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

T. Zhang [a], Y.F. Zhou [a], Y. Zou [a], X.M. Hu [a], L.F. Zheng [a], H.K. Wei [a], I. Giannenas [b], L.Z. Jin [c], J. Peng [a,d,*], S.W. Jiang [d,e,**]

[a] Department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China
[b] Laboratory of Nutrition, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece
[c] Guangzhou Meritech Biological Engineering Technology Co., Ltd., Guangzhou 510080, PR China
[d] The Cooperative Innovation Center for Sustainable Pig Production, Wuhan 430070, PR China
[e] Key Laboratory of Swine Genetics and Breeding of Agricultural Ministry, and Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China

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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

* Corresponding author at: Department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China. Fax: +86 27 87280408.
** Corresponding author at: Key Laboratory of Swine Genetics and Breeding of Agricultural Ministry, and Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, PR China.
Fax: +86 27 87280408.
E-mail addresses: pengjian@mail.hzau.edu.cn (J. Peng), jiangsiwen@mail.hzau.edu.cn (S.W. Jiang).

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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,