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Transient effects of weaning on the health of newly weaning piglets

X. Tao, Z. Xu, X. Men

Institute of Animal Husbandry and Veterinary Science, Zhejiang Academy of Agricultural Sciences, Hangzhou, P.R. China

ABSTRACT: The transient effects of weaning on the health of newly weaning piglets were investigated. In this study, 24 piglets were randomly assigned to weaning and suckling (control) groups. Weaning piglets were sacrificed on days 1, 4, and 7 post-weaning; suckling piglets were slaughtered at the corresponding time points. Blood samples and internal organs were collected for the determination of hematological traits, relative organ weights, and serum biochemical, antioxidant, enzyme, and immune indexes. The results revealed that piglets' growth performance significantly decreased on days 4 and 7 post-weaning. White blood cell and lymphocyte counts changed on days 1 and 4 post-weaning. On day 4, serum glucose levels decreased and urea nitrogen levels increased. Thymus gland weight significantly decreased on day 7, while adrenal gland weight significantly increased on days 4 and 7. Malondialdehyde level increased on day 4 post-weaning and superoxide dismutase, catalase, and alkaline phosphatase activities decreased on days 4 and 7 post-weaning. Serum IgA concentrations decreased from day 1 to day 4 post-weaning. The results showed that the adverse effects of weaning on the serum biochemical indexes gradually weakened on day 4 post-weaning. However, during the first week post-weaning, there were damaging effects on the endocrine, antioxidant, intestinal, and immune systems of piglets.

Keywords: hematological traits; serum biochemical indexes; enzyme; immune

List of abbreviations: W1d, W4d, W7d = weaning piglets slaughtered on days 1, 4, and 7 post-weaning, respectively, S1d, S4d, S7d = suckling piglets slaughtered on days 1, 4, and 7, respectively, ADG = average daily gain, WBC = white blood cell count, GRAN = neutrophilic granulocyte count, LYMF = lymphocyte count, MONO = monocyte count, GR = granulocyte percentage, LY = lymphocyte percentage, MO = monocyte percentage, RBC = red blood cell, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red blood cell volume distribution width, PLT = blood platelet counts, PCT = platelet trait, MPV = mean platelet volume, PDW = platelet distribution width, TP = total protein, ALB = albumin, Glu = glucose, BUN = serum urea nitrogen, TCH = total cholesterol, LDL = low-density lipoprotein, MDA = malondialdehyde, T-AOC = total antioxidant capacity, SOD = superoxide dismutase, CAT = catalase, XOD = xanthine oxidase, CP = ceruloplasmin, AKP = alkaline phosphatase, 5'-NT = 5'nucleotidase

INTRODUCTION

Weaning is one of the most stressful events in the life of pigs, which affects animal health and growth performance, especially during the first week post-weaning (Campell et al. 2013; Pluske 2013; Yin et al. 2013). Even though advances in nutrition, animal handling, and breeding technology have greatly reduced weaning stress, it is impossible to completely eliminate the adverse physiological

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effects. Therefore, a better understanding of the effects of weaning on piglets' health and growth is useful to prevent weaning stress. Studies have reported that abrupt weaning practices reduce feed intake, lower growth performance, damage the immune system, and contribute to diarrhea in piglets (Funderburke and Seerley 1990; Kelly et al. 1990; Morrow-Tesch et al. 1994). However, these studies were conducted more than two decades ago. Additionally, most researchers have evaluated the physiological effects of weaning stress more than one week post-weaning (Bonnette et al. 1990a, b; Ching et al. 2002); earlier times points, when weaning stress is more significant, have not been studied. Furthermore, only a shared control group of suckling piglets has been evaluated in previous studies (Colson et al. 2006; van der Meulen et al. 2010).

The objective of this study was to investigate the effects of weaning on growth performance, relative organ weights, hematological traits, and serum biochemical parameters of piglets within the first week post-weaning.

MATERIAL AND METHODS

Animals and treatments. All animal studies were conducted in accordance with the principles and procedures established by the Zhejiang Farm Animal Welfare Council of China and approved by the ethics committee of the Zhejiang Academy of Agricultural Sciences.

Four litters of newborn piglets (Duroc × Yorkshire × Landrace) and their respective sows were separately housed in pens with farrowing crates. The seven-day-old piglets had *ad libitum* access to water and ground pre-starter feed (digestible energy 14.53 MJ/kg; crude protein (21.6%), made from a commercial feed and consisting of 35% corn, 20% soybean, 5% sugar, and 40% nucleus components. At 25 days of age, 24 piglets (6 per litter) with average body weight of 7.15 ± 0.17 kg were randomly selected. Twelve piglets (3 per litter) were randomly assigned to the weaning group. These piglets were removed from the sows and housed in nursery pens with *ad libitum* access to water and ground starter feed (Table 1). The remaining 12 piglets were allowed to continue suckling the sows (control group). Four piglets from the weaning group were sacrificed on days 1, 4, and 7 post-weaning (referred to as W1d, W4d, and W7d groups, respectively). Four piglets from the control (suckling) group were simultaneously slaughtered at the corresponding time points (S1d, S4d, and S7d groups). To eliminate genetic background differences, a piglet was selected from each litter based on its body weight and slaughtered. Piglets from the weaning group were slaughtered 3 h after the last meal; piglets from the control group were slaughtered 1 h after the last suckling period.

Sample collection. Prior to slaughter, blood samples were collected from the jugular vein into 10-ml EDTA-containing vacuum tubes. The meas-

Table 1. Compositions and nutrient contents of the starter diet

Ingredients (%)	Diet
Swelled corn	51.52
Soybean meal	12.0
Swelled soybean	10.0
Whey meal	10.0
Fish meal	4.0
Fermented soybean meal	3.0
Plasma protein meal	2.0
Dicalcium phosphate	1.20
Limestone	4.0
Salt (NaCl)	0.35
L-Lysine	0.16
DL-Methionine	0.10
L-Threonine	0.11
L-Trypotophan	0.06
Premix[1]	1.50
Total	100
Calculated analysis[2]	
CP (%)	19.61
DE (MJ/kg)	14.23
Ca (%)	0.74
Total P (%)	0.66

CP = crude protein, DE = digestible energy

[1]vitamin and mineral mixture supplied per kg of diet: vitamin A 3950 IU, vitamin D_3 595 IU, vitamin E 23 IU, vitamin B_2 5.5 mg, vitamin B_{12} 0.03 mg, biotin 0.15 mg, nicotinic acid 30 mg, folacin 1 mg, choline choride 600 mg, vitamin K_3 1 mg, pantothenic acid 15 mg, thiamin 1.5 mg, vitamin B_6 8 mg, linoleic acid 0.15%, Cu ($CuSO_4·5H_2O$) 200 mg, Fe ($FeSO_4·7H_2O$) 110 mg, Zn ($ZnSO_4·7H_2O$) 120 mg, Mn ($MnSO_4·H_2O$) 40 mg, Se ($NaSe_2O_3$) 0.3 mg, I (IO_3) 1 mg

[2]based on nutrient contents of ingredients listed in National Research Council (2012)

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urement of hematological traits was finished 4 h after the piglets were sacrificed. Serum from whole blood samples was obtained by centrifugation at 3500 *g* for 10 min at 4°C and stored at –80°C. All samples were tested in duplicate; mean values were reported.

The piglets were sedated with a combination of xylazine and ketamine and euthanized using intravenous pentobarbital via a catheterized ear vein. Internal organs of the piglets (liver, kidneys, heart, spleen, adrenal glands, and thyroid glands) were removed and weighed. Relative organ weights were expressed as g/kg live body weight.

Measurements of hematological traits. Eighteen hematological traits including seven leukocyte traits (white blood cell count (WBC), neutrophilic granulocyte count (GRAN) and percentage (GR%), lymphocyte count (LYMF) and percentage (LY%), and monocytes count (MONO) and percentage (MO%)), seven erythrocyte traits (red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell volume distribution width (RDW)), and four platelet traits (blood platelet counts (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and platelet trait (PCT)) were measured.

Assessment of serum biochemical parameters. The activities of alkaline phosphatase (AKP), superoxide dismutase (SOD), total antioxidant capacity (T-AOC), xanthine oxidase (XOD), catalase (CAT), 5'-nucleotidase (5'-NT), and ceruloplasmin (CP), and the concentrations of serum urea nitrogen (BUN), malondialdehyde (MDA), total protein (TP), albumin (ALB), total cholesterol (TCH), low-density lipoprotein (LDL), and glucose (Glu) were determined with commercial kits (Nanjing Jiancheng Biochemical Reagent, Nanjing, China). Enzyme activity was expressed as units per ml of serum. One unit of SOD activity was defined as the amount of enzyme that reduced absorbance at 550 nm by 50%. One unit of T-AOC was defined as the amount necessary to increase the absorbance at 520 nm by 0.01 in 1 min/ml serum. One unit of XOD was defined as the amount of enzyme that catalyzed 1 μmol/min substrate at 530 nm. One unit of CAT was defined as the amount of enzyme that quenched 1 μmol/s H_2O_2 at 405 nm. One unit of AKP was defined as the amount of enzyme that produced 1 mg phenol at 520 nm in 15 min/100 ml serum. One international unit of CP was defined as the amount of enzyme that catalyzed 1 μmol/min substrate at 540 nm. One unit of 5'-NT activity was defined as the amount of enzyme that produced 1 mmol phosphorus (phosphoric acid) at 680 nm.

The serum concentrations of immunoglobulins IgA, IgG, and IgM were measured with ELISA kits (Nanjing Jiancheng Biochemical Reagent, Nanjing, China).

Statistical analyses. Statistical analyses were performed by Student's *t*-test using SPSS software package (Version 17.0, 2009). Results were expressed as mean ± SEM. Statistical significance was set at $P < 0.05$.

RESULTS

Effects of weaning on growth performance. All piglets remained healthy during the study. There were no cases of diarrhea. The body weights and average daily gain (ADG) of the piglets are shown in Table 2. Compared with S4d, W4d piglets had reduced body weights and ADG ($P < 0.01$). Similarly, growth performance of W7d was significantly lower ($P < 0.01$) than that of S7d. Even though the body weights of the piglets decreased during the first week post-weaning, the adverse effects on growth performance weakened on day 7.

Table 2. Effects of weaning on growth performance

Items	Treatments					
	S1d	W1d	S4d	W4d	S7d	W7d
Initial weight (kg)	7.06 ± 0.21	7.06 ± 0.21	7.07 ± 0.20	7.06 ± 0.17	7.07 ± 0.21	7.05 ± 0.20
Final weight (kg)	#	#	8.51 ± 0.29	6.42 ± 0.24**	9.15 ± 0.41	6.47 ± 0.16**
ADG (g)	#	#	360.0 ± 27.3	–160.0 ± 20.3**	297.1 ± 38.8	–82.9 ± 8.74**

W1d, W4d, W7d = weaning piglets slaughtered on days 1, 4, and 7 post-weaning, respectively; S1d, S4d, S7d = suckling piglets slaughtered on days 1, 4, and 7, respectively; ADG = average daily gain

#piglets were slaughtered after weighing

**$P < 0.01$

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Table 3. Effects of weaning on organ weight indexes

Items	Treatments					
	S1d	W1d	S4d	W4d	S7d	W7d
Liver index	22.84 ± 0.85	21.52 ± 0.83	23.52 ± 0.96	21.82 ± 0.68	22.51± 1.06	23.26 ± 0.70
Kidney index	6.15 ± 0.48	6.50 ± 0.39	6.32 ± 0.33	6.80 ± 0.41	5.27 ± 0.26	6.25 ± 0.41
Heart index	5.67 ± 0.12	5.92 ± 0.06	5.18 ± 0.22	6.19 ± 0.44	4.94 ± 0.18	5.42 ± 0.24
Spleen index	1.95 ± 0.08	2.15 ± 0.15	1.98 ± 0.23	1.96 ± 0.08	2.18 ± 0.12	2.24 ± 0.30
Thyroid index	2.09 ± 0.19	2.39 ± 0.32	2.24 ± 0.34	1.60 ± 0.20	2.35 ± 0.32	1.04 ± 0.34*
Adrenal gland index	0.14 ± 0.01	0.16 ± 0.02	0.10 ± 0.01	0.14 ± 0.01*	0.11 ± 0.01	0.15 ± 0.01*

W1d, W4d, W7d = weaning piglets slaughtered on days 1, 4, and 7 post-weaning, respectively; S1d, S4d, S7d = suckling piglets slaughtered on days 1, 4, and 7, respectively

*$P < 0.05$

Effects of weaning on relative organ weights. The relative organ weights of the piglets are shown in Table 3. The results revealed no significant differences in the relative weights of the liver, kidney, heart, and spleen between the weaning and corresponding control groups ($P > 0.05$). Compared to S7d, W7d had lower thymus gland weight (–55.74%, $P < 0.05$). Adrenal gland weight

Table 4. Effects of weaning on hematological traits

Items	Treatments					
	S1d	W1d	S4d	W4d	S7d	W7d
WBC (10^9/l)	12.00 ± 1.40	11.88 ± 0.52	7.38 ± 1.66	13.38 ± 1.89	9.90 ± 0.67	17.30 ± 1.14**
GRAN (10^9/l)	0.80 ± 0.22	0.84 ± 0.13	1.15 ± 0.48	0.84 ± 0.18	3.04 ± 1.61	2.89 ± 0.82
LYMF (10^9/l)	9.53 ± 1.09	10.38 ± 0.42	5.02 ± 1.05	11.07 ± 1.70*	6.25 ± 1.39	13.36 ± 1.10 **
MONO (10^9/l)	0.93 ± 0.31	0.65 ± 0.21	1.18 ± 0.37	0.97 ± 0.28	0.56 ± 0.66	0.91± 0.25
GR (%)	5.03 ± 1.21	7.15 ± 1.25	15.08 ± 3.72	9.18 ± 1.11	11.15 ± 3.50	16.80 ± 4.26
LY (%)	89.13 ± 1.86	87.45 ± 0.79	68.95 ± 1.73	82.98 ± 1.77**	83.08 ± 3.92	78.18 ± 4.29
MO (%)	5.85 ± 1.92	5.30 ± 1.51	15.98 ± 4.15	7.85 ± 2.71	5.78 ± 0.76	5.03 ± 1.16
RBC (10^{12}/l)	5.95 ± 0.34	5.16 ± 0.20	4.59 ± 0.60	5.73 ± 0.29	5.63 ± 0.55	6.00 ± 0.17
HGB (g/l)	108.75 ±7.47	87.45 ± 0.79	83.75 ± 11.43	112.50 ± 7.50	101.25 ± 10.87	111.25 ± 4.27
HCT (%)	42.75 ± 4.13	43.00 ± 2.27	38.88 ± 5.25	48.50 ± 4.22	44.38 ± 3.47	50.13 ± 2.29
MCV (fl)	72.25 ± 6.33	83.50 ± 2.22	85.00 ± 3.24	84.25 ± 3.04	79.75 ± 4.78	83.75 ± 2.25
MCH (Pg)	18.30 ± 0.88	19.83 ± 0.46	18.23 ± 0.45	19.55 ± 0.68	17.95 ± 0.31	18.55 ± 0.59
MCHC (g/l)	256.75 ± 11.21	238.50 ± 5.48	215.25 ± 4.87	233.75 ± 10.42	227.50 ± 15.33	222.25 ± 3.97
RDW (%)	23.43 ± 3.26	24.70 ± 1.84	26.58 ± 1.26	26.28 ± 1.79	24.10 ± 1.10	30.83 ± 1.91*
PLT (10^9/l)	586.25 ± 115.42	321.25 ± 106.45	304.25 ± 123.00	420.00 ± 117.93	406.75 ± 119.59	395.00 ± 36.91
PCT (%)	0.13 ± 0.03	0.14 ± 0.05	0.08 ± 0.03	0.14 ± 0.03	0.10 ± 0.01	0.11 ± 0.02
MPV (fl)	10.98 ± 0.36	10.60 ± 0.17	9.15 ± 0.50	10.28 ± 0.77	9.60 ± 0.54	10.08 ± 0.74
PDW (%)	14.85 ± 0.50	15.28 ± 1.42	11.63 ± 0.43	14.55 ± 1.13	12.20 ± 0.93	16.93 ± 0.72

W1d, W4d, W7d = weaning piglets slaughtered on days 1, 4, and 7 post-weaning, respectively; S1d, S4d, S7d = suckling piglets slaughtered on days 1, 4, and 7, respectively; WBC = white blood cell count, GRAN = neutrophilic granulocyte count, LYMF = lymphocyte count, MONO = monocytes count, GR = granulocyte percentage, LY = lymphocyte percentage, MO = monocytes percentage, RBC = red blood cell, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red blood cell volume distribution width, PLT = blood platelet counts, PCT = platelet trait, MPV = mean platelet volume, PDW = platelet distribution width

*$P < 0.05$, **$P < 0.01$

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Table 5. Effects of weaning on serum biochemical indexes

Items	Treatments					
	S1d	W1d	S4d	W4d	S7d	W7d
TP (g/l)	62.09 ± 3.74	54.21 ± 1.43	56.42 ± 1.95	53.61 ± 3.12	55.58 ± 2.04	52.16 ± 2.14
ALB (g/l)	49.34 ± 2.11	43.94 ± 1.35	43.32 ± 1.71	40.34 ± 1.88	46.05 ± 2.43	43.15 ± 4.40
Glu (μmol/ml)	8.45 ± 0.54	7.54 ± 0.73	7.94 ± 0.32	5.49 ± 0.51**	7.81 ± 0.73	6.50 ± 0.31
BUN (mmol/ml)	9.36 ± 0.69	10.22 ± 0.68	7.95 ± 0.50	13.58 ± 1.52*	6.58 ± 0.41	8.98 ± 1.31
TCH (mmol/l)	6.00 ± 0.49	4.33 ± 0.41*	4.35 ± 0.31	3.54 ± 0.26	4.18 ± 0.41	2.88 ± 0.66
LDL (mmol/l)	1.16 ± 0.22	0.76 ± 0.16	0.57 ± 0.06	0.69 ± 0.09	0.64 ± 0.05	0.49 ± 0.08

W1d, W4d, W7d = weaning piglets slaughtered on days 1, 4, and 7 post-weaning, respectively; S1d, S4d, S7d = suckling piglets slaughtered on days 1, 4, and 7, respectively; TP = total protein, ALB = albumin, Glu = glucose, BUN = serum urea nitrogen, TCH = total cholesterol, LDL = low-density lipoprotein

*P < 0.05, **P < 0.01

increased (P < 0.05) by 40.0% and 36.36% in W4d and W7d, respectively, with no significant differences (P > 0.05) between W1d and S1d.

Effects of weaning on hematological traits. As shown in Table 4, there were no significant differences in hematological traits between W1d and S1d (P > 0.05). LYMF and LY were by 120.52% (P < 0.05) and 20.35% (P < 0.01) higher, respectively, in W4d than in S4d, with no significant differences between the two groups in the remaining hematological traits (P > 0.05). WBC, LYMF, and RDW were by 74.75% (P < 0.01), 113.76% (P < 0.01), and 27.93% (P < 0.05) higher, respectively, in W7d than in S7d.

Effects of weaning on serum biochemical indexes. The serum biochemical indexes of piglets are shown in Table 5. TCH was lower (–27.8%) in W1d than in S1d, with no significant differences between the two groups in TP, ALB, Glu, BUN, or LDL levels. In W4d, Glu decreased by 30.86% (P < 0.01) and BUN increased by 70.82% (P < 0.05); no significant differences (P > 0.05) were detected between W4d and S4d in TP, ALB, TCH, or LDL levels. There were no significant differences in biochemical indexes between W7d and S7d (P > 0.05).

Effects of weaning on antioxidant function and enzyme activities. Serum antioxidant indexes and enzyme activities are shown in Table 6. There were no significant differences (P > 0.05) in antioxidant indexes or enzyme activities between W1d and S1d. Compared with S4d, W4d had higher MDA levels (+96.10%, P < 0.01) and lower SOD (–17.29%, P < 0.05) and CAT activities (–62.76%, P < 0.01). There were no significant differences (P > 0.05) in

Table 6. Effects of weaning on antioxidant function and enzyme activities

Items	Treatments					
	S1d	W1d	S4d	W4d	S7d	W7d
MDA (nmol/l)	2.91 ± 0.25	3.64 ± 0.20	2.31 ± 0.16	4.53 ± 0.13**	2.85 ± 0.37	3.10 ± 0.40
T-AOC (U/ml)	2.68 ± 0.46	4.07 ± 0.50	1.82 ± 0.45	2.62 ± 0.58	2.50 ± 0.25	1.63 ± 0.44
SOD (U/ml)	125.00 ± 6.43	122.19 ± 4.03	126.52 ± 4.78	104.65 ± 6.31*	136.81 ± 6.19	95.65 ± 13.25*
CAT (U/ml)	6.27 ± 0.64	7.01 ± 0.77	6.38 ± 0.56	3.92 ± 0.12**	5.08 ± 0.40	5.75 ± 0.83
XOD (U/l)	15.72 ± 3.84	7.85 ± 0.50	13.99 ± 2.96	8.18 ± 0.66	9.15 ± 1.10	6.44 ± 0.27
CP (U/l)	418.33 ± 31.72	442.91 ± 61.22	371.98 ± 27.14	500.14 ± 69.28	370.77 ± 16.47	508.20 ± 70.00
AKP (U/100 ml)	12.87 ± 2.24	12.86 ± 2.88	9.89 ± 1.35	6.53 ± 2.10	9.71 ± 0.85	5.00 ± 1.49*
5'-NT (U/l)	1.29 ± 0.12	1.17 ± 0.07	1.68 ± 0.29	1.09 ± 0.08	1.69 ± 0.32	1.25 ± 0.22

W1d, W4d, W7d = weaning piglets slaughtered on days 1, 4, and 7 post-weaning, respectively; S1d, S4d, S7d = suckling piglets slaughtered on days 1, 4, and 7, respectively; MDA = malondialdehyde, T-AOC = total antioxidant capacity, SOD = superoxide dismutase, CAT = catalase, XOD = xanthine oxidase, CP = ceruloplasmin, AKP = alkaline phosphatase, 5'-NT = 5'-nucleotidase

*P < 0.05, **P < 0.01

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Table 7. Effects of weaning on plasma immunoglobulin concentrations (μg/ml)

Items	Treatments					
	S1d	W1d	S4d	W4d	S7d	W7d
IgA	67.63 ± 10.70	74.75 ± 8.74	67.74 ± 1.55	61.27 ± 8.18	59.79 ± 6.64	57.34 ± 4.28
IgG	320.84 ± 46.86	362.36 ± 26.72	300.93 ± 48.84	366.01 ± 22.60	307.23 ± 42.15	324.30 ± 15.29
IgM	30.39 ± 4.02	31.90 ± 3.28	20.82 ± 1.71	26.28 ± 3.15	22.11 ± 2.33	24.38 ± 1.31

W1d, W4d, W7d = weaning piglets slaughtered on days 1, 4, and 7 post-weaning, respectively; S1d, S4d, S7d = suckling piglets slaughtered on days 1, 4, and 7, respectively; IgA, IgG, IgM = immunoglobulins

the activities of T-AOC, XOD, CP, AKP, or 5'-NT between the two groups. Compared with S7d, W7d had lower SOD and AKP activities (−30.09% and −48.51%, respectively, $P < 0.05$), with no significant differences ($P > 0.05$) in the activities of the remaining enzymes.

Effects of weaning on serum immunoglobulin concentrations. The levels of serum immunoglobulins IgA, IgG, and IgM are shown in Table 7. Weaning had no significant effects on the levels of serum immunoglobulins in piglets. The concentrations of IgA, IgG, and IgM were not significantly different between the three pairs of groups: W1d and S1d, W4d and S4d, and W7d and S7d.

DISCUSSION

Growth performance is an indicator of the health status of piglets. Decreased body weight after weaning is an indicator of weaning stress. In this study, piglet body weight decreased during the first week post-weaning, especially on day 4. However, the reduction in body weight was gradually recovered on day 7 post-weaning. Le Dividich and Seve (2000) reported that the recovery in piglet body weight was achieved 4 days post-weaning regardless of the animal age at weaning. This finding was consistent with our results. Even though we did not record feed intake in the weaning group, the reduction in body weight was mainly attributed to low feed intakes (Hampson and Kidder 1986; Appleby et al. 1991; Fraser et al. 1994; Bruininx et al. 2002; Boudry et al. 2004; Yin et al. 2015).

Most studies have focused on the effects of weaning stress on the digestive system of piglets (Lindemann et al. 1986; Cera et al. 1988). In recent years, an increasing number of studies have reported that the immune and neuroendocrine systems are extensively activated when the body is under stress (Liu et al. 2013). Our results revealed that the adrenal and thyroid glands of piglets were affected by weaning stress. In this study, low thyroid gland and high adrenal gland weight indexes were probably related to metabolic and immune-neuroendocrine disorders on days 4 and 7 post-weaning. Although our findings only preliminarily revealed effects of weaning on the neuroendocrine system of piglets, these results maybe indicated we should pay more attention to the health of the neuroendocrine system of weaning piglets in future studies. And it is also helpful to improve piglets' health by alleviating the adverse effects of weaning stress on the neuroendocrine system with some nutritional or other manipulations.

In our study, the changes in hematological traits mainly involved white blood cell count, lymphocyte count, and red blood cell volume distribution on day 7 post-weaning. Davis et al. (2006) reported that the total count of white blood cells and lymphocytes increases on days 2, 10, and 27 post-weaning. Similarly, Sugiharto et al. (2014) reported that the white blood cell count of piglets increases from day 0 to days 4 and 11 post-weaning. Concentrations of total white blood cells are closely related to porcine health status, and their variation might mean the occurrence of inflammation and the decrease of immunology in newly weaning piglets. It was pointed out that the changes of lymphocyte concentrations were likely age-related and not altered due to weaning, and the percentages of lymphocytes were unaffected by weaning (Davis et al. 2006). Sugiharto et al. (2014) also found that although the percentages of lymphocytes varied, the ratio of neutrophils to lymphocytes did not change in the post-weaning period. Even though our results revealed differences in lymphocyte count and percentage on day 4 between suckling and weaning piglets, the difference between the two groups was attributed to abnormal changes in the control group. Therefore, the results of

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lymphocyte counts could not indicate the piglets' immunological response to weaning stress. However, to some extent we showed it is necessary for detecting hematological traits of piglets to have a corresponding control for each time point when the sample sizes are small.

Our serum biochemical index results revealed that weaning mainly decreased glucose concentrations and increased urea nitrogen concentrations on day 4 post-weaning. This result is closely related to the negative energy balance in piglets due to underfeeding during the post-weaning period (Le Dividich and Seve 2000; Xiong et al. 2014; Wang et al. 2015). Funderburke and Seerley (1990) reported that pigs that are abruptly weaned have lower plasma glucose concentrations within 132 h post-weaning. In our study, weaning had no significant effects on serum biochemical parameters on days 1 or 7 post-weaning. Even though the concentration of total cholesterol significantly changed on day 1, it was also a false-positive result due to the small sample size. Furthermore, our results suggested that the effects of weaning on serum biochemical indexes were transient and could be corrected to normal levels within one week post-weaning.

Studies have shown that weaning induces oxidative stress in piglets. Zhu et al. (2012) reported that serum MDA levels increase on day 14 post-weaning, while Yin et al. (2014) showed that plasma MDA levels peak on day 3 post-weaning. Additionally, the authors reported that plasma SOD activity decreases on day 1 post-weaning and normalizes on days 3, 5, and 7 post-weaning (Yin et al. 2014). Consistent with these results, our study showed that the concentration of serum MDA was the highest on day 4 post-weaning. Similarly, CAT activity was the lowest on day 4; however, SOD activity continuously decreased from day 1 to day 7, which may be attributed to differences in weaning periods between the two studies. Additionally, in this study, serum AKP activity decreased after weaning, suggesting that the intestinal health of piglets suffered adverse effects during the first week post-weaning.

Serum immunoglobulin concentrations are indicative of the innate immune system of weaning piglets. Even though the interruption of colostrum intake reduces serum immunoglobulin levels in weaning piglets, weaning age affects the immune responses of piglets due to the development of the immune system. Low IgA concentrations were observed in ultra-early and early weaning piglets (Levast et al. 2010; Smith et al. 2010). Sugiharto et al. (2014) reported that the concentration of plasma IgA was higher on day 11 than on days 0 and 4 post-weaning, with no significant changes in IgG levels. However, Kick et al. (2012) demonstrated that early weaning did not negatively affect the adaptive immunological competence of piglets. Our results showed that the concentrations of IgA, IgG, and IgM did not significantly change during the first week post-weaning; however, IgA levels had a decreasing trend with increasing weaning time. This result may be related with the limited number of samples, and the values of IgA in S7d were lower than those in groups S1d and S4d. In other words, the statistical analysis was influenced by the limited number of samples and the control differences.

In this study, some novel findings regarding the transient effects of weaning on the health of newly weaned piglets were obtained. However, the limited number of samples might compromise the conclusions. Therefore, future studies should include larger sample sizes to confirm these results.

In conclusion, the adverse effects of weaning on growth performance and serum biochemical indexes of piglets were gradually reduced from day 1 to day 7 post-weaning. However, the effects of weaning on the endocrine, antioxidant, intestinal, and immune systems were intensified during the first week post-weaning. Furthermore, our results suggest that a corresponding control at each post-weaning time point is necessary when the sample sizes are small.

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Corresponding Author

Ziwei Xu, Zhejiang Academy of Agricultural Sciences, Institute of Animal Husbandry and Veterinary Science, Hangzhou, Zhejiang 310021, P.R. China
Phone: +86 571 864 190 28, e-mail: xzwfyz@sina.com