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Effects of Dietary Supplementation of Chitosan on Stress Hormones and Antioxidative Enzymes in Weaned Piglets

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Abstract: This experiment was conducted to investigate the effects of dietary supplementation of chitosan on stress hormones and antioxidative enzymes in weaned piglets. One hundred and eighty piglets weaned at 28 days (Duroc x Large white x Landrace) were assigned randomly to 5 dietary treatments with 6 repetitions in each treatment. The piglets in the 5 treatments were fed on the basal diet supplemented with 0 (control), 100, 500, 1000 and 2000 mg chitosan/kg feed. Results showed that dietary chitosan decreased serum Adrenocorticotropic Hormone (ACTH) concentrations in a linear dose-dependent manner ($p<0.05$) on day 28 and declined serum Cortisol (COR) concentrations in a linear or quadratic dose-dependent manner ($p<0.05$) on day 14 and 28. With increasing chitosan, serum Glutathione Peroxidase (GSH-Px) was enhanced both in a quadratic manner ($p<0.05$) on day 14 and a linear manner on day 28 ($p<0.05$) and Serum Glutathione Peroxidase (SOD) and Catalase (CAT) were improved quadratically on day 28 ($p<0.05$). These results implied that dietary chitosan mitigated weaning stress and improved the activity of antioxidative enzymes in weaned piglets.

Key words: Chitosan, weaned piglet, stress hormone, antioxidative enzyme, stress

INTRODUCTION

In modern pig husbandry, early weaning was one of the most stressful events that resulted in intestinal, immunological and behavioral changes. During this period, pigs are subjected to a number of stressors such as an abruptly separation from the sow, diet switched, unfamiliar pigs in a new environment (Dudink *et al*., 2006). As a consequence, feed intake, growth and immune function were reduced and weaning syndrome occured in piglets. Campbell *et al*. (2013) indicated that weaning stress in pigs could contribute to intestinal and immune system dysfunctions that resulted in the reduction of pig health, growth and feed intake. Moeser *et al*. (2007) found that stress signaling pathways activated by weaning mediated intestinal dysfunction in piglets. Traditionally, determination of Hypothalamus Pituitary Adrenal (HPA) axis activity is the standard procedure to evaluate stress conditions in farm animals (Mormede *et al*., 2007), because the release of Adrenocorticotropin Hormone (ACTH) and Cortisol (COR) concentrations were increased via HPA axis in animals or human under stress conditions (Gaillet *et al*., 1991; Fisher *et al*., 1996). Plasma ACTH and/or COR were measured to identify and quantify tress or acute pain related to tail docking and/or castration in lambs and pigs (Mellor and Murray, 1989; Prunier *et al*., 2005). Moreover, Yang (1989) demonstrated that stress results in a decrease in intracellular anti-oxidant ability and an increase in the production of Reactive Oxygen Species (ROS). Leng and Zhu (2010) reported that the levels of SOD and GSH-Px were significantly decreased by weaning stress in piglets. The free radicals would not be cleared in time due to the decrease of these antioxidant enzymes activities which would destroy cell embrane structure or cause some diseases in weaned piglets.

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). It possesses many beneficial biological properties such as antimicrobial activity, immune stimulation activity, biocompatibility, biodegradability and wound healing property (Yen *et al*., 2008). Moreover, mitigating stress and improving antioxidant activity are well-known function of chitosan. The earlier studies found that dietary supplementation with chitosan decresed the level of ACTH or COR in broilers (Shi, 2005; Li, 2009). Many studies have shown that chitosan inhibit ROS and prevent the lipid oxidation in food and biological systems. Anandan *et al*. (2013) indicated chitosan significantly attenuated the oxidative stress in the heart tissue of aged rats through the counteraction of free radical formation by maintaining the enzymatic

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(glutathione peroxidase, glutathione reductase) and nonenzymatic (reduced glutathione) status at levels comparable to that of normal young rats. Yang *et al.* (2009) have reported that chitosan enhanced resistance to oxidative stress caused by drought stress in crop plants.

In piglets however, there were limited studies available in evaluating the effect of chitosan on stress hormones and antioxidative enzymes. Therefore, the present study aimed to investgate the effect of chitosan on the stress hormones and antioxidative enzymes of weaned piglets. This finding may provide a useful evidence for the application of chitosan in diets to mitigate stress and improve antioxidant activity in weaned piglets.

MATERIALS AND METHODS

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

Experimental design and animals management: A total of 180 healthy piglets (DurocxLarge whitexLandrace) with an initial average body weight of 7.6 kg (at weaning) were assigned randomly to 5 treatments with 6 repetitions (3 pens of males and 3 pens of females) in each treatment with 6 piglets in each pen (4.0×4.2 m^2). The formation of experimental diets referenced to the Nutrient Requirements Standards of NRC (1998) (Table 1). All diets were offered in meal form. Five dietary treatments supplemented with 0 (control), 100, 500, 1000 or 2000 mg chitosan/kg feed on the basal diet, respectively. Chitosan used in this trial was provided by Jinan Haidebei Marine Bioengineering Limited Company. The deacetylation degree of chitosan was determined to be 85.09% and the viscosity was 45 cps.

Table 1: Composition and nutrient levels of the basal diet (air dry basis, %)

Ingredients	Content	Nutrients	Level
Corn	51.9	Digestible energy (MJ kg^{-1})	14.32
Soybean meal	16.0	Crude protein	20.02
Wheat	20.0	Crude fat	3.00
Fish meal	2.5	Crude fibre	4.20
Corn gluten meal	2.0	Calcium	0.72
Whey powder	2.0	Phosphorus	0.56
Soya bean oil	2.0	Lysine	1.35
Limestone	0.7	Methionine+Cystine	0.82
$CaHPO_4$	1.0	Threonine	0.74
NaCl	0.3		
Premix[1]	1.6		
Total	100.0		

[1]The premix provides following nutrients per kg diet: Vitamin A 16, 000 IU; Vitamin D3 2500 IU; Vitamin E 60 IU; Vitamin K3 4.5 mg; Vitamin B1 2.6 mg; Vitamin B2 8.7 mg; Vitamin B6 7.0 mg; Vitamin B12 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; Iodin 0.35 mg; Cobalt 0.2 mg; Selenium 0.25 mg; Choline chloride 750 mg; Phytase 500 FTU

Piglets were weaned at the age of 28 days, penned in a temperature-controlled nursery building where temperature was maintained 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 was 28 days. Feed and water were freely available during the experimental period.

Sample collection: On day 14 and 28 of experiment, 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 3,000×g for 10 min at 4°C to yield serum. Serum was stored at -20°C until analysis of concentrations of ACTH and COR as well as activity of GSH-PX, SOD and CAT.

Determination of ACTH and COR contents: Plasma ACTH concentrations were determined using a commercially available RIA kit (Beijing sino-uk institute of biological technology, China) according to the manufacturer's instructions. The quantification limit of the assay was 5 pg mL^{-1} plasma and the intra and interassay CV were 3.0 and 7.8%, respectively. Plasma cortisol concentrations were determined using a commercially available RIA kit (Beijing sino-uk institute of biological technology, China). The intra and interassay CV for samples were 3.8 and 10.7%, respectively.

Assay for antioxidative enzymes activity: Serum GSH-Px enzyme activity was determined using a commercially available assay kit A005 (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. The GSH-Px activity was expressed as units mL serum and 1 unit was defined as a decrease in GSH of 1 mM per minute after the decrease in GSH per minute of the non-enzymatic reaction was subtracted. SOD was determined by the method described by Misra and Fridovich (1972) and CAT was estimated by the method described by Sinha (1972).

Statistical analysis: Regression analysis was conducted to evaluate linear and quadratic effects of chitosan on the various response criteria in piglets by using the SAS software (SAS, 2003). Differences were considered significant if probability values of $p<0.05$ were obtained.

RESULTS

Serum ACTH and COR: With increasing addition of chitosan, serum ACTH concentrations were declined in a linear dose-dependent manner ($p<0.05$) on day 28 and

Table 2: Effects of chitosan on the level of stress hormones in serum of weaned piglets

	Level of chitosan (mg kg^{-1})						p-value	
Items	0	100	500	1000	2000	SEM	Linear	Quadratic
ACTH (pg mL^{-1})								
14 days	31.57	25.37	26.47	29.23	25.98	1.471	0.355	0.655
28 days	26.84	25.09	23.94	23.88	22.30	1.315	0.026	0.070
COR (ng mL^{-1})								
14 days	117.26	109.27	104.63	109.01	108.85	2.396	0.229	0.047
28 days	116.86	115.19	112.48	111.94	108.25	2.567	0.016	0.036

ACTH = Adreno-Cortico-Tropic-Hormone. COR = Cortisol. SEM = Standard Error of the Mean; $p<0.05$ means significant regression relation; $p<0.01$ means extremely significant regression relation

Table 3: Effects of chitosan on the level of antioxidative enzymes acitivity in serum of weaned piglet

	Level of chitosan (mg kg^{-1})						p-value	
Items	0	100	500	1000	2000	SEM	Linear	Quadratic
GSH-PX (U mL^{-1})								
14 days	742.61	727.17	801.86	816.78	754.80	24.651	0.410	0.033
28 days	697.77	762.45	845.31	739.93	703.19	25.405	0.039	0.090
SOD (U mL^{-1})								
14 days	50.55	54.82	55.32	55.10	51.86	1.744	0.802	0.227
28 days	50.05	50.42	55.81	59.27	55.52	2.818	0.111	0.040
CAT (U mL^{-1})								
14 days	51.82	54.47	57.23	56.92	55.85	1.742	0.191	0.097
28 days	50.91	52.92	56.41	60.46	52.35	1.573	0.812	0.041

GSH-PX = Glutathione Peroxidase. SOD = Superoxide Dismutase. CAT = Catalase. SEM = Standard Error of the Mean; $p<0.05$ means significant regression relation, $p<0.01$ means extremely significant regression relation

serum COR concentrations were decreased in a linear or quadratic dose-dependent manner ($p<0.05$) on day 14 and 28 (Table 2).

Serum antioxidative enzymes activity: Chitosan enhanced serum GSH-Px enzymes activity both in a quadratic manner ($p<0.05$) on day 14 and a linear manner on day 28 ($p<0.05$) and improved quadratically the activity of serum SOD and CAT enzymes on day 28 ($p<0.05$) (Table 3). In addition, piglets fed diets containing 500 and 1000 mg kg^{-1} chitosan had higher activity of serum GSH-Px, SOD and CAT compared with those in other treatments. However, positive effects of chitosan tended to be suppressed when the additional dose of chitosan was increased to 2000 mg kg^{-1}.

DISCUSSION

The HPA axis is a stress-responsive neuroendocrine system of organism that connects the central nervous system with the hormonal system. Under stress, the hypothalamus secretes corticotropin releasing hormone and this provokes the release of ACTH from the pituitary. ACTH triggers the secretion of glucocorticoids from the adrenal cortex (Kudielka and Kirschbaum, 2005). Therefore, determination of HPA axis activity is the standard procedure to evaluate stress conditions in farm animals (Mormede *et al*., 2007). Shi (2005) found that chitosan could inhibit increase of COR induced by LPS in broilers. Li (2009) indicated dietary supplementation of chitosan decreased serum ACTH and COR concentrations in broilers. the results agreed with these of earlier studies. In present study, dietary supplied with appropriate amount of chitosan decreased serum ACTH and COR concentrations in weaned piglets. However, serum ACTH and COR contents in piglets tended to increase when the additional dose of chitosan was increased to 2000 mg kg^{-1} which suggested chitosan mitigated stress in a dose-dependent manner. Although, this mechanism is still not clear, decrease of serum ACTH and COR concentrations in piglets implied that organism could save some energy was used to resist stress and more energy might be used to improve growth or health.

ROS are produced by abnormal metabolic processes as well as sunlight, ultraviolet light and chemical reactions and can change the structure of DNA, membrane lipids and protein which may result in disease such as cancer, aging, inflammation and atherosclerosis (Moskovitz *et al*., 2002). Cells are possessed of enzymatic system including SOD, GSH-Px, CAT and so on, as a part of the cellular defense system to preserve stability between oxidant and antioxidant (Rosen and Rauckman, 1984). The antioxidant enzymes SOD, GSH-Px and CAT play an important role as protective enzymes against reactive oxtgen species in tissues and comprise the cellular antioxidant defense system (Huang *et al*., 2005). In the study, with increasing chitosan, the activity of serum GSH-Px, SOD and CAT was enhanced in a dose-dependent manner in piglets. Anandan *et al*. (2013) indicated chitosan significantly attenuated the oxidative stress in the heart tissue of aged

rats through the counteraction of free radical formation by maintaining the enzymatic (glutathione peroxidase, glutathione reductase) and nonenzymatic (reduced glutathione) status at levels comparable to that of normal young rats. Yen *et al.* (2007) demonstrated that chitosan gallate stimulated the protein expression of antioxidant enzymes such as SOD-1 and GSR. Tao *et al.* (2013) reported chitosan and water-soluble chitosan increased the serum SOD levels significantly. Qiao *et al.* (2011) found that administration of chitosan prevented LPS-induced lipid peroxidation and reserved depletion of anti-oxidants including GSH levels and CAT activity. The findings of the current study were similar with the previous studies. However, the mechanisms of chitosan increasing the activity of serum GSH-Px, SOD and CAT was not clear this improvement of those enzymes could scavenge free radicals and preserve stability between oxidant and antioxidant more effectively.

In addition, some recent researches showed the antioxidant activities of chitosan and its derivatives can react with free radicals since, chitosan chains have active hydroxyl and amino groups (Huang *et al.*, 2005; Fenga *et al.*, 2008). Hydroxyl and amino groups of chitosan can react with unstable free radicals which protected cells from damage. Although, the level of free radicals in pigs was not measured in this tudy, researchers speculated that chitosan might improve antioxidant activity of piglet via following pathways: improved activity of antioxidant enzymes activity and /or scavenged the free radicals by itself. So, more researches were needed to explore its antioxidant mechanisms further.

CONCLUSION

It is concluded from this study that chitosan mitigated weaning stress and improved antioxidative enzymes activity in weaned piglets. The diets containing 500-1000 mg kg^{-1} chitosan had a better regulative action on stress hormones and antioxidative enzymes activity in piglets compared with other dietary treatments. However, this positive effect of chitosan tended to be suppressed when the additional dose of chitosan was increased to 2000 mg kg^{-1} which suggested that the effect of chitosan on the stress hormones and antioxidative enzymes activity was dose-dependent in weaned piglets.

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REFERENCES

Anandan, R., B. Ganesan, T. Obulesu, S. Mathew, K.K. Asha, P.T. Lakshmanan and A.A. Zynudheen, 2013. Antiaging effect of dietary chitosan supplementation on glutathione-dependent antioxidant system in young and aged rats. Cell Stress Chaperones, 18: 121-125.

Campbell, J.M., J.D. Crenshaw and J. Polo, 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol., Vol. 4. 10.1186/2049-1891-4-19.

Dudink, S., H. Simonse, I. Marks, F .H. de Jonge and B.M. Spruijt, 2006. Announcing the arrival of enrichment increases play behaviour and reduces weaning-stress-induced behaviours of piglets directly after weaning. Applied Anim. Behav. Sci., 101: 86-101.

Fenga, T., Y. Du, J. Li, Y. Hu and J.F. Kennedy, 2008. Enhancement of antioxidant activity of chitosan by irradiation. Carbohydr. Polym., 73: 126-132.

Fisher, A.D., M.A. Crowe, M.E.A. de la Vega and W.J. Enright, 1996. Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth and feed intake of bull calves. J. Anim. Sci., 74: 2336-2343.

Gaillet, S., J. Lachuer, F. Malaval, I. Assenmacher and A. Szafarczyk, 1991. The involvement of noradrenergic ascending pathways in the stress-induced activation of ACTH and corticosterone secretions is dependent on the nature of stressors. Exp. Brain Res., 87: 173-180.

Huang, R., E. Mendis and S.K. Kim, 2005. Factors affecting the free radical scavenging behavior of chitosan sulfate. Int. J. Biol. Macromol., 36: 120-127.

Kudielka, B.M. and C.K. Kirschbaum, 2005. Clemens Kirschbaum, Sex differences in HPA axis responses to stress: A review. Biol. Psychol., 69: 113-132.

Leng, J. and R.J. Zhu, 2010. Effect of weaning stress in different ages on serum antioxidant enzymes in piglet. Guandong Agric. Sci., 2: 125-127.

Li, H.Y., 2009. Effects of chitosan on immune function in broilers chickens and the underlying molecular mechanisms. Ph.D. Thesis, Inner Mongolia Agricultural University, Huhhot, Inner Mongolia.

Mellor, D.J. and L. Murray, 1989. Changes in the cortisol responses of lambs to tail docking, castration and ACTH injection during the first seven days after birth. Res. Vet. Sci., 46: 392-395.

Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.

Moeser, A.J., C.V. Klok, K.A. Ryan, J.G. Wooten, D. Little, V.L. Cook and A.T. Blikslager, 2007. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. Am. J. Physiol. Gastrointest. Liver Physiol., 292: 173-181.

Mormede, P., S. Andanson, B. Auperin, B. Beerda and D. Guemene *et al*., 2007. Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. Physiol. Behav., 92: 317-339.

Moskovitz, J., K.A. Yim and P.B. Chok, 2002. Free radicals and disease. Arch. Biochem. Biophys., 397: 354-359.

NRC, 1998. Nutrient Requirements of Swine. 10th Edn., National Research Council, Washington, DC., USA.

Prunier, A., A.M. Mounier and M. Hay, 2005. Effects of castration, tooth resection, or tail docking on plasma metabolites and stress hormones in young pigs. J. Anim. Sci., 83: 216-222.

Qiao, Y., X.F. Bai and Y.G. Du, 2011. Chitosan oligosaccharides protect mice from LPS challenge by attenuation of inflammation and oxidative stress. Int. Immunopharmacol., 11: 121-127.

Rosen, G.M. and E.J. Rauckman, 1984. Spin trapping of superoxide and hydroxyl radicals. Methods Enzymol., 105: 198-209.

SAS, 2003. SAS User's Guide: Statistics. Version 9.0. SAS Institute, Cary, North Carolina.

Shi, B.L., 2005. Effects of chitosan on growth performance and immune function in broilers and the underlying mechanisms. Ph.D. Thesis, China Agricultural University, Beijing.

Sinha, A.K., 1972. Colorimetric assay of catalase. Anal. Biochem., 47: 389-394.

Synowiecki, J. and N.A. Al-Khateeb, 2003. Production, properties and some new applications of chitin and its derivatives. Crit. Rev. Food. Sci. Nutr., 43: 145-171.

Tao, Y., H.L. Zhang, Y.M. Hu, S. Wan and Z.Q. Su, 2013. Preparation of chitosan and water-soluble Chitosan microspheres via spray-drying method to lower blood lipids in rats fed with high-fat diets. Int. J. Mol. Sci., 14: 4174-4184.

Yang, F., J. Hu, J. Li, X. Wu and Y. Qian, 2009. Chitosan enhances leaf membrane stability and antioxidant enzyme activities in apple seedlings under drought stress. Plant Growth Regul., 58: 131-136.

Yang, Y., 1989. The mechanism and meaning of oxygen free radical produced by lick-up cells. Chin. Physiol. Sci., 9: 10-13.

Yen, M.T., J.H. Yang and J.L. Mauc, 2008. Antioxidant properties of chitosan from crab shells. Carbohydr. Polym., 74: 840-844.

Yen, M.T., Y.H. Tseng, R.C. Li and J.L. Mau, 2007. Antioxidant properties of fungal chitosan from shiitake stipes. LWT Food Sci. Technol., 40: 255-261.