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

Running head: Oregano essential oil and transport stress in pigs

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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.

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 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 closet 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 cm × 112.5 cm × 120 cm (length × width × height) and covers an area of approximately 2.138 m^2. Twenty-four pigs were selected from control group of pigs, according to low stocking density (4 pigs per vehicle pen or 187 kg/m^2), 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

115 stocking density (6 pigs per vehicle pen or 280 kg/m^2) and taken on a continuous 5-hr
116 journey on a country road at a speed of 60–90 km/hr with no feed or water provided
117 before slaughter. The weather was hot, with outdoor temperature ranging from 27 °C
118 to 34 °C during the 5-hr transportation period (from 9:00 to 14:00).
119
120 *Sampling and processing:* After arrival in the abattoir, twelve pigs from each
121 group (two pigs per vehicle pen) were randomly selected from each treatment group
122 (control, TS, TS-vit E or TS-OEO) with final BW closest to 100 kg and were selected
123 for slaughter for meat quality evaluation. Pigs were slaughtered quickly by severance
124 of the jugular veins after electrical stunning (75V, 1.5A, 3–4 sec) on a single day to
125 avoid interference arising from lairage time and treatment during lairage. *Longissimus*
126 *thoracis et lumborum* (LTL) samples with backfat layer (30 cm around the 13th rib)
127 were removed from the left side of pig carcasses [15]. Each sample was sliced into
128 two sections. One section was divided into five pieces (about 1 g), vacuum packed
129 and snap frozen in liquid nitrogen and then stored at −80 °C until analysis; the other
130 (about 2 kg) was kept for meat quality studies. Samples of the jejunum itself were
131 removed from the middle jejunum segment and then rinsed with ice-cold
132 physiological saline. Jejuna (3 cm) were kept in 4% neutral buffered formalin for gut
133 morphological analysis. Blood samples were collected by beaker during
134 exsanguination and then quickly separated into five tubes. A 10 m*l* sample was placed
135 on ice immediately and subsequently centrifuged at 1,300 × g at 4 °C for 15 min to
136 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, pHi) and 24 hr (pH ultimately, pHu) 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 12 th and 13 th ribs was sliced across the fibers, left exposed to the air at room temperature for 45 min and stored at 4 ^{o}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 for 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 ^{o}C for 24 hr. Meat EC 45 min and EC 24 hr were measured for 3 times per sample with the electrode inserted 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 were weighed on the scale used for the taring procedure. All drip loss measurements was 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 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 analyser (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.

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 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 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

247 significantly different between pigs receiving Vit E supplementation and control pigs.
248 Expression of *Hsp 90* did not change significantly after 5 hr transportation ($P > 0.05$),
249 and no significant differences in *Hsp 90* mRNA levels were observed amongst the
250 dietary treatments ($P > 0.05$).
251
252 DISCUSSION
253 Stress caused by transportation is one of the major factors influencing intestine
254 and meat quality in pigs [28, 31, 33]. Deterioration of meat quality during the
255 transport period is of particular economic importance in animal and meat production
256 [25]. Effects of transport stress on the gastrointestinal tract are of great interest,
257 because these effects may increase the risk of bacterial translocation, leading to
258 carcass damage, poor meat quality and high morbidity [13, 14, 22, 23]. The status of
259 the gut and its microscopic structure are good indicators of the stress response of the
260 intestinal tract [30]. In the present study, transportation resulted in increased stress
261 status accompanied by lower LTL pHi values and high drip loss values. Additionally,
262 the villi were scattered and seriously desquamated in the jejunum of the negative
263 group, while lower villi were observed in the jejunum of transport stress pigs.
264 Blood parameters are considered to be the most effective marker of a
265 physiological condition or disease [3]. Serum cortisol concentrations in serum are a
266 hallmark of stress in pigs, especially transport stress [6]. Creatine kinase (CK) is
267 released into the blood when there is muscle damage or when there is vigorous
268 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* mRNA 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, ρ-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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335 REFERENCES

336 1. Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. 2008. Biological effects of essential oils–

337 a review. *Food Chem. Toxicol.* **46**:446-475.

338 2. Bruce, J. L., Price, B. D., Coleman, C. N. and Calderwood, S. K. 1993. Oxidative injury rapidly

339 activates the heat shock transcription factor but fails to increase levels of heat shock proteins. *Cancer*

340 *Res.* **53**:12-15.

341 3. Buckham-Sporer, K., Weber, P., Burton, J., Earley, B. and Crowe, M. 2008. Transportation of

342 young beef bulls alters circulating physiological parameters that may be effective biomarkers of stress.

343 *J. Anim. Sci.* **86**:1325-1334.

344 4. Buckley, D., Morrissey, P. and Gray, J. 1995. Influence of dietary vitamin E on the oxidative

345 stability and quality of pig meat. *J. Anim. Sci.* **73**:3122-3130.

346 5. Cannon, J., Morgan, J., Schmidt, G., Tatum, J., Sofos, J., Smith, G., Delmore, R. and Williams, S.,

347 1996. Growth and fresh meat quality characteristics of pigs supplemented with vitamin E. *J. Anim. Sci.*

348 **74:** 98-105.

349 6. Chai, J., Xiong, Q., Zhang, C., Miao, W., Li, F., Zheng, R., Peng, J. and Jiang, S. 2010. Effect of

350 pre-slaughter transport plant on blood constituents and meat quality in halothane genotype of NN Large

351 White× Landrace pigs. *Livest. Sci.* **127**:211-217.

352 7. Christians, E. S., Ishiwata, T. and Benjamin, I. J. 2012. Small heat shock proteins in redox

353 metabolism: implications for cardiovascular diseases. *Int. J. Biochem. Cell. B.* **44**:1632-1645.

354 8. Clarkson, P. M. and Ebbeling, C. 1988. Investigation of serum creatine kinase variability after

355 muscle-damaging exercise. *Clin. Sci.* **75**:257-261.

356 9. Collier, R. J., Collier, J., Rhoads, R. and Baumgard L. 2008. Invited review: genes involved in the

357 bovine heat stress response. *J. Dairy Sci.* **91**:445-454.

358 10. Folch, J., Lees, M. and Sloane-Stanley, G. 1957. A simple method for the isolation and
359 purification of total lipids from animal tissues. *J. Biol. Chem.* **226**:497-509.

360 11. Giannenas, I., Bonos, E., Christaki, E. and Florou-Paneri, P. 2013. Essential oils and their
361 applications in animal nutrition. *Med. Aromat. Plants* **2**:1-12.

362 12. Guo, Q., Richert, B., Burgess, J., Webel, D., Orr, D., Blair, M., Fitzner, G., Hall, D., Grant, A. and
363 Gerrard, D. 2006. Effects of dietary vitamin E and fat supplementation on pork quality. *J. Anim. Sci.*
364 **84**:3089-3099.

365 13. Hartung, J. 2003. Effects of transport on health of farm animals. *Vet. Res. Commun.* **27**:525-527.

366 14. Hassoun, H. T., Kone, B. C., Mercer, D. W., Moody, F. G., Weisbrodt, N. W. and Moore, F. A.
367 2001. Post-injury multiple organ failure: the role of the gut. *Shock* **15**:1-10.

368 15. Kauffman, R., Habel, R., Smulders, F., Bergstrom, P. and Hartman, W. 1990. Recommended
369 terminology for the muscle commonly designates Longissimus dorsi. *Meat Sci.* **28**:259-265.

370 16. Kawana, K. I., Miyamoto, Y., Tanonaka, K., Han-no, Y., Yoshida, H., Takahashi, M. and Takeo, S.
371 2000. Cytoprotective mechanism of heat shock protein 70 against hypoxia/reoxygenation injury. *J. Mol.*
372 *Cell. Cardiol.* **32**:2229-2237.

373 17. Lee, W., Lin, K., Chiu, Y., Lin, J., Cheng, H., Huang, H., Yang, P., Liu, S. and Mao, S. 1996.
374 Substantial decrease of heat shock protein 90 in ventricular tissues of two sudden-death pigs with
375 hypertrophic cardiomyopathy. *Faseb. J.* **10**:1198-1204.

376 18. Locke, M. and Noble, E. G. 1995. Stress proteins: the exercise response. *Can. J. Appl. Physiol.*
377 **20**:155-167.

378 19. Peeters, E., Driessen, B. and Geers, R. 2006. Influence of supplemental magnesium, tryptophan,

379 vitamin C, vitamin E, and herbs on stress responses and pork quality. *J. Anim. Sci.* **84**:1827-1838.

380 20. Peeters, E., Driessen, B., Steegmans, R., Henot, D. and Geers, R. 2004. Effect of supplemental
381 tryptophan, vitamin E, and a herbal product on responses by pigs to vibration. *J. Anim. Sci.*
382 **82**:2410-2420.

383 21. Peeters, E., Neyt, A., Beckers, F., De, Smet S., Aubert, A. and Geers, R. 2005. Influence of
384 supplemental magnesium, tryptophan, vitamin C, and vitamin E on stress responses of pigs to vibration.
385 *J. Anim. Sci.* **83**:1568-1580.

386 22. Rostagno, M. H. 2009. Can stress in farm animals increase food safety risk? *Foodborne Pathog.*
387 *Dis.* **6**:767-776.

388 23. Rotstein, O. D. 2000. Pathogenesis of multiple organ dysfunction syndrome: gut origin, protection,
389 and decontamination. *Surg. Infect.* **1**:217-225.

390 24. Santoro, M. G. 2000. Heat shock factors and the control of the stress response. *Biochem.*
391 *Pharmacol.* **59**:55-63.

392 25. Schwartzkopf-Genswein, K., Faucitano, L., Dadgar, S., Shand, P., González, L. and Crowe, T.
393 2012. Road transport of cattle, swine and poultry in North America and its impact on animal welfare,
394 carcass and meat quality: A review. *Meat Sci.* **92**:227-243.

395 26. Simitzis, P., Deligeorgis, S., Bizelis, J., Dardamani, A., Theodosiou, I. and Fegeros, K. 2008.
396 Effect of dietary oregano oil supplementation on lamb meat characteristics. *Meat Sci.* **79**:217-223.

397 27. Tang, S., Bao, E., Sultan, K. R., Nowak, B. and Hartung, J. 2013. Transportation stress and
398 expression of heat shock protein affecting pork quality. *Pak. Vet. J.* **34**:112-115.

399 28. Tarrant, P. 1989. The effects of handling, transport, slaughter and chilling on meat quality and
400 yield in pigs: A review. *Irish J. Agr. Food Res.* **13**:79-107.

401 29. Todini, L. 2007. Thyroid hormones in small ruminants: effects of endogenous, environmental and
402 nutritional factors. *Animal* **1**:997-1008.
403 30. Viveros, A., Chamorro, S., Pizarro, M., Arija, I. Centeno, C. and Brenes, A. 2011. Effects of
404 dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks.
405 *Poultry Sci.* **90**:566-578.
406 31. Wan, C., Yin, P., Xu, X., Liu, M., He, S., Song, S., Liu, F. and Xu, J. 2014. Effect of simulated
407 transport stress on the rat small intestine: A morphological and gene expression study. *Res. Vet. Sci.*
408 **96**:355-364.
409 32. Wei, H. K., Chen, G., Wang, R. J. and Peng, J. 2015. Oregano essential oil decreased
410 susceptibility to oxidative stress-induced dysfunction of intestinal epithelial barrier in rats. *J. Funct.*
411 *Foods* **18**:1191-1199.
412 33. Wilson, L. M. and Baldwin, A. L. 1999. Environmental stress causes mast cell degranulation,
413 endothelial and epithelial changes, and edema in the rat intestinal mucosa. *Microcirculation* **6**:189-198.
414 34. Zou, Y., Xiang, Q., Wang, J., Peng, J. and Wei, H. 2016. Oregano Essential Oil Improves
415 Intestinal Morphology and Expression of Tight Junction Proteins Associated with Modulation of
416 Selected Intestinal Bacteria and Immune Status in a Pig Model. *Biomed. Res. Int.* Article ID 5436738,
417 11 pages. http://dx.doi.org/10.1155/2016/5436738.
418 35. Zou, Y, Xiang, Q, Wang, J, Wei, H. and Peng, J. 2016. Effects of oregano essential oil or quercetin
419 supplementation on body weight loss, carcass characteristics, meat quality and antioxidant status in
420 finishing pigs under transport stress. *Livest. Sci.* **192**:33-38.
421 36. Zou, Y, Wei, H, Xiang, Q, Wang, J, Zhou, Y. and Peng, J. 2016. Protective effect of quercetin on
422 pig intestinal integrity after transport stress is associated with regulation oxidative status and

inflammation. *J. Vet. Med. Sci.* **78**:1487-1494.

FIGURE LEGENDS：

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 × 100. Bars represent 200 μm. CT, control; TS, transport stress; OEO, oregano essential oil; Vit E, vitamin E.

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 ± 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 ± 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.

445 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).

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

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

459 Table 3. Effect of dietary OEO and Vit E supplementation on growth performance in
460 finishing pigs[1, 2]

	Treatments				
Parameters	**Control**	**Vit E**	**OEO**	***SEM***	***P - Value***
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[ab]	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[ab]	2.54[b]	0.04	0.02

461 OEO, oregano essential oil; Vit E, vitamin E; BW, body weight; ADG, average daily
462 gain; ADFI, average daily feed intake; FCR, feed conversion ratio.
463 [1]Pigs were fed *ad libitum*; control group was fed with a pelleted diet, whereas the Vit
464 E and OEO groups were fed the same diet, with the only difference that feed was
465 uniformly supplemented with 200 mg/kg Vit E and 25 mg/kg OEO, respectively.
466 [2]Data were expressed as pooled standard error (SEM).
467 [3]sixteen pigs per pen, n =6.
468 [a, b] Letters means means within a row that do not have a common superscript letter
469 differ, $P < 0.05$.
470
471 Table 4. Effect of dietary supplementation with OEO and Vit E on meat quality
472 characteristics in transport stress pigs[1]

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 h)	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 h)	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 h) mS/cm	3.0	2.9	2.9	3.0	0.03	0.12
Drip loss (24 h, %)	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, b, 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 pigs[1]

Parameters	***CT***	***TS***	***TS-Vit E***	***TS-OEO***	***SEM***	***P - value***
Villous height (μm)	393.99a	316.36[b]	346.16[ab]	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, $P < 0.05$.

Figure 1

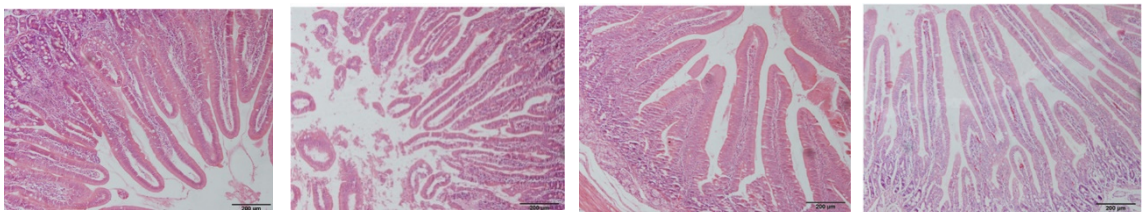


Figure 2

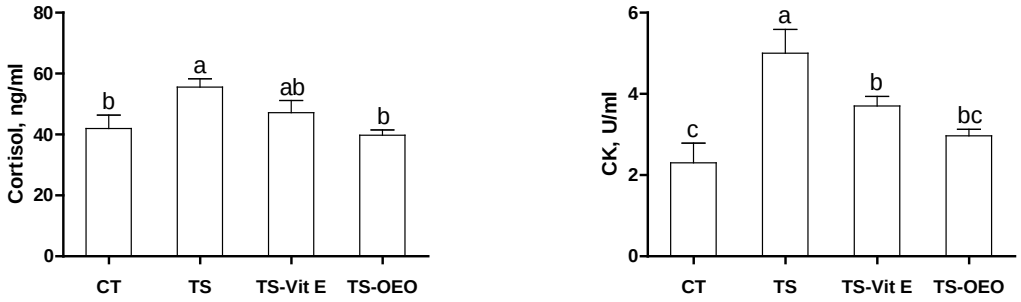
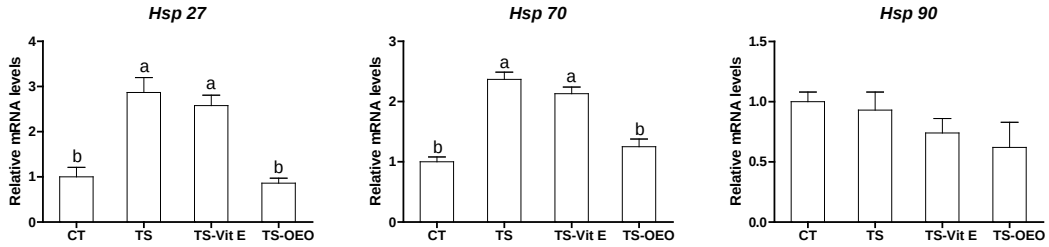


Figure 3

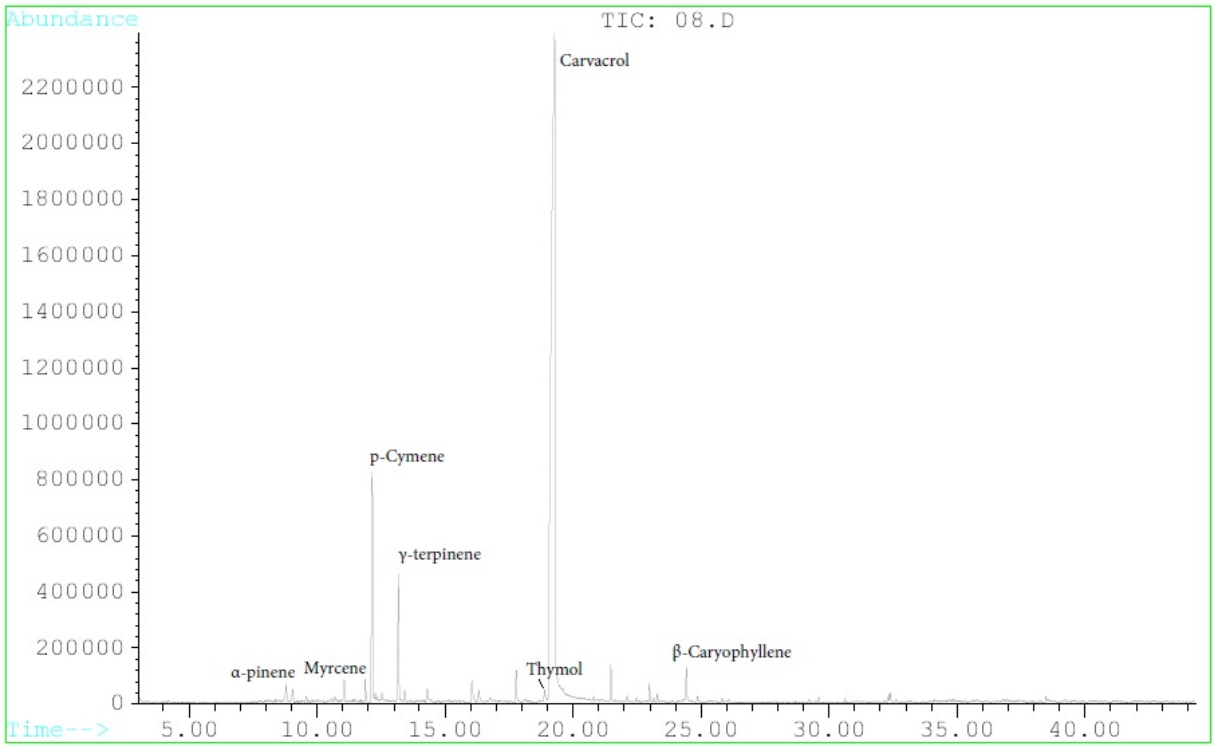


Supplementary Table 1. ANALYSIS OF OREGANO OIL GC-MS

Component	**Oregano oil (MGZ-008)**
	%
α-Thujene/α-Pinene	0.56
Camphene	0.08
β-Pinene	0.09
Sabinene	0.03
Myrcene	0.91
α-Phellandrene	0.09
A-Terpinene	0.50
Limonene	0.15
1,8-Cincole+β-phellandrene	0.07
β-Ocimene	0.07
r-Terpinene	4.54
3-Ocimene	0.07
P-Cymene	3.11
Terpinoiene	0.05
3-Octanoi	0.11
1-Octen-3-ol	0.22
Dimethyl styrene	0.10
Trans-Sabinene hydrate	0.14
Linalool	0.32
cis-Sabinene hydrate	0.03
1-Terpincol	0.05
Terpinen-4-ol	0.22
Carvacrol methyl ether	0.33
B-Caryophyllene	1.43
Dihydrocarvone	0.09
α-Humulene	0.08
α-Terpineol	0.21
Borneol	0.33
β-Bisabolene	0.71
Caryophylene oxide	0.16
Thymol	1.90
Carvacrol	79.92

These results relate only to the sample(s) tested and do not guarantee the bulk of the mentioned to the equal quality.

Supplementary Figure. 1. Typical chromatogram of oregano essential oil components.



The Figure were provided by Meritech Bioengineering Co. Ltd.