, and decontamination. *Surg. Infect. (Larchmt)* **1**: 217–223, discussion 223–225. [[Medline](#)] [[CrossRef](#)]
24. Santoro, M. G. 2000. Heat shock factors and the control of the stress response. *Biochem. Pharmacol.* **59**: 55–63. [[Medline](#)] [[CrossRef](#)]
25. Schwartzkopf-Genswein, K. S., Faucitano, L., Dadgar, S., Shand, P., González, L. A. and Crowe, T. G. 2012. Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: a review. *Meat Sci.* **92**: 227–243. [[Medline](#)] [[CrossRef](#)]
26. Simitzis, P. E., Deligeorgis, S. G., Bizelis, J. A., Dardamani, A., Theodosiou, I. and Fegeros, K. 2008. Effect of dietary oregano oil supplementation on lamb meat characteristics. *Meat Sci.* **79**: 217–223. [[Medline](#)] [[CrossRef](#)]
27. Tang, S., Bao, E., Sultan, K. R., Nowak, B. and Hartung, J. 2013. Transportation stress and expression of heat shock protein affecting pork quality. *Pak. Vet. J.* **34**: 112–115.
28. Tarrant, P. 1989. The effects of handling, transport, slaughter and chilling on meat quality and yield in pigs: A review. *Ir. J. Agric. Food Res.* **13**: 79–107.
29. Todini, L. 2007. Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors. *Animal* **1**: 997–1008. [[Medline](#)] [[CrossRef](#)]
30. Viveros, A., Chamorro, S., Pizarro, M., Arija, I., Centeno, C. and Brenes, A. 2011. Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. *Poult. Sci.* **90**: 566–578. [[Medline](#)] [[CrossRef](#)]
31. Wan, C., Yin, P., Xu, X., Liu, M., He, S., Song, S., Liu, F. and Xu, J. 2014. Effect of simulated transport stress on the rat small intestine: A morphological and gene expression study. *Res. Vet. Sci.* **96**: 355–364. [[Medline](#)] [[CrossRef](#)]
32. Wei, H. K., Chen, G., Wang, R. J. and Peng, J. 2015. Oregano essential oil decreased susceptibility to oxidative stress-induced dysfunction of intestinal epithelial barrier in rats. *J. Funct. Foods* **18**: 1191–1199. [[CrossRef](#)]
33. Wilson, L. M. and Baldwin, A. L. 1999. Environmental stress causes mast cell degranulation, endothelial and epithelial changes, and edema in the rat intestinal mucosa. *Microcirculation* **6**: 189–198. [[Medline](#)] [[CrossRef](#)]
34. Zou, Y., Xiang, Q., Wang, J., Peng, J. and Wei, H. 2016. Oregano Essential Oil Improves Intestinal Morphology and Expression of Tight Junction Proteins Associated with Modulation of Selected Intestinal Bacteria and Immune Status in a Pig Model. *Biomed. Res. Int.* Article ID 5436738, 11 pages. <http://dx.doi.org/10.1155/2016/5436738>.
35. Zou, Y., Xiang, Q., Wang, J., Wei, H. and Peng, J. 2016. Effects of oregano essential oil or quercetin supplementation on body weight loss, carcass characteristics, meat quality and antioxidant status in finishing pigs under transport stress. *Livest. Sci.* **192**: 33–38. [[CrossRef](#)]
36. Zou, Y., Wei, H. K., Xiang, Q. H., Wang, J., Zhou, Y. F. and Peng, J. 2016. Protective effect of quercetin on pig intestinal integrity after transport stress is associated with regulation oxidative status and inflammation. *J. Vet. Med. Sci.* **78**: 1487–1494. [[Medline](#)] [[CrossRef](#)]