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# Effects of dietary oregano essential oil supplementation on the stress response, antioxidative capacity, and *HSPs* mRNA expression of transported pigs

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ABSTRACT

Transportation stress affects carcass quality, metabolism and immune function. The utilization of feed additives is a possible strategy of mitigating physical and psychological stresses after animal transportation. Oregano essential oil (OEO) is an aromatic plant extract that mainly contains carvacrol and thymol. However, the effects of dietary supplementation with OEO for the welfare of transported pigs are limited. This study aimed to investigate the effect of OEO on alleviating stress and increasing antioxidative capacity after the transportation of finishing pigs. 180 crossbred pigs were randomly allocated to 1 of 3 diets: the basal diets, 200 mg $kg^{-1}$ vitamin E (VE), or 25 mg $kg^{-1}$ OEO. Each group was divided into two subgroups: no stress (NS) or transportation stress (TS) after 28 days. Here we report that serum cortisol and norepinephrine concentrations of transported pigs were significantly reduced ($P<0.05$) in OEO diet. Reactive oxygen species (ROS) and malondialdehvde (MDA) were significantly increased in the serum ($P<0.05$) and liver ($P<0.05$) of TS pigs. Serum glutathione peroxidase (GSH-Px) was markedly raised ($P=0.01$) in dietary treatment. Liver SOD was dramatically raised no matter transportation ($P<0.01$) or dietary treatment ($P=0.01$). Liver heat shock protein (HSP) *27* and *HSP90* were significantly increased ($P<0.01$) after transportation. These results indicated that OEO is beneficial in alleviating transportation stress and improving antioxidative activity, similar to VE.



## 1. Introduction

Road transport of livestock is considered a major stressor and might have deleterious influence on the behavior, welfare and carcass quality of the animals. Several researchers have confirmed that transportation for a short or a long period can impose a variety of physical and psychological stimuli that disrupt homeostasis of different animal species. Both over-stocking and long-distance transportation of slaughter pigs are known to increase the stress of the animal (Kim et al., 2004), which increases the serum concentrations of glucocorticoid (Gupta et al., 2007; Buckham Sporer et al., 2008). In addition, the activities of antioxidant enzymes may change during transportation (Adenkola and Ayo, 2010; Hu et al., 2011), although studies have yielded conflicting results.

The utilization of feed additives in animal nutrition is a possible strategy of mitigating physical and psychological stresses after transportation. These feed additives have an antioxidant function and include vitamins C (Pion et al., 2004) and vitamin E (VE) (Guo et al., 2006), which are believed to alleviate stress. Several aromatic feed additives are plant-derived products used in animal feeding to improve the performance of agricultural livestock. Among these additives, oregano (*Origanum vulgare* L.) essential oil (OEO) is an aromatic plant extract (Vokou et al., 1993) that mainly

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contains carvacrol and thymol, which comprise approximately 78–82% of the total oil (Pandey et al., 2003), and possesses significant in vitro antimicrobial (Dorman and Deans, 2000), antifungal (Daouk et al., 1995), and antioxidant properties (Cervato et al., 2000). Oregano and its extracts can reportedly improve the oxidative stability of cooked chicken breast meat when added to the diet of broiler chickens (Roofchaee et al., 2011; Avila-Ramos et al., 2012). The increased growth performance of weaned pigs (Marcin et al., 2006; Neill et al., 2006), growing lambs (Bampidis et al., 2005), and broilers (Hernandez et al., 2004) has also been reported. Furthermore, dietary supplementation with OEO improved feed efficiency, reduced serum cholesterol level, and increased lipoprotein response in broilers (Hong et al., 2012). Interestingly, our group has discovered that OEO effectively improves pork quality and increases the water-linking capacity of meat (unpublished results). However, OEO supplementation for easing stress and enhancing antioxidative capacity after the transportation of finishing pigs has not yet been reported.

The combination of the potential effects of dietary supplementation with OEO is beneficial in terms of production. However, to our knowledge, reports on the effects of dietary supplementation with OEO for the welfare of transported and slaughtered pigs are limited. Therefore, this study aimed to assess the potential of OEO as a dietary supplement for finishing pigs. The effects of OEO on stress hormones, serum and liver antioxidant activities, and liver HSP gene expression in pigs were investigated.

## 2. Materials and methods

### 2.1. Chemicals

VE was purchased from Zhejiang New Weipu Additive Co., Ltd. (China), and oregano essential oil (OEO) was in the form of a powder called Orego-Stim [Meriden Animal Health Ltd. (Northampton, UK)] that contains 5% OEO of *O. vulgare* subsp. *hirtum* plants and 95% natural feed grade inert carrier. The components of OEO are shown in Table 1, and derived from Meriden Animal Health Ltd.

**Table 1**
Components of the oregano essential oil[a].

| Chemical constituents | % |
|---|---|
| α-Thujene/α-pinene | 0.66 |
| Camphene | 0.09 |
| β-Pinene | 0.07 |
| Sabinene | 0.04 |
| Myrcene | 0.86 |
| α-Phellandrene | 0.08 |
| α-Terpinene | 0.58 |
| Limonene | 0.13 |
| 1,8-Cineole + β-phellandrene | 0.09 |
| β-Ocimene | 0.07 |
| γ-Terpinene | 4.49 |
| 3-Octanone | 0.07 |
| ρ-Cymene | 3.07 |
| Terpinolene | 0.04 |
| 3-Octanel | 0.01 |
| 1-Octen-3-ol | 0.24 |
| Dimethyl styrene | 0.01 |
| Trans-sabinene hydrate | 0.10 |
| Linalool | 0.28 |
| Cis-sabinene hydrate | 0.06 |
| 1-Terpilool | 0.04 |
| Terpine-4-ol | 0.34 |
| Carvacrol methyl ether | 0.22 |
| β-Caryophyllene | 1.41 |
| Dihydrocarvone | 0.08 |
| α-Humulene | 0.14 |
| α-Terpineol | 0.16 |
| Bomeol | 0.30 |
| β-Bisaholene | 0.70 |
| Caryophyllene oxide | 0.14 |
| Thymol | 3.5 |
| Carvacrol | 81.92 |
| Total | 99.99 |

[a] The dates were provided by Meriden Animal Health Ltd.

### 2.2. Animals, diets and treatments

All animal handling protocols were approved by the Huazhong Agricultural University Animal Care and Use Committee guidelines. A total of 180 crossbred pigs (Landrace × Yorkshire) were randomly allotted to 3 treatments arrangement based on initial body weight (initial body weight BW = 77.80 ± 4.25 kg) and 6 replicates with 10 pigs per pen in each replication. Each group was randomly allocated to 1 of 3 finishing diets containing commercial basal diets (the control), 200 mg $kg^{-1}$ VE, or 25 mg $kg^{-1}$ OEO of feed. Pigs were provided ad libitum access to feed and water. The ingredients and the composition of the commercial basal diet are presented in Table 2, and all diets were formulated to meet, or exceed, the NRC AA, energy, and other nutrient requirements for growing-finishing swine (NRC, 2012). The experiment lasted for 28 days.

### 2.3. Transport and slaughter

At the end of the 28 days trial period, each group was divided into two subgroups, and per subgroup from each dietary treatment was selected randomly and subjected either to no stress (NS) (low stocking density and rest for 20 h) or 5 h of transportation stress (TS) (high stocking density and ordinary roads). Pigs were transported using the method described by Chai et al. (2010). The truck has threelayers and contain 10 small pens each layer of specification which is 1900 mm × 1125 mm each, covering an area of approximately 2.138 $m^2$. One day prior to slaughter, 24 pigs per dietary supplementation in the NS pigs, according to low stocking density (6 replicates, 4 pigs per vehicle pen or 187 kg/$m^2$, and come from the same pen), were transported to the abattoir a day prior to slaughter about one hour's drive at a speed of 60–80 km/h (from 17:00 to 18:00, temperature from 26 °C to 28 °C) and housed in resting pens for 20 h (18:00 to next day 14:00) with water ad libitum before slaughter.

On the day of slaughter, 36 pigs per dietary supplementation in the TS group were transported in an open truck at an average ambient temperature ranging from 27 °C to 35 °C for 5 h via ordinary roads (from 9:00 to 14:00), highways and bumpy road at a speed of 30–40 km/h before slaughter by high stocking density (6 replicates, 6 pigs per pen or 280 kg/$m^2$, and come from the same pen). After arrival in the abattoir, 12 pigs from each supplementation group (control, VE and OEO, 2 pigs per vehicle pen) were randomly selected in both NS and TS conditions; thus a total of 72 pigs were electrically stunned (75 V, 1.5 A, 3–4 s) and slaughtered immediately after weighing by exsanguination using conventional methods.

### 2.4. Sampling and processing

Blood samples were collected immediately after slaughter. Blood samples from the selected pigs were collected into a 500-ml glass beaker. Blood samples (20 ml per pig) were transferred into collection tubes containing heparin anti-coagulant (50 IU $ml^{-1}$), and kept on ice until centrifugation. Serum samples was separated within 2 h of collection by centrifugation at 4 °C for 10 min at 3000 rpm placed in 1.5 ml Eppendorf tubes and stored at −80 °C,

**Table 2**
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 |
| **Composition**[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 (C) was fed with the above basal diet, whereas the VE and OEO groups consumed the basal diet supplemented with 200 mg $kg^{-1}$ VE and 25 mg $kg^{-1}$ OEO.
[b] Provided per kg of diet: Cu 21.20 mg; Fe 121.36 mg; Zn 141.23 mg; Mn 57.59 mg; Zn 141.23 mg; I 0.84 mg; Se 0.30 mg; VitE 60 IU; VitA 13.50 kIU; VitD 3.00 kIU; VitK 3.00 mg VitB1 3.00 mg; VitB2 7.5 mg; VitB3 15 mg; VitB6 4.5 mg; VitB9 1.50 mg; VitB12 0.03 mg; Vpp 30.00 mg; and VH 0.23 mg.
[c] Metabolism energy was calculated from data provide by Feed Database in China (1999).

until the day of analysis. The samples of liver were removed and snap frozen in liquid nitrogen and then stored at −80 °C before use for the isolation of total RNA and lipid oxidation assays.

### 2.5. Measurement of cortisol and noradrenaline in plasma

Plasma cortisol and noradrenaline concentration was analyzed by a commercially available ELISA kit (R&D, America). The methods were according to the manufacturer's instructions.

### 2.6. Measurement of ROS and lipid oxidation

The reactive oxygen species (ROS) concentration analysis was performed by the double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) Kit (R&D, America). Immunological detection of the ROS was performed essentially according to the manufacturer's instruction.

Lipid oxidation was assessed on the basis of the malondialdehyde (MDA) in the examined samples. MDA, the compound used as an index of lipid peroxidation, was determined by a selective third-order derivative spectrophotometric method previously developed by some of the authors (Botsoglou et al., 1994). In brief, 2 g liver samples were homogenized (Polytron homogenizer, D-9, Germany) in the presence of 8 ml of 5% aqueous trichloroacetic acid and 5 ml of 0.8% butylated hydroxytoluene in hexane, and the mixture was centrifuged. The top layer was discarded, and a 2.5 ml aliquot from the bottom layer was mixed with 1.5 ml of 0.8% aqueous 2-thiobarbituric acid to be further incubated at 70 °C for 30 min. Following incubation, the mixture was cooled under tap water and submitted to conventional spectrophotometry (UV-2102C, China) in the range of 400–650 nm. The concentration of MDA (nmol/mg protein) in analyzed samples was calculated on the basis of the height of the third-order derivative peak at 521.5 nm by referring to slope and intercept data of the computed least-squares fit of a standard calibration curve prepared using 1,1,3,3-tetraethoxypropane.

All of the antioxidant enzyme activities were expressed as units per milliliter of serum (U/ml) and units per milligram of protein of liver (U/mg). The protein content of the tissues was measured using the Bicinchoninic acid (BCA) assay. The superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px) activity and total antioxidant capacity (T-AOC) of serum and liver were assayed following the standard kit protocol (Nanjiang Jianchen Bioengineering Instiute, China).

### 2.7. Fluorescence quantitative real time PCR

Real-time PCR technology was employed to verify changes in the mRNA levels of *HSP27*, *HSP70* and *HSP90* genes. Total RNA was isolated from the liver tissue using TRIZOL reagent (Invitrogen, USA) according to the manufacturer's recommendations. Primer sets for all genes were designed using Primer 6.0 Software (Applied Biosystems, Foster City, CA) and synthesized commercially by Sangon (Shanghai, China). These genes and their primer sequences are described in Table 3, and *β-actin* was selected as the control gene for all qRT-PCR reactions. cDNA was amplified in a real-time PCR System (LC480, Roche) by using iTaq Universal SYBR Green Supermix (Bio-Rad). The profile was 95 °C for 3 m for enzyme activation, followed by denaturing at 95 °C for 20 s, and annealing at 59 °C for 20 s and elongation at 72 °C for 20 s, repeated for a total of 40 cycles. The results were analyzed by the mode of $2^{-\Delta\Delta Ct}$.

### 2.8. Statistical analysis

Serum cortisol and norepinephrine concentrations, ROS levels, MDA levels, antioxidant enzyme activities, and *HSPs* gene expressions of liver were analyzed by ANOVA with a $2 \times 3$ factorial design, with pig as the experimental unit, including dietary treatment (the control, VE or OEO), stressor treatment (LS or TS), as well as interactions between factors. When a significant interaction detected (*F*-test, $P < 0.05$), data were compared using one way-ANOVA test. All model analyses were performed using SAS 8.0 Institute, Inc. (2005) software. Results are provided as mean ± SEM. Differences were considered significant at $P < 0.05$.

## 3. Results

### 3.1. Serum stress-response hormones

Table 4 shows the effect of transport and diet on the serum levels of cortisol and norepinephrine. Serum cortisol concentrations ($P=0.09$) increased in TS pigs regardless of diet type. Conversely, serum cortisol concentrations were significantly reduced

**Table 3**
Primers used for real-time PCR.

| Gene | Accession no. | Primers (sense/antisense 5′–3′) | Product length (bp) |
|---|---|---|---|
| *HSP27* | NM_001007518 | Forward: CCGGTGTTTCACTCGAAAATACA Reverse: GCTTTTCCGACTTTCCAGCTTCT | 200 |
| *HSP70* | NM_001123127 | Forward: GCCCTGAATCCGCAGAATA Reverse: TCCCCACGGTAGGAAACG | 152 |
| *HSP90* | U94395.1 | Forward: AATCGCCCAGTTGATGTCG Reverse: TGTCCACTATCGTGAGGGTCC | 206 |
| *β-actin* | DQ452569.1 | Forward: CCAGGTCATCACCATCGG Reverse: CCGTGTTGGCGTAGAGGT | 158 |

**Table 4**
Effect of stress hormones in serum of NS and TS pigs by dietary supplementation of OEO and VE.

| Item | NS | | | TS | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | VE | OEO | Control | VE | OEO | | Transport | Diet | Transport × diet* |
| No. of pigs | 6 | 6 | 6 | 6 | 6 | 6 | | | | |
| Cortisol, ng/ml | $47.76^{b}$ | $45.55^{bc}$ | $37.20^{c}$ | $66.35^{a}$ | $48.3^{b}$ | $41.33^{bc}$ | 10.04 | 0.09 | 0.01 | < 0.01 |
| Norepinephrine, ng/ml | $1.16^{abc}$ | $1.12^{abc}$ | $1.10^{bc}$ | $1.20^{a}$ | $1.18^{ab}$ | $1.07^{c}$ | 0.05 | 0.51 | 0.85 | < 0.01 |

NS, low stocking density and rest for 20 h; TS, high stocking density and ordinary roads for 5 h; Control, commercial basal diets; VE, control diets add 200 mg $kg^{-1}$ vitamin E; OEO, control diets add 25 mg $kg^{-1}$ oregano essential oil VE; SEM, standard error of mean. Means values in the same row with different superscripts differ significantly ($P < 0.05$).

* Transport × diet interaction effect.

($P=0.01$) in pigs fed with VE and OEO. Moreover, cortisol concentrations of TS pigs were markedly lower ($P < 0.05$) in those fed with OEO and VE than in those fed with the control diet (transport × diet interaction, $P < 0.01$). Serum norepinephrine concentrations were unaffected by type of transportation ($P=0.51$) or diet ($P=0.85$). Furthermore, circulating norepinephrine concentrations of TS pigs were significantly lower ($P < 0.05$) in those fed with OEO than those in fed with the VE and control diets (transport × diet interaction, $P < 0.01$).

### 3.2. Serum lipid peroxidation and antioxidant activity

Table 5 shows the effect of TS and diets on the levels of ROS and MDA and on the activity of antioxidant enzymes in serum. ROS concentrations significantly increased ($P < 0.01$) in TS pigs. Serum ROS levels were unaffected ($P=0.34$) by diet, and transport × diet interaction ($P=0.91$) effects on serum ROS concentrations were also not observed. MDA is a terminal product of lipid peroxidation; thus, the MDA content can be used to estimate the extent of lipid peroxidation. In serum, MDA levels were unaffected by transportation ($P=0.29$) or diet ($P=0.46$). SOD activity slightly increased ($P=0.08$) in pigs fed with VE and OEO. GSH-Px activity was markedly higher ($P=0.01$) in pigs fed with VE and OEO than in those fed with the control diets. However, the SOD and GPX-Px activities were unaffected by transportation. Similar to the results of T-AOC activity, neither transportation ($P=0.30$) nor diet ($P=0.14$) affected GSH-Px activity. Low transport × diet interactions were observed for MDA levels and antioxidant enzyme activity.

### 3.3. Liver lipid peroxidation and antioxidant activity

The data for ROS, MDA production, and enzyme activities in liver are presented in Table 6. The liver ROS concentrations were significantly higher ($P=0.01$) in TS pigs than in NS pigs. No significant change in the ROS level was found in pigs fed with VE or OEO ($P=0.92$). Compared with NS pigs, liver MDA levels increased ($P=0.02$), whereas dietary VE and OEO supplementation were markedly reduced ($P < 0.01$). SOD activity was dramatically higher ($P < 0.01$) in TS pigs than in NS pigs, whereas feeding OEO decreased SOD activity ($P=0.01$) in liver. GSH-Px activity was unaffected by transportation ($P=0.27$) or diet ($P=0.16$), whereas T-AOC activity was significantly higher ($P < 0.01$) after transportation than NS but did not differ ($P=0.44$) among diets. No transport × diet interaction was observed for ROS levels, MDA levels, and antioxidant enzyme activity.

### 3.4. HSPs mRNA Levels

The levels of *HSP27*, *HSP70*, and *HSP90* mRNA in the liver were measured by real-time polymerase chain reaction (Fig. 1). In the liver, the levels of *HSP27* mRNA significantly increased ($P < 0.01$) after transportation compared with NS pigs. VE treatment decreased ($P=0.01$) the levels of *HSP27* mRNA, whereas OEO treatment resulted in no difference. *HSP70* levels had no significant differences between TS and NS pigs ($P > 0.82$) as well as among the types of diet ($P=0.38$). The expression levels of *HSP90* mRNA in the liver of the transported pigs were higher ($P < 0.01$) than in those of NS pigs. However, the levels of *HSP90* had no significant ($P=0.26$) differences among the VE, OEO, and control diets. No transport × diet interaction was observed for *HSP27* ($P=0.28$), *HSP70* ($P=0.88$), and *HSP90* ($P=0.92$) mRNA levels in the liver.

## 4. Discussion

Transportation stress can affect carcass quality, behavior, metabolism, immune function, and animal mortality (Chirase et al., 2004; Yagi et al., 2004; Gupta et al., 2007). Estimates reported that approximately 0.2% of all slaughter pigs die annually during transport in Germany (Schütte, 1994). Some studies have shown that increased levels of VE in finishing pig diets can alleviate stress and increase lipid stability in pork (Peeters et al., 2005; Guo et al., 2006). Supplementation with 200 IU of VE $kg^{-1}$ of feed for 6 wk

**Table 5**
Effect of ROS, MDA and antioxidant enzymes activities in serum of NS and TS pigs by dietary supplementation of OEO and VE.

| Item | NS | | | TS | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | VE | OEO | Control | VE | OEO | | Transport | Diet | Transport × diet[a] |
| No. of pigs | 6 | 6 | 6 | 6 | 6 | 6 | | | | |
| ROS, IU/ml | 444.17 | 440.49 | 403.70 | 539.77 | 508.87 | 490.36 | 16.64 | < 0.01 | 0.34 | 0.91 |
| MDA, nmol/ml | 3.09 | 2.53 | 2.5 | 3.61 | 3.53 | 3.16 | 0.48 | 0.29 | 0.46 | 0.97 |
| SOD, U/ml | 140.46 | 143.44 | 159.42 | 136.18 | 143.35 | 187.1 | 19.07 | 0.47 | 0.08 | 0.56 |
| GSH-Px, U/ml | 1073.61 | 849.11 | 975.04 | 947.53 | 755.86 | 1218.25 | 163.32 | 0.90 | 0.01 | 0.11 |
| T-AOC, U/ml | 3.49 | 4.14 | 4.31 | 3.34 | 3.03 | 4.27 | 0.55 | 0.30 | 0.14 | 0.37 |

NS, low stocking density and rest for 20 h; TS, high stocking density and ordinary roads for 5 h; Control, commercial basal diets; VE, control diets add 200 mg $kg^{-1}$ vitamin E; OEO, control diets add 25 mg $kg^{-1}$ oregano essential oil; SEM, standard error of mean; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; and T-AOC, total antioxidant capacity.

[a] Transport × diet interaction effect.

**Table 6**
Effect of ROS, MDA and antioxidant enzymes activities in liver of NS and TS pigs by dietary supplementation of OEO and VE.

| Item | NS | | | TS | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | VE | OEO | Control | VE | OEO | | Transport | Diet | Transport × diet[a] |
| No. of pigs | 6 | 6 | 6 | 6 | 6 | 6 | | | | |
| ROS, IU/ml | 1112.94 | 900.01 | 832.4 | 1223.58 | 1351.78 | 1189.78 | 199.24 | < 0.01 | 0.92 | 0.19 |
| MDA, nmol/mg protein | 1.10 | 0.46 | 0.49 | 1.89 | 0.89 | 0.83 | 0.52 | 0.02 | < 0.01 | 0.25 |
| SOD, U/mg protein | 107.81 | 122.45 | 124.84 | 150.95 | 151.2 | 184.79 | 27.66 | < 0.01 | 0.01 | 0.19 |
| GSH-Px, U/mg protein | 94.31 | 98.15 | 57.4 | 79.25 | 71.24 | 75.84 | 15.08 | 0.27 | 0.16 | 0.10 |
| T-AOC, U/mg protein | 0.86 | 1.21 | 1.07 | 1.39 | 1.35 | 1.43 | 0.22 | < 0.01 | 0.44 | 0.12 |

NS, low stocking density and rest for 20 h; TS, high stocking density and ordinary roads for 5 h; Control, commercial basal diets; VE, control diets add 200 mg $kg^{-1}$ vitamin E; OEO, control diets add 25 mg $kg^{-1}$ oregano essential oil; SEM, standard error of mean; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; and T-AOC, total antioxidant capacity.

[a] Transport × diet interaction effect.

before market is beneficial in improving lipid stability and pork quality (Guo et al., 2006). Therefore, 200 IU of VE $kg^{-1}$ was as a positive control of the diet treatment in this research. The major ingredients of OEO are carvacrol and thymol that have a potent antioxidant (Pandey et al., 2003). Dietary supplementation with OEO improves growth performance of weaned pigs (Marcin et al., 2006). In addition, OEO have been reported to posses antimicrobial activity (Dorman and Deans, 2000), and antifungal activity (Daouk et al., 1995) in animal production. Thus, OEO may create more value-added products. OEO supplementation (25 mg $kg^{-1}$) was lower than VE (200 mg $kg^{-1}$), and could save costs.

Blood parameters are considered the most effective marker for a physiological condition or disease (Anderson, 2002; Ginsburg and Haga, 2006; Sporer et al., 2008). Serum concentrations of glucocorticoid cortisol is a hallmark of stress in livestock species (Grandin, 1997; Von Borell, 2001; Möstl and Palme, 2002; Sporer et al., 2008), particularly TS (Gupta et al., 2007; Buckham Sporer et al., 2008; Sporer et al., 2008). Catecholamines have a central function in a myriad of stress-related phenomena, from acute psychological stress to actual traumatic injury (Freestone et al., 2008). In the present study, serum cortisol concentrations were elevated in TS pigs but significantly reduced in pigs fed with VE and OEO compared with the control. Results of a previous study showed that VE administration decreases the cortisol levels in stressed animals (Arnott et al., 2012). There was a significant interaction of cortisol concentrations. Although serum norepinephrine concentrations were unaffected by type of transportation or diet, a transport × diet interaction is found to be significant. The result indicated that OEO and VE can significantly reduce the levels of stress hormone under stress condition. It is widely accepted that the response of hypothalamic–pituitary–adrenal (HPA) axis was accompanied by the change of stress hormones when animals are subjected to stressors. However, whether OEO administration affected cortisol and norepinephrine levels in TS pigs has not yet been determined. It is likely to be because OEO and VE may exert a protective effect on the hypofunction or dysfunction of HPA axis. Our results suggested that dietary OEO administration may mitigate TS in finishing pigs.

Lipid peroxidation is an important parameter that can indicate the occurrence of oxidative stress. Transportation can induce lipid peroxidation (Wernicki et al., 2006), increase ROS concentrations and MDA levels (Chirase et al., 2004), as well as cause oxidative stress in animals (Wernicki et al., 2006). Once ROS production levels exceed the scavenging capacity of the antioxidant system, superfluous ROS cause lipid peroxidation, DNA oxidation damage and DNA strand break (Tan et al., 2008). MDA is the main oxidation product of peroxidised polyunsaturated fatty acids (Sahin et al., 2010), and increased MDA level is usually a sign of lipid peroxidation (Onmaz et al., 2011). Antioxidant enzymes such as SOD, GSH-Px, and CAT are the first-line defense antioxidants (Yildirim et al., 2011). Normally, a balance exists between free radicals and antioxidant defense systems (Onmaz et al., 2011).

In this study, ROS concentrations were significantly higher in the liver of TS pigs than in NS pigs (Table 6). Liver MDA levels were noticeably higher in the TS than in the NS group. In addition, MDA levels significantly increased in the TS group compared with the NS group, whereas the addition of VE or OEO to the diets of finishing pigs significantly decreased MDA levels in the liver compared with the control diet. Sahin et al. (2009) reported a very high level of MDA after transportation. Similarly, Celik et al. (2012) determined a significant increase in MDA concentration in the liver. Furthermore, synthetic VE (200 mg $kg^{-1}$) supplementation was used as a positive control treatment. Dunshea et al. (2005) conducted a review of literature and found that 200 mg $kg^{-1}$ synthetic VE added to pig feed for periods ranging from 84 to130 days preslaughter resulted in significantly reduced muscle oxidation levels. Furthermore, our study showed that transportation induced significantly increased levels of SOD and T-AOC in the liver without markedly enhancing the serum concentration of antioxidant enzymes. However, OEO administration significantly increased the serum levels of SOD and GSH-Px, as well as SOD in the liver of TS finishing pigs. These findings were consistent with those of previous studies. Alarcon-Rojo et al. (2013) reported that

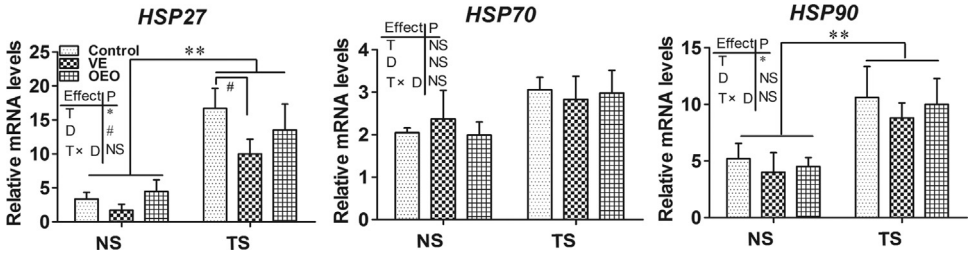


**Fig. 1.** *HSF27*, *HSF70* and *HSP90* mRNA levels in the liver of finishing pigs. Values indicated are Mean ± SEM ($n=6$). T, transportation factor; D, diet factor; T × D, transportation × diet interaction. For all graphs, NS = no stress; TS = transportation stress. *Effect of transportation factor was significant ($^{*}P<0.05$). #Effect of diet factor was significant ($P<0.05$).

dietary supplementation with OEO significantly reduces oxidation in pork. Roofchaee et al. (2011) demonstrated that dietary OEO increases the serum antioxidant activity of broiler chickens. Bakkali et al. (2008) reviewed the related literature on biological effects and indicated that essential oils can act as pro-oxidants affecting inner cell membranes, cell organelles, and intracellular redox potential. OEO antioxidant activity is mainly attributed to the presence of the phenolic compounds thymol and carvacrol (Undeger et al., 2009). Thus, dietary OEO could also activate SOD in serum and liver, as well as decrease the contents of MDA and the product of lipid peroxidation, which suggest free-radical scavenging to some extent.

During transportation, heat stress response (HSR) also occurs; as a signal of HSR, the expression of HSP may change after transportation (Zhu et al., 2009). Our investigation showed that the liver levels of *HSP27* and *HSP90* mRNA were enhanced after transportation compared with NS pigs whereas the liver levels of HSP70 exhibited no obvious changes after transportation compared with NS pigs. The reason for this finding was that different HSPs may differ in their capabilities to protect tissue in pigs responding to TS (Hao et al., 2010). Furthermore, the levels of HSP were unaffected by OEO diet. OEO administration affects HSP mRNA levels, which has not been described in TS pigs. We believed that the cause might be the lack of sensitivity of HSP gene expression regulation to OEO, which requires further investigation.

Modern consumers are increasingly taking other aspects into account, such as the welfare and meat quality (Gregory, 2008). But welfare measurement is not always easy (Pérez et al., 2002). The duration of the trip is an evident indicator of poor welfare during transport (Pérez et al., 2002). In our previous projects, we found that the level of blood stress hormone is also an indicator of animal welfare (Chai et al., 2010). Transportation can influence animal welfare during road transport and subsequent meat quality, such as occurrence of dark, firm and dry (DFD) and pale, soft and exudative (PSE) meat. Increase in the drip loss is related to preslaughter stress (Apple et al., 2005; Dunshea et al., 2005; Guo et al., 2006; Chai et al., 2010). Therefore, the conditions during transport and the welfare and meat quality of transported animals are more and more the subject of discussion.

## 5. Conclusions

The results of the present investigation showed that dietary OEO and VE supplementation may alleviate TS and reduce lipid oxidation. Supplementation with OEO and VE both reduced serum stress-response hormones cortisol levels compared with the control diet, but only OEO can reduced norepinephrine levels in TS pigs. Dietary VE and OEO supplementation reduced MDA levels and partly increased antioxidant enzyme activity. However, supplemental VE and OEO did not significantly affect *HSP* mRNA expression in the liver of finishing pigs. Overall, OEO may be a potential alternative for mitigating TS and enhancing antioxidative capacity, similar to VE.

## Conflict of interest

The authors report that there is no conflict of interest relevant to this publication.

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