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GROWTH PERFORMANCE AND ANTIOXIDANT CAPACITIES IN PIGLETS WITH DIFFERENT WEANING AGE

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ABSTRACT

In order to study the effects of weaning ages on antioxidant capacities, total 24 piglets were randomly assigned to four treatments, with six replicates in each treatment witheach piglet asa replicate. Piglets from different treatment groups were weaned at 14, 21, 28 and 35 ages, respectively, and slaughtered at the age of 42days, blood and tissue samples of liver, kidney and lung tissue were obtained. Contents of Malondialdehyde (MDA) and activities of total superoxide dismutase (T-SOD), Glutathione peroxidase (GSH-Px) andcatalase (CAT) were determined. The results showed that plasma MDA levels ofpiglets weaned at 21, 28 and 35 days were significantly lower than those weaned at the age of 14 days (P<0.05). With the weaning age postponed, plasma T-SOD and GSH-Px activities in piglets increased gradually, where T-SOD and GSH-Px activities of piglets weaned at 28 and 35 days were significantly higher than those weaned at 14 days (P<0.05).Tissue MDA contents were affected by the weaning age, where liver(P<0.05), kidney(P<0.05) and lung(P<0.05) MDA contents weaned at 35 days were decreased in comparison with those weaned at 14 ages, respectively. The T-SOD and GSH-Px activities in the liver, kidney and lung tissues gradually increased with the delayed weaning ages. Activities of T-SOD (P<0.05) and GSH-Px(P<0.05) were higher in tissues of the piglets weaned at 35 ages than those weaned at 14 ages (P< 0.05). The CAT activities did not show significant differences in blood and tissues of liver, kidney and lung among each weaning groups. Those results indicated that the antioxidant function was markedly affected by weaning ages in piglets, and early weaning could decrease antioxidant capacity in piglets.

Key words: Weaning age; Piglet; Blood; Liver; Kidney; Lung; Antioxidant capacity.

INTRODUCTION

Weaning age in commercial pig farms has gradually decreased over the last 50 years as animal production has become more intensive. It has been driven not only by the demands for greater reproductive rates in sows but also by advances in nutrition. Weaning ages around 12 days and sometimes as early as 7 days are reported (Collins *et al*., 2013; Worobec *et al*., 1999; Leliveld *et al*., 2013; Hohenshell *et al*., 2000; Levastb *et al*., 2010). Early weaning leads to adaption problems in piglets, such as diarrhea, growth retardation, *et al*. The problem may be getting worse when antibiotic growth promoters are banned because ofpublic health concerns. Due to animal welfare concerns, the current EU minimum weaning age has been raised to 28 days and organic production of pork in the UK recommends 42 days.

Very early weaning has immediate negative effect on physiology, performance and behavior of piglets (Salmon *et al*., 2009; Jarvis *et al*., 2008). The early weaned piglet's digestive system is under developed and low feed intake and diarrhea are common when weaning occurs at this age. Compared to piglets weaned at a later age (d14 and d28), Worobec *et al*. (1999) reported that piglets weaned at 7days spent only 1%of their time at the feeder during the first 2 days after weaning. Orgeur*et al*. (2001) also found a starvation period ranging from 12 to 48h after very early weaning. The emotional distress and acute food deprivation could lead to elevate urinary cortisol levels the day after weaning and prolonged suppression of the release of catecholamines in early weaned piglets (Hay *et al*., 2001).

Reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (·OH), are produced during aerobic metabolism (Schieber *et al*., 2014).Generally, the capability of oxidation and antioxidation of the bodykeeps a balance (Monaghan *et al*., 2014). If reactive oxygen species (ROS) are not removed ina timely manner by the antioxidant system, an imbalancebetween free radical generation and the removal would lead tooxidative stress. Gluthathione peroxidases (GSH-Px), along withsuperoxide dismutases (SOD) and catalase (CAT), areconsidered the main antioxidant enzymes in mammals. Mammalian cells may encounter oxidativestress that causes destruction of macromolecules andabnormal functions (Evans *et al*., 1997). Sequentially, animals mayshow alterations in physiology and behavior,

displaying poorgrowth performance and suffering from various kinds of diseases.

At present, majority of studies were to investigate the effects of weaning on the physiological functionsof piglets at a single age (Molist *et al*., 2014; Xu *et al*., 2014; Tao *et al*., 2015; Liu *et al*., 2014; Jiang*et al*., 2014; Liang *et al*., 2011), and few were focusing on the comparison of different weaning ages (Leliveld *et al*., 2013; Jarvis*et al*., 2008;Ganelang *et al*., 2014).It is known that the antioxidant function of piglets is related to the anti-stress ability, production performance and immune function of piglets, however, the information about how different weaning ages could possibly affect the antioxidant function of piglets is rare. Therefore, the current study is aimed to investigate the antioxidant capacity in piglets, as reflected by the changes of the malondialdehyde (MDA) contents and antioxidant enzyme activities when piglets were weaned at different ages.

MATERIALS AND METHODS

Animalsand treatments: Twenty four healthy Duroc× Landrace × Yorkshire pigletsaged 7 days old were selected from 8 litters based upon the similar body weight. All piglets were divided into 4 groups with 6 piglets per group, which were weaned at 14, 21, 28, 35 days of age, respectively.

Feeding management and experimental diets: These experimental piglets after birth were vaccinated according to the conventional procedures. Two-stage feed for nursery pigs was provided from the age of 8 days and the nutritional composition was listed in Table 1(Li *et al*., 2014). All piglets are allowed free access to water, and sow milk before weaning or creep feed after weaning. Initial body weight (7 days old) and final body weight (42 days old) of piglets were takenin the morning, respectively. The health status of piglets was observed during the whole experiment.

Samples collection: At the age of 42 days, all experimental piglets were killed by jugular bloodingafter a 12 h fast.Blood samples were collected to heparinized tubes and immediately placed on ice. Plasma was separated by centrifugation at 1300 g at 4 °C for 15 min. Plasma samples were stored at -20 °C until analysis. The samples of liver, kidney and lung tissues were taken immediately and rapidly frozen in liquid nitrogen, then stored at -80°C.The experiment was undertaken following the guidelines of regional Animal Ethics Committee.

Assay of antioxidant indices in plasma and tissues: Determination of total protein, MDA, total superoxide dismutase (T-SOD), GSH-Px and CAT were conducted using the assay kits according to manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). For biochemical assays, eachtissue was homogenized in ice-cold isotonic physiological saline (v/w = 1/10) and supernatant were collected by centrifugation at 1300 g. The supernatants andprepared plasma were then subjected to the measurement of MDA, T-SOD, GSH-Px and CAT levels by spectrophotometric methods (Model UV-1100; Shanghai MAPADA Instruments, Shanghai, China). The activity of the enzyme was expressed as units permg protein (for tissues) and units per ml (for plasma).

Table 1. Composition and nutrient levels of piglets diets (air-dry basis)

Compositions (%)	Diet Ⅰ (Day 8-28)	Diet Ⅱ (Day 29-42)
Extruded soybean	34.90	31.40
Corn	37.60	50.00
Whey powder	15.00	5.50
Fish meal	8.00	6.70
Soybean oil	--	0.90
Wheat bran	2.00	3.50
$CaHPO_4$	0.85	0.60
Limestone	0.65	0.40
Premix[a]	1.00	1.00
Nutrients		
DE/(MJ/kg)	15.09	14.15
Crude Protein	23.78	20.45
Ca	0.93	0.69
Total phosphorus	0.79	0.65
Available phosphorus	0.61	0.39
Na	0.62	0.72
Lys	1.86	0.29
Met + Cys	0.79	0.68
Thr	0.99	0.85

[a]The premix provides following a kilogram of diets: Fe 150 mg, Cu 150 mg, Mn 50 mg, Zn 150 mg, Co 1 mg, Se 0.5 mg, I 0.5 mg, VA 12 000 IU, VD_3 5 000 IU, VE 40 IU, VB_{12}26 μg, VB_2 5 mg, VB_3 12 mg, niacin 28 mg.

Statistical analysis: All statistical analyses were performed with SPSS 12.0 for Windows.All data were expressed as mean ±SEM. The general linear model (GLM) procedure was used to determine treatment effects using one way analysis of variance. The level of significancewas set at $P<0.05$ in all analyses.

RESULTS

Growth performance of piglets: During the experimental period, only a few piglets haddiarrhea occasionally. As shown in Table 2, there wasno significant difference in body weight among different weaning groups($P>0.05$).

Table 2. Effect of weaning age on growth performance of piglets

Items	14 d weaning	21 d weaning	28 d weaning	35 d weaning	*P*-value
Initial body weight (kg)	2.64±0.12	2.67±0.11	2.62±0.08	2.65±0.11	0.628
Final body weight (kg)	8.03±0.25	8.45±0.51	8.08±0.44	8.17±0.49	0.185

Note: Values represent mean ±SEM of 6 piglets from each group.

Plasma antioxidant capacity: As shown in Table 3, plasma MDA levels of the piglets weaned at 21, 28 and 35 days were significantly lower than those weaned at 14day of age (P<0.05). Levels of MDA in piglets weaned at 28 and 35 days were also significantly lower than that of 21 days weaned piglets (P<0.05). Plasma T-SOD and GSH-Px activities gradually increased in piglets when the weaning age was postponed. Activities of T-SOD and GSH-Px in piglets weaned at 28 and 35 days were significantly higher than that of piglets weaned at 14 days (P<0.05). Activity of T-SOD was significantly higher in piglets weaned at 21days than those weaned at 14 days (P< 0.05), and while piglets weaned at 28 and 35 days had higher T-SOD activity than those weaned at 21 days (P<0.05). Greater GSH-Px activity was found in piglets weaned at 35 days than those weaned at 21 days (P<0.05). Plasma CAT activities of piglets did not show significant difference among each weaning age.

Table 3. Effect of weaning age on antioxidant capacity of plasma in piglets

Items	14 d weaning	21 d weaning	28 d weaning	35 d weaning	*P*-value
MDA (nmol/mL)	7.42^{a}±2.04	5.63^{b}±1.23	4.18^{c}±1.21	4.25^{c}±0.48	0.025
T-SOD (U/mL)	103.36^{a}±22.51	138.78^{b}±25.07	170.09^{c}±19.49	173.89^{c}±17.46	0.013
GSH-Px (U/mL)	151.55^{a}±5.24	156.70^{ab}±6.51	172.39^{bc}±14.99	179.44^{c}±9.68	0.038
CAT (U/mL)	31.61±4.25	34.09±2.16	35.40±3.90	34.88±3.59	0.246

Note: Values represent mean ±SEM of 6 samples from each group. Means in the same column without common superscript differ significantly(P < 0.05). The same as below.

Antioxidant capacity in the liver: The antioxidant capacity of the liver was presented in Table 4. Liver MDA levels of the piglets weaned at 28 and 35 days were significantly lower than those weaned at 14 and 21 days (P< 0.05). Liver T-SOD and GSH-Px activities increased gradually when piglets were weaned at an elder age. T-SOD and GSH-Px activities were significantly higherinpiglets weaned at 35 days than those weanedat 14 days (P<0.05). GSH-Px activity of piglets weaned a 28 days was significantly higher than those weaned at 14 days (P<0.05), while its activitywassignificantly higher in piglets weaned at 35 days than those weaned at 21and 28 days (P<0.05). Liver CAT activities did not differ among each group of piglets.

Table 4. Effect of weaning age on antioxidant capacity of the liver in piglets

Items	14 d weaning	21 d weaning	28 d weaning	35 d weaning	*P*-value
MDA (nmol/mL)	0.45^{a}±0.07	0.39^{ab}±0.09	0.29^{c}±0.06	0.31^{c}±0.05	0.017
T-SOD (U/mL)	263.59^{a}±35.32	280.32^{ab}±33.16	286.23^{ab}±20.55	302.89^{b}±26.03	0.039
GSH-Px (U/mL)	19.50^{a}±3.63	21.89^{ab}±4.19	25.36^{b}±2.47	31.44^{c}±7.81	0.028
CAT (U/mL)	69.31±8.20	69.54±9.79	67.49±2.46	72.06±9.78	0.597

Antioxidant capacity in the kidney: As shown in Table5, MDA levels in the kidney of the piglets weaned at 21, 28 and 35 days were significantly lower than that of 14days weaned piglets (P<0.05), while its content in piglets weaned at 28 and 35 days were significantly lower than those weaned at 21 days (P<0.05). Kidney T-SOD and GSH-Px activities in piglets increased graduallywhen the weaning age was delayed. Kidney activity of T-SOD in piglets weaned at 35 days was significantly higher than that of piglets weaned at 14 days (P<0.05). GSH-Px activities of piglets weaned at 28 and 35 days were significantly higher than that of 14 days weaned piglets (P<0.05), while GSH-Px activity of piglets weaned at 35 days was also significantly higher than that of 21 days weaned piglets (P<0.05). Kidney CAT activities of piglets was similar among different weaned groups.

Antioxidant capacity in the lung: As shown in Table6,the lung content of MDA in the piglets weaned at 35 days was significantly lower than that of 14days weaned piglets (P<0.05). The lung T-SOD and GSH-Px activities in piglets increased gradually with the weaning daysdelayed. Lung T-SOD activity in piglets weaned at

28 and 35 days were significantly higher than that found in piglets weaned at 14 days (P<0.05),whereas GSH-Px activity was higher in piglets weaned at 35 days than those weaned at 14 days (P<0.05). The lung CAT activities of piglets in all weaned groups only increased slightly withdelayed weaning, but notstatistically non-significant.

Table 5. Effect of weaning age on antioxidant capacity of the kidney in piglets

Items	14 d weaning	21 d weaning	28 d weaning	35 d weaning	*P*-value
MDA (nmol/mL)	$0.42^{a}\pm0.02$	$0.34^{b}\pm0.04$	$0.27^{c}\pm0.03$	$0.29^{c}\pm0.04$	0.019
T-SOD (U/mL)	$129.63^{a}\pm11.65$	$145.28^{ab}\pm15.06$	$150.46^{ab}\pm9.76$	$160.41^{b}\pm20.71$	0.024
GSH-Px (U/mL)	$13.86^{a}\pm2.21$	$16.16^{ab}\pm2.01$	$19.07^{bc}\pm1.70$	$22.28^{c}\pm3.39$	0.033
CAT (U/mL)	60.57 ± 6.57	62.21 ± 5.03	63.44 ± 4.14	64.48 ± 7.57	0.528

Table 6. Effect of weaning age on antioxidant capacity of the lung in piglets

Items	14 d weaning	21 d weaning	28 d weaning	35 d weaning	*P*-value
MDA (nmol/mL)	$1.93^{a}\pm0.33$	$1.63^{ab}\pm0.43$	$1.67^{ab}\pm0.46$	$1.48^{b}\pm0.53$	0.017
T-SOD (U/mL)	$103.20^{a}\pm16.06$	$118.89^{ab}\pm14.22$	$130.22^{b}\pm9.78$	$140.36^{b}\pm12.23$	0.014
GSH-Px (U/mL)	$10.05^{a}\pm2.05$	$11.12^{ab}\pm2.02$	$12.69^{ab}\pm2.94$	$14.20^{b}\pm2.81$	0.041
CAT (U/mL)	58.34 ± 3.47	59.97 ± 6.41	61.94 ± 6.58	63.39 ± 5.62	0.492

DISCUSSION

Abrupt changes in the environment and feed supply at weaning reduce weight gainand induce immediate or subsequent adaptive changes in the behavior and physiology ofpiglets compared to those suckling counterparts. Colson *et al*. (2006) showed that weaning at 21 or 28 days induced a reduction in growth rate compared to nursed piglets and more negative consequences were found when piglets were weaned at 21 days compared with those weaned at 28 days.Despite of the reduction in growth rate, body weight did not differ between piglets weaned at 21 and 28 days and the control group weaned at 40 days (Colson *et al*., 2006, Landrain *et al*., 1997). Growth performance was improved between weaning and 10 weeks of age when weaning was postponed, but no effect was found on the resulting body weight (Leliveld *et al*., 2013). In our present study, our results showed that no significant difference in body weight among each weaning group were found at the end of the 42 days, which is consistent with former researches (Landrain *et al*., 1997; Colson, 2006; Leliveld *et al*., 2013).Few effect of weaning age was speculated on body weights of late finishing pigs because of catch-up growth.

It is well known that MDA is a terminal product oflipid peroxidation, so the content of MDA can be used toestimate the extent of lipid peroxidation, which indirectly reflects the metabolism of freeradicals (Hanson *et al*., 2015). Previous results indicated thatthe concentrations of piglets serum MDA, NO, and H_2O_2 was increased (P<0.05) after weaning, and there was an inhibition in the antioxidant system (Zhu *et al*., 2012). Yin *et al*. (2014) found that the plasma MDA level increased significantly at 3 days after weaning and speculated that weaning disrupted oxidative balance and caused oxidative injury in piglets. In the presentstudy, plasma, liver, kidney and lung tissues MDA levels of the piglets weaned at 35 days were significantly lower than that of 14 days weaned piglets, plasma liver and kidney MDA levels of the piglets weaned at 28 and 35 days were also significantly lower than that of 21 days weaned piglets. Contents of MDA in the plasma and these tissues showed a gradually decreased tendency when the weaning was delayed. It suggested that piglets weaned at a very early age could suffer from excessive free radicals and heavier lipid peroxidation.

Enzymes, such as SOD, GSH-Px and CAT, are the main components in the antioxidantsystem (Shi *et al*., 2014; Ma *et al*., 2014), which scavengeunwanted O_2^- and H_2O_2, and ROOH produced by freeradicals. For example, SOD catalyzes superoxide radical dis-mutation: $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. The resultinghydrogen peroxide in turn is decomposed by the enzymes GSH-Px and CAT. Decreasedactivity of those enzymes would induce accumulation of freeradicals, and injuries in corresponding tissues (Li *et al*., 2015; Hou *et al*., 2014).The liver, kidney and lung are important metabolic organs in pigs, playingimportant roles in maintaining physicalhealth.In this study, when the weaning age was delayed, T-SOD and GSH-Px activities in the plasma, liver, kidney and lung tissuesincreased gradually. T-SOD and GSH-Px activities of piglets weaned at 35 days were significantly higher than that of 14 days weaned piglets (P<0.05). Activities of liver and kidney GSH-Px and lung T-SOD were also higher in piglets weaned at 28 days than those weaned at 14 days (P<0.05). These findings

are consistent with previous study, which demonstrated that early weaning suppressed the plasma activity of SOD and GSH-Px at 1 or 3days after weaning, respectively (Yin *et al*., 2014). The concentrations of serum SOD activity decreased piglets (P<0.05) after weaning, and there was an inhibition of the antioxidant system(Zhu*et al*., 2012). Decreased SOD activity may lead to the increased O_2^- radicals, while decreased GSH-Px and CAT activities could increase tissue H_2O_2(Jiang *et al*., 2014). This study showed that very-early weaning (at 14 days) reduced the T-SOD and GSH-Px activities of plasma and in the liver, kidney and lung, which weaken the antioxidant capacity of piglets. It may be one of the reasonswhich early weaned piglets are more susceptible to early weaning stress syndrome.

Activity of CAT did not change in the plasma, liver, kidney and lung. However, the inconsistencyeffects of weaning age on CAT and other antioxidant enzymesneeds further investigation.

Conclusions: The antioxidant capacity in piglets can be markedly affected by weaning ages and the decreasing degree ofantioxidant capacity is growing when the weaning was advanced.

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