



Effects of Oxidative Stress on Growth Performance, Nutrient Digestibilities and Activities of Antioxidative Enzymes of Weanling Pigs*

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ABSTRACT : This study was undertaken to investigate the effects of oxidative stress on growth performance, nutrient digestibilities and activities of antioxidant enzymes of weanling pigs. In the experiment, 24 male Landrace×Yorkshire weanling pigs were allotted to three groups of 8 animals each. Pigs were fed individually. According to a single factorial arrangement, pigs received diets with 5% of either fresh (group 1 and group 3) or oxidized fish oil (peroxide value was 786.50 meq O₂/kg before inclusion in the diet, group 2). At the beginning of the experiment, pigs in group 3 received an intraperitoneal injection of diquat at 12 mg/kg of body weight. The trial lasted for 26 d. A metabolism test was carried out during the last 4 days of the second week. The results showed that feeding diets containing oxidized fish oil or injection with diquat depressed the growth performance and nutrient digestibilities of weanling pigs, decreased activities of antioxidant enzymes and increased concentration of malondialdehyde in plasma and liver. Intraperitoneal injection of diquat would induce more serious oxidative stress than oral intake of oxidized fish oil in the diet. In conclusion, administration of oxidized fish oil or diquat could induce oxidative stress in weanling pigs, and oxidative stress could depress growth performance and impact anti-oxidative ability of young pigs. (**Key Words :** Oxidative Stress, Piglets, Performance, Nutrient Digestibility, Antioxidant Enzyme)

INTRODUCTION

Reactive oxygen species (ROS), such as superoxide (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radicals (·OH), are produced during aerobic metabolism (Ha et al., 1998). Superoxide anion is believed to be the first radical formed, mainly by the electron transport chain when O₂ picks up a single electron. Radicals such as OH, O₂⁻ and H₂O₂ are formed from O₂⁻ (Nappi et al., 1998). Generally, the capability of oxidation and antioxidation of the body keeps balance (Wen et al., 2002). If ROS are not removed in a timely manner by the antioxidant system, an imbalance between free radical generation and removal would lead to oxidative stress. Mammalian cells may encounter oxidative stress that causes destruction of macromolecules and abnormal function (Evans et al., 1997). The animals may show alteration of physiology and behavior and poor growth performance, and suffer from various kinds of

diseases. Glutathione peroxidases (GPX), along with superoxide dismutases (SOD) and catalase (CAT), are considered the main antioxidant enzymes in mammals.

Lipids contain a large number of polyunsaturated fatty acids (PUFAs). When lipids are heated at the existence of oxygen, oxidative reactions occur and primary lipid peroxidation products such as peroxides and hydroperoxides are produced. Because the primary lipoxyl radical is unstable, it could easily produce secondary lipid peroxidation products such as aldehyde, ketone, hydrocarbonyl and carbonyls when there are metal ions (Nadya et al., 2002). Thermally oxidized fat is generally considered to contain potentially toxic lipid peroxidation products and induce oxidative stress in animals (Klaus et al., 2003).

Diquat is a bipyridyl herbicide that utilizes molecular oxygen to produce superoxide anion radical and subsequently hydrogen peroxide. The major target organ of diquat is liver (Burk et al., 1995). Administering diquat to the animals provides us an ideal model to study the effects of oxidative stress on metabolism of nutrients, activities of antioxidant enzymes and the related biochemical and physiological changes. Our objectives were to study the effects of oxidative stress on growth performance, nutrient

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Table 1. Composition and nutrient levels of basal experimental diets

Ingredients	%	Nutrition levels	%
Corn	41.4	Digestible energy (MJ/kg)	14.0
Soybean	12.2	Crude protein	20.9
Soybean meal	23.5	Calcium	0.88
Rice bran	4.5	Phosphorus available	0.44
Fish meal (CP 62.5%)	5.0	Lysine	1.15
Fish oil	5.0	Threonine	0.72
Lactose	5.0	Tryptophan	0.24
L-lys-HCl (78%)	0.09	Methionine+cystine	0.66
DL-methionine	0.06		
CaHPO ₄	0.94		
CaCO ₃	0.88		
Vitamins ^a	0.03		
Salt	0.3		
Chloride choline	0.1		
Premix ^b	1.0		

^a Vitamin mixture supplied as the following (per kg diet): Vitamin A, 15,000 IU; Vitamin D₃, 3,000 IU; Vitamin E, 7.5 IU; Vitamin K₃, 1.5 mg; Vitamin B₁, 0.6 mg; Vitamin B₂, 4.8 mg; Vitamin B₆, 1.8 mg; Vitamin B₁₂, 0.009 mg; Nicotinic acid, 10.5 mg; Pantothenic acid, 7.5 mg; Folic acid, 0.15 mg; Biotin, 80.0 mg.

^b Mineral mixture provided as the following (per kg diet): Fe²⁺, 100 mg (FeSO₄·7H₂O); Zn²⁺, 100 mg (ZnSO₄·7H₂O); Cu²⁺, 6 mg (CuSO₄·5H₂O); Mn²⁺, 4 mg (MnSO₄·H₂O); Se⁶⁺, 0.3 mg (Na₂SeO₃); I, 0.14 mg (KI).

digestibilities and activities of antioxidant enzymes of weanling pigs by using oxidized fish oil and diquat to induce oxidative stress.

MATERIALS AND METHODS

Experimental animals and diets

This experiment was conducted according to protocols approved by the Sichuan Agricultural University Animal Care and Use Committee. 24 male Landrace×Yorkshire weanling pigs (28 d, provided by Sichuan Provincial Swine Stock Farm) weighted 6.77±0.20 kg were allotted to three groups of 8 animals each. Animals were given free access to distilled water and diets, and housed individually in wire cages in a constant temperature (25-27°C) animal rooms with a 12 h light-dark cycle. The feeding trial was carried out in the animal research farm of Animal Nutrition Institute, Sichuan Agricultural University. The diets were prepared based on corn-soybean meal. Nutrient levels met the requirements of 10-20 kg bodyweight of pigs according to NRC (1998) (Table 1).

Experimental design

According to a single factorial arrangement, pigs received diets with 5% of either fresh (group 1 and group 3) or oxidized fish oil (group 2, POV of the oil was 786.50 meq O₂/kg before inclusion in the diet). At the beginning of the experiment, pigs in group 3 received an intraperitoneal

injection of diquat at 12 mg/kg of body weight. Diquat (dibromide monohydrate, Chem Service, West Chester, PA) was dissolved in isotonic saline and filter-sterilized. The injection volume was controlled at 10 ml per head. Group 1 and group 3 were injected the same volume of isotonic saline. The trial lasted for 26 d, metabolism test was carried out during the last 4 days of the second week.

Record the bodyweight, feed intake of the pigs at the beginning, the 14th and the 26th day of the experiment. Average daily feed intake (ADFI), average daily gain (ADG) and the rate of feed intake to gain (F/G) were calculated. All the feces and urine were collected during the metabolism test period. Crude protein (CP), gross energy (GE), dry matter (DM) and ether extract (EE) in diets and feces, nitrogen and energy in the urine were detected to calculate nutrient digestibilities or availabilities (Yang, 1991).

Preparation of the test oil

Ten kilograms of fish oil (provided by Sichuan Tongwei Co., Ltd) was added with 1,435.4 mg of FeSO₄·7H₂O, 589.4 mg CuSO₄·5H₂O, 6,000 mg H₂O₂ and 30 ml H₂O, and heated for 60 h at a constant temperature of 37°C. Throughout the heating process, air was continuously bubbled through the oil (Ren et al., 2001). Before inclusion into the diet, the extent of peroxidation of the fresh and oxidative fish oil was determined by assaying the peroxide value (POV), acid value (AV), thiobarbituric acid-reactive substances (TBARS), iodine value (IV) and saponification value (SV) (Huang et al., 2000). After inclusion in the diet, extract the fat (Yang, 1991) and detect dietary POV, AV, IV and SV.

Sample collection and analytical methods

The blood was collected from the portal vein precava into heparinized polyethylene tubes after the pigs weighted at the 14th day. Plasma was prepared by centrifuging the blood (4,000 rpm, 5 min) and immediately stored at -20°C.

At the end of the feeding period, pigs were feed-deprived for 12 h, and were anesthetized with intravenous injection of phenobarbital (0.25 mg/kg bodyweight). Fresh liver sample was collected and frozen in liquid nitrogen then stored at -20°C for biochemical assays.

Activities of SOD, GPx, capability of inhibiting hydroxy radical (CIHR) and concentration of malondialdehyde (MDA) in plasma and liver were measured by assay kit (Nanjing Jiancheng Bioengineering Institute).

Statistical analysis

For statistical evaluation, means of the data from the three groups were compared by Duncan test. Values in the

Table 2. Characteristics of the experimental oil

	Fresh fish oil	Oxidized fish oil
Before inclusion in the diet		
POV (meq O ₂ /kg)	2.37±0.21 ^A	786.50±15.33 ^B
AV (mg KOH/kg)	2.70±0.10 ^A	5.83±0.73 ^B
IV (g I/100 g)	135.16±2.67 ^a	118.22±2.29 ^b
TBARS (mg MDA/kg)	67.35±8.82 ^A	1317.60±179.71 ^B
SV (mg KOH/g)	199.09±2.04 ^A	227.89±2.87 ^B
After inclusion in the diet		
POV (meq O ₂ /kg)	32.90±0.10 ^A	122.63±1.58 ^B
AV (mg KOH/kg)	18.81±0.67 ^A	28.31±0.46 ^B
IV (g I/100 g)	129.36±6.65 ^a	107.98±1.00 ^b
SV (mg KOH/g)	191.06±3.87 ^a	204.79±2.43 ^b

n = 3. Mean values in a row without the same superscript small letter are different (p<0.05), those without the same superscript capital letter are significantly different (p<0.01).

text are means±SEM. All analyses were conducted using SPSS 11.0.

RESULTS

Characterization of the experimental oil

The characterization of the fresh fish oil and the oxidized fish oil before and after inclusion into the diet

were shown in Table 2. Before inclusion in the diet, the POV of the oxidized fish oil was 332 times higher than that of fresh fish oil. AV and SV of the oxidized fish oil were increased compared with the fresh fish oil. The concentration of TBARS of oxidized fish oil was 20 times higher than that of the fresh fish oil. IV of the oxidized fish oil decreased compared to the fresh fish oil. After inclusion in the diet, POV and AV of the fresh oil group were a 14 and 7-fold higher, whereas POV of the oxidized fish oil group were a 6-times lower compared with the value before inclusion into the diet.

Growth performance

The effect of oxidized fish oil and diquat on performance of weanling pigs were shown in Table 3. Compared with group 1, ADG and ADFI of group 2 and 3 were decreased, F/G of those groups were increased. Compared to group 2, ADG and ADFI of group 3 were decreased, F/G of group 3 was increased.

Digestibilities of nutrients

The digestibilities of nutrients were influenced by the type of oxidative stress (Table 4). Pigs fed oxidized fish oil had significantly lower digestibility of CP (p<0.05) than

Table 3. Effect of oxidative stress on growth performance of weanling pigs

	Group 1	Group 2	Group 3
Initial BW (kg)	6.76±0.21	6.76±0.23	6.81±0.81
BW of 14 th day (kg)	11.84±0.40 ^A	11.01±0.35 ^B	9.80±0.48 ^C
BW of 26 th day (kg)	17.27±0.82 ^{Aa}	16.39±0.51 ^{Ab}	14.01±0.87 ^B
ADG (g/head/d)			
0-14 d	362.86±24.79 ^A	303.04±15.78 ^B	213.56±29.34 ^C
15-26 d	452.50±60.00 ^{Aa}	448.54±38.55 ^{Aa}	350.84±75.01 ^B
0-26 d	404.23±30.91 ^{Aa}	370.19±20.71 ^{Aa}	276.92±36.60 ^B
ADFI (g/head/d)			
0-14 d	510.66±26.91 ^A	449.69±26.64 ^B	338.46±46.95 ^C
15-26 d	693.84±86.53 ^a	721.05±64.87 ^a	589.11±129.5 ^b
0-26 d	595.19±42.51 ^{Aa}	574.93±34.99 ^{Aa}	454.15±66.72 ^B
F/G			
0-14 d	1.41±0.07 ^{Aa}	1.48±0.03 ^{Bb}	1.59±0.07 ^B
15-26 d	1.54±0.05 ^A	1.61±0.04 ^{AB}	1.68±0.11 ^B
0-26 d	1.47±0.03 ^A	1.55±0.03 ^B	1.64±0.05 ^C

n = 8. Mean values in a row without the same superscript small letter are different (p<0.05), those without the same superscript capital letter are significantly different (p<0.01).

Table 4. Effect of oxidative stress on nutrient digestibility and utilization of weanling pigs (%)

	Group 1	Group 2	Group 3
DCP	67.96±2.68 ^a	61.11±3.69 ^b	58.16±2.28 ^b
DDM	66.43±2.21	57.82±5.04	57.26±1.88
DEE	69.99±3.78 ^a	73.63±2.95 ^a	58.20±4.13 ^b
BV of protein	65.63±3.38	66.37±3.26	61.13±3.75
AME	66.70±2.18 ^a	58.29±4.17 ^{ab}	57.41±1.99 ^b

n = 8. Mean values in a row without the same superscript small letter are different (p<0.05), those without the same superscript capital letter are significantly different (p<0.01).

DCP = Digestibility of CP; BV = Biological value; AME = Apparent metabolizable energy.

DDM = Digestibility of dry matter; DEE = Digestibility of ether extract.

Table 5. Effect of oxidative stress on activities of antioxidant enzymes and MDA in plasma of weanling pigs (14th day of the test)

	Group 1	Group 2	Group 3
SOD (U/ml)	87.01±6.21 ^A	74.13±3.77 ^B	61.59±5.29 ^C
CIHR (U/ml)	704.21±48.47 ^{Aa}	663.43±49.61 ^{Ab}	523.69±44.92 ^{Bc}
GPx (U)	576.67±54.66 ^A	489.40±37.04 ^B	360.00±66.63 ^C
MDA (nmol/ml)	2.11±0.266 ^A	2.73±0.41 ^B	3.57±0.49 ^C

n = 8. Mean values in a row without the same superscript small letter are different (p<0.01), those without the same superscript capital letter are significantly different (p<0.001).

SOD = Superoxide dismutase; CIHR = Capability of inhibiting hydroxy radical; GPx = GSH peroxidase; MDA = Levels of malondialdehyde.

Table 6. Effect of oxidative stress on activities of antioxidant enzymes and MDA in liver of weanling pigs (26th day of the test)

	Group 1	Group 2	Group 3
SOD (U/mg protein)	392.05±21.18 ^A	262.42±17.44 ^B	232.38±19.09 ^C
CIHR (U/mg protein)	131.80±24.22 ^{Aa}	98.28±7.77 ^{Bb}	111.06±24.30 ^{ABb}
GPx (U)	5.04±0.93 ^A	3.70±0.38 ^B	2.96±0.52 ^C
MDA (nmol/mg protein)	1.09±0.27 ^A	1.41±0.23 ^B	2.04±0.22 ^C

n = 8. Mean values in a row without the same superscript small letter are different (p<0.01), those without the same superscript capital letter are significantly different (p<0.001).

SOD = Superoxide dismutase; CIHR = Capability of inhibiting hydroxy radical; GPx = GSH peroxidase; MDA = Levels of malondialdehyde.

pigs fed fresh fish oil. The digestibilities of energy and DM of group 2 were decreased, but showed no significant difference between both dietary fats; Compared to group 1, diquat treatment decreased digestibilities of CP (DCP) and ether extract (DEE) and apparent metabolizable energy (AME) significantly (p<0.05). Biological value (BV) of protein and digestibility of dry matter (DDM) of that group were decreased with no significant difference (p>0.05).

Activities of antioxidant enzymes and MDA concentrations in plasma and liver

The activities of antioxidant enzymes and concentration of MDA in plasma (Table 5) and liver (Table 6) were influenced by oxidative stress. The activities of antioxidant enzymes in plasma and liver of group 2 and group 3 were decreased significantly compared with group 1. Pigs fed oxidized fish oil and received diquat had significantly higher concentration of MDA in plasma and liver than those fed fresh fish oil.

DISCUSSION

This study was carried out to investigate the effects of oxidative stress induced by dietary supplementation of oxidized fish oil and intraperitoneal injection of diquat on the performance, nutrient digestibilities and antioxidant enzymes of weanling pigs. Fish oil treated at a low temperature produces a series of different oxidant products, which are unstable and will be decomposed easily during heating and storing (Klaus et al., 2003). POV, AV, SV, TBARS and IV are commonly measured to indicate the oxidation status of oils. In this study, fish oil was heated for 60 h at 37°C and at the presence of metal cations. Results based on POV, AV, SV, TBARS and IV showed that the fish oil had seriously oxidized. Inclusion of this oil at 5% in the

diet resulted in higher POV and TBARS of the diet. Therefore, the diet with the oil could induced oxidative stress and the results in terms of parameters reflecting oxidative status confirmed the hypothesis. This oxidative model is very close to the practical situation. In swine production practice of China, diets for young pigs often contain 3-5% animal fat or plant oil which are normally byproducts of animal or plant processing and contain some peroxidation products. Pigs fed this kind of diets suffer diarrhoea and poor performance. The reason is not very clear. This experiment may be helpful for further understanding and solving the practical problem.

Diquat dibromide is a moderately toxic chemical. The oral half lethal dose (LD50) for diquat in rats is 120 mg/kg. Studies on wild-type mice found that intraperitoneal injection at one-tenth of LD50 could induce the oxidative stress and couldn't kill the animal (Fu et al., 1999). In this study, we applied this dose to induce oxidative stress by intraperitoneal injection on weanling pigs. At the beginning of post-injection, vomiting and anorexia occurred for all the treated pigs. But feed intake of this group increased gradually along with the trial time prolonged. All the pigs survived. Oxidative stress model by intraperitoneal injecting diquat succeeded.

In the experiment, the growth performance and nutrient digestibilities of the pigs fed oxidized fish oil or injected with diquat decreased significantly compared with those pigs fed fresh fish oil. Injection of diquat resulted in more extent decrease of growth performance and nutrient digestibilities than feeding oxidized fish oil. These results were consistent with other studies (Hochgraf et al., 1997; Engberg et al., 1996; Tao, 2005; Wang, 2006). Poor performance and nutrient digestibilities were due to oxidative stress. Oxidation of lipids at low temperatures with catalyst produced mainly primary and secondary lipid

peroxidative products (Andrea et al., 2004), which could damage the antioxidative capability of animals.

The mechanism of poor performance and nutrient digestibilities lies in the oxidative stress. In this experiment, the activities of the antioxidative enzymes and CIHR in the plasma and liver were significantly decreased. GPX and SOD and CAT are the main antioxidative enzymes in mammals, and these enzymes could reduce hydrogen peroxide (H_2O_2) and organic hydroperoxides (Tao, 2005; Wang, 2006). Their activities are commonly used to assess body antioxidative status (Knight et al., 1987). Diquat is a redox cycling bipyridyl herbicide and utilizes molecular oxygen to initiate O_2^- . O_2^- is converted to H_2O_2 by SOD. If H_2O_2 fails to be metabolized to water by catalase and peroxidases in a timely way, it would undergo a series of iron-catalyzed reactions to produce OH \cdot . These highly toxic radicals are extremely reactive with macromolecules and result in pulmonary and /or multiple organ injuries (Shu et al., 1979). The activities of antioxidant enzymes were decreased due to the feedback of H_2O_2 (Bray et al., 1974) or inactivated by the O_2^- (Sinet et al., 1981). These could be regarded as the protective effect of the bodies when encountering the changes of environmental factors (Amstad et al., 1994; Peled-Kamar et al., 1997).

Among the more susceptible targets of OH \cdot are PUFAs. Abstraction of hydrogen atom from a molecule of PUFAs initiates the process of lipid peroxidation. A hydrogen atom is abstracted from a second molecule, leading to a new free radical (Ahsan et al., 2003). Oxidative stress is generally associated with increased peroxidation of membrane PUFAs and formation of lipid peroxidation products (Benzie, 1996). Peroxidation of lipid by ROS can result in the formation of lipid hydroperoxides, and it may also be broken down and result in the formation of lipid alkoxyl radicals that can initiate and propagate the chain reactions of lipid peroxidation (Bucala, 1996). Thus the oxidative damage to biological membranes occurs.

CONCLUSION

Feeding diets with oxidized fish oil or injecting with diquat depressed the growth performance and nutrient digestibilities of weanling pigs due to oxidative stress. A reduced activities of antioxidative enzymes and an increased concentration of MDA in plasma and liver indicated that antioxidative capabilities of pigs were damaged. Further studies are required to clarify the mechanisms underlying the effects of oxidized fats on weanling pigs.

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