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Dietary Chitosan Affects Metabolism of Arachidonic Acid in Weaned Piglets

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ABSTRACT

Li J.-L., Xu Y.-Q., Shi B.-L., Sun D.-S., Yan S.-M., Guo X.-Y. (2017): **Dietary chitosan affects metabolism of arachidonic acid in weaned piglets**. Czech J. Anim. Sci., 62, 58–66.

The effects of chitosan on immune function via arachidonic acid (AA) pathway in weaned piglets were investigated. A total of 180 piglets (Duroc × Yorkshire × Landrace) were randomly assigned to 5 dietary treatments and fed a basal diet supplemented with 0 (control), 100, 500, 1000, and 2000 mg chitosan/kg feed, respectively. Results showed that serum AA, prostaglandin E2 (PGE2), and leukotriene B4 (LTB4) contents in piglets were increased in a linear or quadratic dose-dependent manner with increasing chitosan on day 28 ($P < 0.05$). Chitosan increased serum cytosolic-phospholipase A2 (cPLA2) activity in a linear or quadratic dose-dependent manner on day 14 or 28, and improved 5-lipoxygenase (5-LOX) activity in a linear manner and cyclooxygenase-2 (COX-2) activity quadratically on day 28 ($P < 0.05$). Moreover, chitosan elevated gene expression of *cPLA2* mRNA quadratically in the small intestine on days 14 and 28, increased the *COX-2* mRNA expression in the duodenum or jejunum in a linear or quadratic manner on day 28, and improved the *5-LOX* mRNA expression quadratically in the small intestine ($P < 0.05$). These results implied that the metabolism of AA was regulated by chitosan in a dose-dependent relationship, which may be one reason why chitosan affected immune function via AA pathway in weaned piglets.

Keywords: cytosolic-phospholipase A2; prostaglandin E2; leukotriene B4; 5-lipoxygenase; cyclooxygenase-2; mRNA expression

Weaning age of pigs has been markedly reduced over the past 30 years. With the decreasing of weaning age, piglets are smaller and have less mature digestive and immune systems at weaning, making them more susceptible to problems in the early post-weaning period. Pigs are weaned between 14 and 28 days of age in modern intensive swine production systems, to optimize production efficiency. But early weaning not only interrupts the supply of important immune factors from sow's milk and increases susceptibility to infection (Varley et al. 1986; Drew and Owen 1988), but also impairs the production of antibodies and compromises cellular immune functions (Touchette et al. 2002; Kick et al. 2012), which leads to piglets being infected more easily by pathogens. Disease is considered to be a major contributing factor associated with poor performance of post-weaning pigs. Thus, reducing immunological challenges to pigs or improving the ability of pigs to cope with an immunological challenge may help improve performance.

Arachidonic acid (AA) is an essential fatty acid for pigs to maintain good growth and normal physi-

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ological activities, and it is noticeably present in immune cell membrane phospholipids (Kew et al. 2003), and likely to influence lymphocyte maturation and function (Field et al. 2000). AA and some of its metabolites may influence the developing of immune system because AA is the precursor of leukotrienes (LTs) and prostaglandins (PGs) which are thought to be important mediators of immune responses (Ma et al. 2003; Calder 2006; Dirix et al. 2009). Over the recent years, considerable work has been conducted extensively due to the fact that AA plays an important role in modulating animal immunity. One possible mechanism is that AA fulfills a variety of roles within immune cells like neutrophils and macrophages (Ma et al. 2003). In addition, through the action of cyclooxygenase (COX) (COX-1, -2, and -3) and 5-lipoxygenase (5-LOX), AA is transformed to metabolites including PGs and LTs that are effective regulators of immune function (Ma et al. 2003; Moodley et al. 2009). Furthermore, AA probably interferes with cytokine release via modulation of eicosanoids synthesis (Baker 1990).

Chitosan, a natural and nontoxic alkaline polysaccharide, is formed by the action of chitin deacetylases and is a key structural component of helminths, arthropods, and fungi (Synowiecki and Al-Khateeb 2003). In the last decade, many reports were concerned with chitosan regulating the animal's immune function. Porporatto et al. (2005) indicated that chitosan enhanced the T helper cell type 2 (Th2)/Th3 microenvironment in the intestinal mucosa of rats. Kim et al. (2007) showed that chitosan acting as an adjuvant enhanced both the mucosal and systemic immune responses of piglets against recombinant transferrin-binding protein B (rTbp B) of *Actinobacillus pleuropneumoniae* via direct tracheal administration. Our previous study also indicated that chitosan increased the humoral and cellular immune function in weaned piglets (Li et al. 2013). Research in our laboratory (Li et al. 2009) utilizing a broiler model also showed that dietary supplementation with chitosan improved immune functions, which was associated with the increase of AA content and cPLA2 activity in serum as well as *cPLA2* mRNA expression in the small intestine. However, the effect of chitosan on immune function via AA pathway in weaned piglet remains poorly understood. Therefore, it was of interest to examine the capacity of chitosan to affect immune function through the AA pathway.

The objective of the present study was to measure the effect of dietary supplementation of chitosan on AA metabolic network involving the important immunomodulators PGE2 and LTB4, and the key enzymes COX-2 and 5-LOX as well as the gene expression of *COX-2* and *5-LOX* in weaned pigs. The findings may provide useful evidence for the application of chitosan as a dietary additive to regulate the immune functions via AA pathway of weaned piglets.

MATERIAL AND METHODS

All procedures described in this study were approved by the Inner Mongolia Agricultural University Animal Care and Use Committee.

Animals and experimental design. A total of 180 piglets (Duroc × Yorkshire × Landrace) initially weighing 7.6 kg were at 28 days of age randomly allotted into five treatment groups with six repetitions (3 pens of males and 3 pens of females) in each treatment, and six piglets in each pen (2.0 × 2.2 m), and penned in a temperature-controlled building where the temperature was maintained at 26–28°C and relative humidity was about 65–70%. The weaned piglets had 1 week of housing and management adaptation before the experimental phase. The experimental period lasted 28 days. Feed and water were provided *ad libitum*.

This study was designed as single factor randomized block arrangement, with five dietary treatment groups supplemented with 0 (control), 100, 500, 1000 or 2000 mg chitosan/kg feed. Basal diets were formulated to meet the NRC requirements for all nutrients (NRC 1998) (Table 1). All diets were offered in meal form. The chitosan was provided by Jinan Haidebei Marine Bioengineering Ltd. Co., China. The deacetylation degree of chitosan was determined to be 85.09% and the viscosity was 45 cps.

Sample collection. On days 14 and 28, one pig from each replicate of each treatment was randomly selected and blood samples were obtained by puncturing the vena cava. The blood samples were centrifuged at 3000 *g* for 10 min at 4°C to obtain serum. Serum was stored at –20°C until further analysis of AA, PGE2, and LTB4 content as well as cPLA2, COX-2, and 5-LOX activity.

Quantification of mRNA. For determining the gene expression of *cPLA2*, *COX-2*, and *5-LOX*

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Table 1. Composition and nutrient levels of experimental diets (air-dry basis, %)

Ingredients	Content	Nutrients	Level
Corn	51.90	digestible energy(MJ/kg)	14.32
Corn gluten meal	2.00	crude protein	20.02
Whey powder	2.00	ether extract	3.00
Soybean meal	16.00	crude fibre	4.20
Wheat	20.00	calcium	0.72
Fish meal	2.50	phosphorus	0.56
Soya bean oil	2.00	lysine	1.35
Limestone	0.70	methionine + cystine	0.82
$CaHPO_4$	1.00	threonine	0.74
NaCl	0.30		
Premix[1]	1.60		
Total	100.00		

[1]premix provided the following nutrients per kg diet: Vitamin A 9000 IU, Vitamin D_3 2500 IU, Vitamin E 60 IU, Vitamin K_3 4.5 mg, Vitamin B_1 2.6 mg, Vitamin B_2 8.7 mg, Vitamin B_6 7.0 mg, Vitamin B_{12} 0.03 mg, Vitamin C 200 mg, pantothenic acid 13 mg, nicotinic acid 35 mg, biotin 0.47 mg, folic acid 0.85 mg, iron 155 mg, copper 35 mg, zinc 100 mg, manganese 25 mg, iodine 0.35 mg, cobalt 0.2 mg, selenium 0.25 mg, choline chloride 750 mg, phytase 500 FTU, lysine 6.20 g, methionine 2.20 g, threonine 1.10 g

mRNA, the piglets which were used to obtain blood samples were sacrificed, and the duodenum, jejunum, and ileum were quickly removed and immediately placed frozen in liquid nitrogen, and then stored at –80°C until extraction for mRNA analysis.

Detection of AA, PGE2, LTB4, cPLA2, COX-2, and 5-LOX in serum. Serum AA, PGE2, and LTB4 content and cPLA2 activity were determined using RIA kits (Beijing Sinouk Institute of Biological Technology, China) as described by Li et al. (2009). Serum COX-2 and 5-LOX activities were determined using RIA kits (Beijing Sinouk Institute of Biological Technology, China) following the manufacturer's instructions.

Total RNA isolation and reverse transcription (RT). For determining the gene expression of *cPLA2*, *COX-2*, and *5-LOX* mRNA by quantitative real-time PCR, RNA was extracted from the duodenum, jejunum and ileum using the RNAiso Reagent (TaKaRa, China). RNA integrity was verified electrophoretically by ethidium bromide staining; RNA purity was assayed by the OD_{260}/OD_{280} ratio using UV-clear microplates; RNA content was detected directly by UV-clear microplates (both TECAN, Switzerland).

For reversely transcripting into cDNA, the PrimeScript RT Reagent Kit (TaKaRa) was used following the manufacturer's directions. The proportionate volume of total RNA of each sample was added to the RT reaction mixture (10 µl), which contained 0.5 µl PrimeScript™ RT Enzyme Mix I, 2 µl 5 × PrimeScript Buffer, 0.5 µl Random hexamer primers, 0.5 µl Oligo-dT Primer, and RNase-free water. The RT reaction parameters were as follows: RT at 37°C for 15 min, and RT inactivation at 85°C for 5 s. The RT products (cDNA) were stored at –20°C for quantitative polymerase chain reaction (PCR) assay.

Relative quantification of real-time PCR for cPLA2, COX-2, and 5-LOX mRNA. The primers were designed specifically using an online primer design tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index) according to gene sequences in GenBank, where *Sus scrofa β-actin* was used as the housekeeping gene (Table 2). Primers were synthesized by the Shanghai Sangon Biological Engineering Technology and Services Co., Ltd., China.

Relative levels of *cPLA2*, *COX-2*, and *5-LOX* mRNA were quantified using SYBR® PrimeScript™ RT-PCR Kit (TaKaRa, China) following the manufacturer's instructions. Reactions were also performed with negative controls (using water instead of cDNA). The PCR reaction system (20 µl) contained 10 µl 2×SYBR® Premix Ex Taq™ and 0.4 µl (10 µM) of forward and reverse specific primers, 2 µl of cDNA template, and 7.2 µl RNase-free water. The following procedure was used for amplification: one cycle at 95°C for 30 s followed by 40 cycles of 5 s at 95°C for denaturation, 30 s at 60°C for annealing, and 30 s at 72°C for extension. The melting curves were determined between 70 and 95°C with a heating rate of 0.5°C/s and continuous fluorescence measurement. Fluorescence data were acquired after the extension step during PCR reactions that contained SYBR® Green. Expression levels of *cPLA2*, *COX-2*, and *5-LOX* mRNA were calculated as relative values using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). The sizes of RT-PCR products were confirmed by 2% agarose gel electrophoresis in the presence of ethidium bromide, and bands were visualized by exposure to ultraviolet light. Sequences were confirmed by the Shanghai Sangon Biological Engineering Technology and Services Co., Ltd.

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Table 2. Genes and their primer sequences

Name	GenBank Acc. No.	Primer sequences	Product size (bp)
cPLA2	NM_001204400.1	F: GACTAGAGAAGGCTGGAGACCA R: CTATCACTGGGATCTCGTCCTC	429
COX-2	NM_214321.1	F: TGCGGGAACATAATAGAGTTG R: GAAGATTCCTACCACCAGCAAC	405
5-LOX	XM_001927671.1	F: AGCACCTGCTGGACAAGCCTTT R: TCTGGTCATCGCAGGCCAACTT	270
β-actin	XM_003124280.2	F: TACACCGCTACCAGTTCGCCAT R: TCTCCATGTCGTCCCAGTTGGT	270

F = forward primer, R = reverse primer

Statistical analysis. Regression analysis was conducted to evaluate linear and quadratic effects of chitosan on the various response criteria in piglets using SAS software (Statistical Analysis System, Version 9.2, 2008). Regression relation was considered significant if probability values of $P < 0.05$ were obtained.

RESULTS

Serum concentration of AA, PGE2, and LTB4. With increasing addition of chitosan, serum AA content of weaned piglets was increased in a linear dose-dependent manner on day 28 ($P = 0.032$) (Table 3). Chitosan increased serum PGE2 ($P = 0.014$)

Table 3. Effects of chitosan on the content of AA, PGE2, and LTB4 and activity of cPLA2, COX-2, and 5-LOX in the serum of weaning piglets (data are means ± SE, n =6)

Items	Level of dietary chitosan (mg/kg)					*P*-value	
	0	100	500	1000	2000	linear	quadratic
AA (ug/ml)							
14 days	18.60 ± 0.79	22.51 ± 1.28	23.72 ± 0.82	21.23 ± 1.22	19.51 ± 0.99	0.330	0.152
28 days	22.10 ± 1.62	23.74 ± 0.98	23.89 ± 0.19	25.84 ± 0.80	25.73 ± 0.88	0.032	0.058
cPLA2 (ug/l)							
14 days	236.18 ± 16.61	261.27 ± 2.00	274.94 ± 20.39	283.04 ± 20.42	304.63 ± 18.74	0.036	0.095
28 days	187.23 ± 14.31	253.09 ± 26.50	243.98 ± 17.80	283.54 ± 27.58	189.59 ± 16.15	0.957	0.039
PGE2 (pg/ml)							
14 days	33.18 ± 0.60	33.52 ± 0.89	34.52 ± 1.34	35.78 ± 0.78	34.63 ± 0.58	0.186	0.102
28 days	30.98 ± 0.97	31.19 ± 1.09	34.42 ± 0.90	33.47 ± 0.62	31.19 ± 0.83	0.832	0.014
COX-2 (U/l)							
14 days	7.68 ± 0.24	8.19 ± 0.24	8.00 ± 0.42	8.26 ± 0.65	7.91 ± 0.43	0.963	0.824
28 days	7.43 ± 0.39	7.63 ± 0.57	8.45 ± 0.39	9.01 ± 0.43	8.54 ± 0.47	0.079	0.034
LTB4 (pg/ml)							
14 days	174.82 ± 2.00	184.60 ± 4.23	188.97 ± 2.76	180.19 ± 4.48	174.03 ± 3.14	0.190	0.139
28 days	179.09 ± 2.66	200.16 ± 4.70	216.20 ± 5.99	216.25 ± 5.41	210.18 ± 7.45	0.045	0.005
5-LOX (ng/ml)							
14 days	1.35 ± 0.10	1.48 ± 0.08	1.45 ± 0.06	1.62 ± 0.06	1.56 ± 0.08	0.130	0.166
28 days	1.49 ± 0.08	1.47 ± 0.02	1.52 ± 0.05	1.65 ± 0.03	1.61 ± 0.03	0.040	0.052

AA = arachidonic acid, cPLA2 = cytosolic phospholipase A2, PGE2 = prostaglandin E2, COX-2 = cyclooxygenase-2, LTB4 = leukotriene B4, 5-LOX = 5-lipoxygenase

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content and increased linearly or quadratically serum LTB4 ($P = 0.045$, $P = 0.005$) concentration on day 28. Serum AA, PGE2, and LTB4 content of weaned piglets in 500 or 1000 mg/kg chitosan treatment were higher compared with other treatments.

Serum activity of cPLA2, COX-2, and 5-LOX. Chitosan increased serum cPLA2 activity in a linear dose-dependent manner ($P = 0.036$) on day 14 and a quadratic manner on day 28 ($P = 0.039$), and increased serum 5-LOX and COX-2 activity in a linear ($P = 0.040$) or quadratic ($P = 0.034$) dose-dependent manner on day 28 (Table 3).

Relative expression of cPLA2, COX-2, and 5-LOX mRNA in small intestine. With increasing chitosan, the *cPLA2* mRNA expression was enhanced quadratically in the duodenum ($P = 0.001$, $P < 0.0001$) on days 14 and 28, as well as in jejunum ($P = 0.003$) and ileum ($P = 0.027$) on day 14 (Table 4). Chitosan also increased the gene expression of *COX-2* mRNA in duodenum ($P = 0.002$, $P < 0.0001$) and jejunum ($P = 0.026$) in a linear

Table 4. Effects of chitosan on the gene expression of *cPLA2*, *COX-2*, and *5-LOX* mRNA in the small intestine of weaning piglets ($2^{-\Delta\Delta Ct}$) (data are means ± SE, n =6)

Items		Levels of dietary chitosan (mg/kg)					*P*-value	
		0	100	500	1000	2000	linear	quadratic
	Duodenum							
	14 days	1.044 ± 0.132	1.234 ± 0.133	1.339 ± 0.161	1.806 ± 0.183	0.917 ± 0.137	0.676	0.001
	28 days	1.024 ± 0.099	2.026 ± 0.138	2.277 ± 0.093	2.853 ± 0.209	1.842 ± 0.199	0.161	< 0.001
	Jejunum							
cPLA2	14 days	1.044 ± 0.136	1.455 ± 0.147	1.618 ± 0.173	2.128 ± 0.089	1.592 ± 0.043	0.097	0.003
	28 days	1.056 ± 0.158	1.990 ± 0.367	2.012 ± 0.337	2.309 ± 0.390	1.839 ± 0.236	0.315	0.073
	Ileum							
	14 days	1.030 ± 0.102	1.288 ± 0.098	1.635 ± 0.103	1.408 ± 0.134	1.309 ± 0.090	0.427	0.027
	28 days	1.047 ± 0.146	2.064 ± 0.096	1.767 ± 0.189	1.955 ± 0.277	1.779 ± 0.243	0.368	0.274
	Duodenum							
	14 days	1.025 ± 0.092	1.291 ± 0.106	1.784 ± 0.161	1.477 ± 0.132	1.356 ± 0.261	0.194	0.057
	28 days	1.105 ± 0.219	3.028 ± 0.101	4.247 ± 0.194	4.970 ± 0.231	4.180 ± 0.148	0.002	< 0.001
	Jejunum							
COX-2	14 days	1.043 ± 0.133	1.400 ± 0.137	1.390 ± 0.102	1.557 ± 0.226	1.479 ± 0.198	0.148	0.153
	28 days	1.071 ± 0.188	1.603 ± 0.108	2.243 ± 0.128	2.069 ± 0.222	2.049 ± 0.123	0.068	0.026
	Ileum							
	14 days	1.104 ± 0.219	1.276 ± 0.219	1.618 ± 0.112	1.498 ± 0.147	1.133 ± 0.155	0.822	0.070
	28 days	1.023 ± 0.094	1.740 ± 0.226	1.914 ± 0.126	1.582 ± 0.121	1.574 ± 0.138	0.475	0.148
	Duodenum							
	14 days	1.024 ± 0.105	1.390 ± 0.204	1.584 ± 0.195	1.603 ± 0.234	1.450 ± 0.184	0.272	0.109
	28 days	1.029 ± 0.114	3.326 ± 0.225	3.173 ± 0.220	2.794 ± 0.313	2.069 ± 0.121	0.916	0.020
	Jejunum							
5-LOX	14 days	1.022 ± 0.094	1.226 ± 0.085	1.270 ± 0.095	1.424 ± 0.168	1.222 ± 0.173	0.390	0.132
	28 days	1.021 ± 0.089	1.278 ± 0.141	2.137 ± 0.123	1.636 ± 0.222	0.806 ± 0.120	0.310	0.004
	Ileum							
	14 days	1.027 ± 0.104	1.426 ± 0.132	1.471 ± 0.116	1.551 ± 0.151	1.492 ± 0.122	0.084	0.045
	28 days	1.015 ± 0.083	1.453 ± 0.044	1.525 ± 0.126	1.449 ± 0.114	0.693 ± 0.063	0.079	0.012

cPLA2 = cytosolic phospholipase A2, COX-2 = cyclooxygenase-2, 5-LOX = 5-lipoxygenase

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or quadratic dose-dependent manner on day 28 (Table 4). Chitosan increased the gene expression of *5-LOX* mRNA in a quadratic dose-dependent manner in duodenum (P = 0.020) and jejunum (P = 0.004) on day 28, and increased it in a quadratic dose-dependent manner in ileum on day 14 (P = 0.045) and day 28 (P = 0.012) (Table 4). In addition, 500 and 1000 mg/kg chitosan treatments had higher *cPLA2*, *COX-2*, and *5-LOX* mRNA expression in the duodenum, jejunum, and ileum compared with the control and other treatments. However, the positive effects of chitosan tended to be suppressed when the additional dose of chitosan was increased to 2000 mg/kg.

DISCUSSION

AA, a precursor of eicosanoids that exist on the cell membrane in the form of phospholipids, is widely distributed in the body and belongs to n-6 series polyunsaturated essential fatty acids. In the normal physiological state, most AA in the body is esterified in the Sn-2 position of phospholipids present in the cell membrane and has no biological activity, while a small amount of AA present in the cytoplasm and body fluids in the free form has normal physiological activity (Li et al. 2012). AA can affect physical stability of cell membrane and activities and signalling pathways of membrane-associated enzymes by modifying fatty acid profiles of cell membrane, which subsequently alters immune functions of macrophages (Calder 2008). Furthermore, AA can be released from phospholipids mainly by cPLA2 under a variety of stimulations including lipopolysaccharides, cytokines, and nitric oxide (Piotrowska-Tomala et al. 2012), and is ultimately converted to PGs and LTs which inhibit inflammatory responses in favour of humoral responses (Tilley et al. 2001; Piotrowska-Tomala et al. 2012). In the present study, the AA concentration in the serum of piglets increased in a significant dose-dependent manner with increasing chitosan supplementation. At the same time, it was also found that chitosan improved the activity of cPLA2 in the serum and gene expression of the *cPLA2* in the small intestine in a significant dose-dependent manner. Li et al. (2009) indicated that immune functions improved by chitosan were associated with increased AA content and cPLA2 activity in serum as well as *cPLA2* mRNA expression in the small intestine of broilers. In addition, it is known that chitosan can adhere to the surface of the intestinal mucosa because of its positively charged amino groups binding with negatively charged sites on the cell surface. This can profoundly affect immune functions of the intestine (Porporatto et al. 2005). It may be speculated that chitosan adheres onto the surface of intestinal mucosa, and its amino groups enhance the gene expression and activity of cPLA2 in mucosa cells, and then increase the serum content of AA.

AA metabolites are key factors involved in the inflammatory process. Free AA in the body which is induced by the cPLA2 can be metabolized via two pathways: PG pathway and LOX pathway. In PG pathway, having been implicated as inflammatory mediators, PGs, particularly PGE2, are produced when COX acts on AA released from cell membranes. In LOX pathway, LOX-5 enzyme can lead to the formation of LTs, which also appear to be important inflammatory mediators (Kuehl and Egan 1980). The present results demonstrated that the PGE2 and LTB4 concentrations as well as the activity in serum and gene expression of *COX-2* and *LOX-5* in the small intestine were enhanced with increasing dietary chitosan. Usami et al. (1998) indicated that chitosan stimulated canine polymorphonuclear cells to release PGE2 and LTB4. Our previous study also showed that chitosan increased the concentrations of AA, PGE2, and LTB4, and improved the activities of cPLA2, COX-2, and 5-LOX as well as the expressions of *cPLA2*, *COX-2*, and *5-LOX* mRNA in peripheral blood lymphocytes of weaned piglets (Li et al. 2014). In the metabolism of AA, COX-2 and 5-LOX are important enzymes for the production of PGE2 and LTB4. The improved PGE2 and LTB4 contents in serum were associated with the increase of COX-2 and 5-LOX activity in serum as well as their mRNA expression in the small intestine. Another reason for the increasing of PGE2 and LTB4 contents in serum may be associated with the increase of AA content. In addition, AA-derived PGE2 could enhance chemotactic reaction of monocytes and neutrophils as well as modulate development and maturation of T cells (Tomobe et al. 2000; Mori and Beilin 2004). LTB4 could induce suppression of T cells and activate natural killer (NK) cells (Tang and Chen 2009). Several studies have shown that chitosan stimulates the migration of polymorphonuclear and mononuclear cells (Shibata et al. 1997a), and up-regulates the production of

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tumor necrosis factor, interleukin-1, and colony-stimulating factor by macrophages (Nishimura et al. 1986, 1987; Shibata et al. 1997b). Published evidence proved that chitosan is able to activate the macrophages for enhanced AA release response (Biancoa et al. 2000). Therefore, PGE2 and LTB4 may be direct participants in the regulation of the immune function by chitosan.

Traditionally, AA and its metabolites are considered as inflammatory mediators. However, studies have shown that AA and its metabolites are still very important intracellular secondary messengers involved in intracellular signal transduction and thus regulate cell activities (Graber et al. 1994; Meves 1994). Dirix et al. (2009) found that AA is considered to be essential in fetal development and some of its metabolites are thought to be important mediators of the immune responses. Our previous study indicated that chitosan could improve growth in weaned pigs (Xu et al. 2014), which might be a response to the properly improved metabolism of AA by chitosan. In addition, Chou et al. (2003) indicated that chitosan significantly inhibited the formation of PGE2 as well as COX-2 protein expression in LPS-treated RAW 264.7 macrophages, which suggested that chitosan could inhibit production of PGE2 by suppressing COX-2 induction. But our previous and present study showed that chitosan significantly increased the serum PGE2 production as well as *COX-2* gene expression in peripheral blood lymphocytes and intestine of piglets. The mechanism of these contradictory results has still been unknown and further researches are required.

Up to date, there has been no evidence showing that chitosan could be digested and absorbed in intestine of mammals, but it can adhere onto the surface of intestinal mucosa and may enhance the activity and gene expression of *cPLA2*, *COX-2*, and *5-LOX* in mucosa cells. Very few data have been reported on the effects of chitosan on the gene expression of key enzymes in AA metabolic network, thus, the exact mechanism for that chitosan affects the metabolism of AA in gastrointestinal tract is unclear. It may be hypothesized as follows: on the one hand, chitosan, as a cationic macromolecule, can link with the anionic components of the proteins on the epithelial cell surface. This interaction may alter the relative concentration of ions within the hydrated interior channels of tight junctions, and increase the epithelial permeability (Artursson et al. 1994; Illum 1998). Therefore, chitosan has been shown to considerably improve the absorption of low molecular weight compounds and large hydrophilic macromolecules (such as mannitol, polyethylene glycol, 9-desglycinamide, 8-L-arginine vasopressin, peptides, and fluorescein dextrans) by increasing their transcellular and/or paracellular transport across mucosal surfaces (Luesen et al. 1997; Illum 1998; Kotze et al.1998), which may regulate the metabolism of AA and its metabolites. On the other hand, chitosan can activate PKC-dependent signal transduction pathways of epithelial cell (Smith et al. 2005), which may regulate the gene expression of key enzymes in AA metabolic network. Interestingly, in the present study, AA metabolites and regulatory enzyme are not always enhancing with the increasing addition of chitosan. For example, the content of LTB4 in serum and the gene expression of *cPLA2* in duodenum, the gene expression of *5-LOX* in jejunum and ileum of 2000 mg/kg treatment were lower than in the control group. The reason might be massive ingestion of chitosan increased the viscosity of the intestinal content and slowed the diffusion of nutrients, which resulted in a highly effective increase in the excretion of nutrients, especially adipose and fat-soluble vitamins, and caused a shift in nutrient partitioning away from gene expression toward other metabolic responses. In addition, the content of PGE2 and activity of regulatory enzyme (cPLA2, COX-2, and 5-LOX) in serum, and gene expression of *5-LOX* in small intestine were not increased over the time. The causes of this result are unclear.

CONCLUSION

In conclusion, chitosan can regulate the contents of AA, PGE2, and LTB4, and the activities of cPLA2, COX-2, and 5-LOX in the serum as well as their gene expression in the small intestine of weaned piglets in a dose-dependent manner. Our results may provide new mechanism by which chitosan accounts for its beneficial effect on immune function via AA pathway in weaned piglets.

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