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Effects of dietary supplementation of chitosan on immune and antioxidative function in beef cattle

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ABSTRACT: The present experiment was conducted to study the effects of chitosan (CHI) on immune and antioxidative function in beef cattle. A total of 24 fattening Simmental cattle with similar body weight and age were divided randomly into three dietary groups, and the three diets contained 0, 500, and 1000 mg/kg CHI, respectively. The feeding trial lasted for 84 days. It was found that: (1) the addition of CHI in diets improved ($P < 0.05$) the levels of IgA and interleukin-1, and decreased ($P < 0.1$) the levels of soluble cluster of differentiation 4 receptor in serum at middle stage except that IgA remained unchanged in 1000 mg/kg CHI group. The levels of IgM and IgA tended to be increased ($P < 0.1$) by dietary CHI at later stage of the experiment; (2) the addition of 500 mg/kg CHI in diets increased ($P < 0.1$) total superoxide dismutase activity and decreased ($P < 0.05$) malondialdehyde content in serum at early and later stages, respectively. In conclusion, these results indicated that addition of 500 mg/kg CHI affected humoral and cellular immune responses, and improved the antioxidative function of beef cattle.

Keywords: dietary chitosan; Simmental cattle; humoral immunity; antioxidative enzyme; malondialdehyde

INTRODUCTION

Previous research indicated that chitosan (CHI), a natural and non-toxic alkaline polysaccharide, could stimulate the production of antibodies in the blood circulation because its amino groups could be recognized by the immune system (Tokura et al. 1999). Some studies suggested that CHI possesses some characteristics such as immune enhancing anti-inflammatory effects and antimicrobial activities, and could be used as an immunostimulant for animals (Siwicki et al. 1994; Yoon et al. 2008; Kong et al. 2014). David et al. (2007) indicated that CHI as an adjuvant enhanced significantly serum IgG titers in mice. Liu et al. (2007) conducted a feeding trial using dried cows and showed the similar result. Our previous study showed that CHI improved the humoral and cellular immune functions in broilers (Li 2009) and weaned piglets (Li et al. 2013).

In addition, the antioxidative capacity of CHI has been given much concern. It showed the effects of scavenging free radicals and protecting the body against damage from peroxides (Tomida et al. 2009). CHI connecting with some groups (Guo et al. 2008; Sun et al. 2008) or being absorbed into nanoparticles (Esumi et al. 2003) exhibited a better antioxidative activity than a single component. Ren (2008) showed that CHI effectively increased the activity of total superoxide dismutase (T-SOD) and total anti-oxidant capability (T-AOC), and enhanced the antioxidative function of dairy cattle. Liu et al. (2011) showed that dietary supplementation with suitable dose of CHI could improve the antioxidative function of broilers. Anandan et al. (2013) suggested that CHI could be an effective therapeutic agent in the treatment of age-associated disorders in rats where oxidative stress was the major causative factor.

Up to now, numerous studies have been focussed on application of CHI in monogastric animals, however, the effects of CHI on immune and antioxidative function in beef cattle have still remained

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unclear. Therefore, our study was conducted to determine the effects of CHI on the immune and antioxidative function in beef cattle and provided the theoretical basis for scientific adding of CHI in diets of beef cattle.

MATERIAL AND METHODS

The protocol of the present experiment was approved by the Animal Care and Use Committee, Inner Mongolia Agricultural University, Huhhot, China.

Experimental animals and material. Healthy fattening Simmental cattle were obtained from Kanghui Beef Farm (Hohhot, Inner Mongolia, P.R. China). The CHI used in this trial was provided by Haidebei Marine Bioengineering Co., Ltd. (Jinan, Shandong, P.R. China) and its deacetylation degree was determined to be 85.09%, and the viscosity was 45 cps (molecular-weight ca. 40–60 kDa). All assay kits were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, P.R. China).

Experimental design and animal management. A total of 24 beef bulls with similar body weight and age were allocated to three dietary groups, and were fed the basal diet supplemented with 0, 500, and 1000 mg/kg CHI, respectively. Each treatment contained eight replicates. The period of the experiment lasted 84 days which were divided into three phases per 28 days each. The basal diet was formulated referring to the nutrient requirements suggested by NRC (2000), with no chitosan (Table 1). During the whole experimental period, the feeding and management condition of the three groups was consistent. Feed and water were provided *ad libitum* throughout the experiment.

Sample collection. On the last day of each phase, 30 ml blood samples were collected through puncturing the tail vein from each experimental animal. Blood samples were allowed to clot for 4 h at room temperature, and then were centrifugated at 1790 *g* for 10 min at 4°C, and the serum was removed and stored at –20°C until analysis of immune and antioxidative parameters.

Determination of immunoglobulins, cytokines, sCD4, and sCD8 in serum. The contents of immunoglobulin (IgG, IgA, and IgM) were determined by bovine ELISA kits, as described previously (Frey et al. 1998). Briefly, the wells on 96-well plate were coated with purified bovine immunoglobulin antibody which was formed into solid-phase antibody. Diluted serum was added to the well, and the immunoglobulin in serum was combined with immunoglobulin antibody labelled with Horse Radish Peroxidase (HRP) and became antibody-antigen-enzyme-antibody complex. TMB (3,3', 5,5'-tetramethylbenzidine) was added to the wells after washing and incubation for coloration. The reaction was terminated by the addition of a sulphuric acid solution. The colour was measured at a wavelength of 450 nm with microplate reader (BioTek Instruments, Inc., Winooski, USA). The concentrations of serum immunoglobulins were calculated by comparing the optical density value to the standard curve. The minimum detectability of IgG, IgA, and IgM was 40, 5, and 8 mg/l, respectively.

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

Ingredients	Early and middle stages (1–56 days)	Later stage (57–84 days)
Hay	54.00	53.00
Corn	27.53	30.48
Corn gluten meal	3.23	3.97
Rapeseed meal	3.22	2.35
Cottonseed meal	10.00	5.12
Soybean meal		3.51
Urea	0.23	
Limestone	0.64	0.60
Dicalcium phosphate	0.14	0.27
NaCl	0.32	0.47
Sodium bicarbonate	0.32	
Magnesium chloride	0.14	
Premix[1]	0.23	0.23
Total	100	100
Nutrients[2]		
NE (MJ/kg)	7.44	7.38
CP	13.12	11.77
NDF	65.78	67.49
ADF	27.38	31.16
Ca	0.93	0.56
P	0.35	0.30

NE = net energy, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre

[1]contents of nutrients per kg diet: vitamin A 9000 IU, vitamin D 2000 IU, vitamin E 16 IU, Fe 50 mg, Cu 10 mg, Zn 30 mg, Mn 20 mg, Co 0.1 mg, I 0.5 mg, Se 0.1 mg

[2]net energy was calculated according to NRC (2000) regulation for beef cattle, and other nutrient compositions were analyzed

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The cytokines interleukin (IL)-1, IL-2, and tumour necrosis factor α (TNF-α) were measured using a commercially available bovine ELISA kits according to the manufacturer's instructions. The minimum detectability of IL-1, IL-2, and TNF-α was 3, 10, and 30 pg/l, respectively. The soluble cluster of differentiation 4 receptor (sCD4) and sCD8 were determined by bovine ELISA kits and the assay ranges were 45–1800 and 16–520 ng/l, respectively. Each of the experiments was performed in duplicate.

Determination of the activities of antioxidative enzymes. The antioxidative parameters were measured with the method of assay kits: the thiobarbituric acid method at 532 nm for malondialdehyde (MDA) levels, the ferric reducing/antioxidant power assay method at 520 nm for T-AOC, the xanthine oxidase method at 550 nm for SOD activity, and the ammonium molybdate method at 405 nm for catalase (CAT) activity in the serum. The OD values were detected using a 722s spectrophotometer (Precision Instrument Co., Ltd., Shanghai, China). Each of the parameters was measured in triplicate. Enzyme activity was expressed as activity unit per 1 ml serum.

Statistical analysis. The data from the trials were analyzed by ANOVA, using the General Linear Models (GLM) procedure of SAS software (Statistical Analysis System, Version 9.2., 2003). Means were compared by the Duncan's multiple range tests. A value of $P < 0.05$ was considered to be statistically significant, whereas $P < 0.10$ was considered to constitute a tendency.

RESULTS

Effects of CHI on immune indices. In general, the levels of IgG, IgA, and IgM in serum of CHI-treated groups, especially the treatment of 500 mg/kg CHI, showed higher scores than those of the control group during the whole period (Table 2). In the middle stage of experiment, the IgA of the 500 mg/kg CHI-treated group was significantly higher than that of the control group ($P < 0.05$) and of the 1000 mg/kg CHI-treated group ($P < 0.05$), but no difference was observed between the latter two groups. In the later stage of experiment, the levels of IgM and IgA in the 500 mg/kg CHI-treated group were higher compared with the control group, and the differences tended to be significant ($P < 0.1$). In addition, dietary supplementation with CHI significantly raised the level of IL-1 compared with the control in mid-experiment ($P < 0.05$) (Table 3), whereas the IL-2 and TNF-α levels exhibited no significant difference among treatments ($P > 0.10$).

As shown in Table 3, the level of sCD4 in CHI-treated groups was higher compared with the control group, and the difference tended to be significant ($P < 0.1$) at the middle stage.

Effects of CHI on the activities of antioxidative enzymes. Dietary supplementation with CHI decreased the content of MDA in serum, especially in the 500 mg/kg CHI-treated group, and significant differences were detected between the 500 mg/kg CHI-treated group and the control group at the middle stage ($P < 0.05$) (Table 4).

Table 2. Effects of chitosan (CHI) on the levels of immunoglobulins in beef cattle

Items	Stages	Levels of CHI (mg/kg)			SEM	*P*-value
		0	500	1000		
IgG (g/l)	early stage	16.24	16.68	16.35	0.367	0.632
	middle stage	16.24	16.92	16.89	0.620	0.676
	later stage	16.47	17.39	16.80	0.460	0.420
IgA (g/l)	early stage	0.98	1.09	1.08	0.073	0.494
	middle stage	0.87[b]	1.09[a]	0.92[b]	0.051	0.015
	later stage	0.86	1.04	0.96	0.049	0.076
IgM (g/l)	early stage	1.54	1.75	1.74	0.184	0.660
	middle stage	1.61	1.94	1.88	0.165	0.352
	later stage	1.49	1.87	1.82	0.115	0.091

Ig = immunoglobulin, SEM = standard error of the mean, early stage = days 1–28, middle stage = days 29–56, later stage = days 57–84

[a,b]data with different superscripts within the same row are significantly different ($P < 0.05$)

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Table 3. Effects of chitosan (CHI) on the concentrations of sCD4, sCD8, and cytokines in the serum of beef cattle

Items	Stages	Levels of CHI (mg/kg)			SEM	*P*-value
		0	500	1000		
IL-1 (ng/ml)	early stage	0.26	0.29	0.27	0.015	0.221
	middle stage	0.24[b]	0.28[a]	0.30[a]	0.012	0.044
	later stage	0.25	0.26	0.25	0.013	0.512
IL-2 (ng/ml)	early stage	6.78	6.99	7.06	0.623	0.938
	middle stage	5.56	6.31	5.91	0.486	0.556
	later stage	6.94	7.79	8.00	0.581	0.479
TNF-α (ng/ml)	early stage	1.90	1.97	1.93	0.090	0.841
	middle stage	2.09	2.13	1.93	0.114	0.629
	later stage	2.01	2.08	1.96	0.192	0.900
sCD4 (U/ml)	early stage	83.54	82.50	77.68	4.417	0.643
	middle stage	88.49	79.95	78.09	3.361	0.080
	later stage	84.04	76.04	81.84	2.709	0.152
sCD8 (U/ml)	early stage	43.77	46.64	43.17	2.505	0.557
	middle stage	45.58	43.41	45.21	1.738	0.646
	later stage	44.37	42.48	40.60	2.961	0.673

IL = interleukin, TNF-α = tumour necrosis factor α, sCD4(8) = soluble cluster of differentiation 4(8) receptor, early stage = days 1–28, middle stage = days 29–56, later stage = days 57–84

[a,b]data with different superscripts within the same row are significantly different ($P < 0.05$)

The T-SOD activity was significantly reduced by the supplementation with 1000 mg/kg CHI at the early stage ($P < 0.05$), but treatment at 500 mg/kg had no such effect. Both the 500 and 1000 mg/kg treatments increased the T-SOD activities to a certain extent compared with the control at the later stage of experiment ($P < 0.1$) (Table 4). Dietary supplementation with CHI had no effect on the T-AOC, but the average activities of CAT were by 7.2–18.2% higher for the 500 mg/kg CHI treat-

Table 4. Effects of chitosan (CHI) on antioxidative activity in the serum of beef cattle

Items	Stages	Levels of CHI (mg/kg)			SEM	*P*-value
		0	500	1000		
MDA (U/ml)	early stage	3.14[a]	1.79[b]	2.46[ab]	0.380	0.031
	middle stage	2.48	1.83	2.44	0.244	0.169
	later stage	3.78	2.76	3.17	0.586	0.493
T-SOD (U/ml)	early stage	130.43[a]	130.41[a]	108.99[b]	4.982	0.028
	middle stage	124.55	131.80	129.37	5.735	0.654
	later stage	100.22	124.67	120.95	6.751	0.059
T-AOC (U/ml)	early stage	3.26	2.94	3.13	0.280	0.670
	middle stage	2.94	3.17	3.91	0.369	0.344
	later stage	3.33	3.49	3.85	0.570	0.727
CAT (U/ml)	early stage	3.18	3.76	3.01	0.386	0.308
	middle stage	2.99	3.42	3.30	0.220	0.406
	later stage	3.75	4.02	3.19	0.448	0.478

MDA = malondialdehyde, T-SOD = total superoxide dismutase, T-AOC = total anti-oxidant capability, CAT = catalase, early stage = days 1–28, middle stage = days 29–56, later stage = days 57–84

[a,b]data with different superscripts within the same row are significantly different ($P < 0.05$)

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ments compared with the control, although the differences were not significant ($P > 0.05$) (Table 4).

DISCUSSION

Dietary immunomodulators (e.g. CHI) were recognized as indirect growth promoters by enhancing immune function of animals. The published data showed that CHI was helpful in improving the growth of beef cattle (Yu et al. 2012). To investigate the effects of CHI on immune functions, the levels of immunoglobulins and cytokines in serum were determined in the experiment. The results showed that CHI dose of 500 mg/kg increased remarkably serum IgA, and improved IgM to a slight extent, however, the effects were weakened with increasing the dose of CHI.

Specific immunoglobulins in combination with lymphokines could indicate collectively the status of humoral immunity playing essential role in protecting against bacterial as well as viral infections (McKee et al. 2007). Amino groups of CHI could be recognized by the immune system, then stimulate immune cells to proliferate and differentiate, and to release immunoglobulins (Tokura et al. 1999). Lim et al. (1997) showed that concentrations of IgG, IgA, and IgM in mesenteric lymph node (MLN) lymphocytes were generally higher in rats fed CHI than in those fed cellulose. Furthermore, dietary supplementation of CHI in piglets increased serum immunoglobulins and recouped a loss which was caused by early weaning (Yin et al. 2008; Li et al. 2013). Xiao et al. (2013) added 50 mg/kg chlortetracycline or 300 mg/kg CHI in the diet of weaned piglets and showed that the expression of jejunal mucosal secretory IgA (sIgA) protein was higher in the CHI group than in both control and chlortetracycline groups, which indicated CHI could improve the intestinal mucosal immune function. The current results have been similar to these reports, especially on IgA which plays an important role in the prevention of allergic reactions by the inhibition of allergen absorption (Metcalfe 1991), which is beneficial for the improvement of humoral immunity in beef cattle.

Cytokines in response to immunological challenge were important to reflect the status of cellular immunity in a host's defense against microbial infection (Renckens et al. 2006). IL-1, a prototypic multifunctional cytokine, was linked to the proliferation or differentiation of T and pre-B cells. The results of increasing in immunoglobulins levels were due to a change in the cytokine-mediated (e.g. IL-1) microenvironment (Schley and Field 2002). The study showed that CHI enhanced the serum level of IL-1 and its gene expression in lymph nodes and jejunal mucosa compared to supplementation with lincomycin in early-weaned piglets (Yin et al. 2008). The sCD4 and sCD8 in peripheral blood represented the soluble form of $CD4^+$ and $CD8^+$, which could reflect the activation of T cells (Uehara et al. 2003). When T cells expressed surface activation markers, generally in the time of inflammation or disease, the sCD4 and sCD8 were released into blood fluid. In the present experiment, it was observed that dietary supplementation of 500 mg/kg CHI enhanced the level of IL-1 and decreased the sCD4 content in serum, which implied 500 mg/kg CHI enhanced cell-mediated immunity in beef cattle.

The high immunity strengthened the anti-stress ability of the body (Zhang 2012); the stresses mainly included environmental stress, managerial stress, and immunological stress. There was a reduction in feed intake and a shift in the partitioning of dietary nutrients away from body fat/protein deposition and skeletal muscle accretion toward metabolic responses that supported the immune system when animals were under stress conditions (Johnson 1997). In addition, Song et al. (2010) showed that immunological stress decreased plasma level of growth hormone (GH) which could stimulate the growth and differentiation of chondrocytes and promote the anabolism of protein. Taken together, the above conclusions support the idea that the improved immune function of beef cattle could reflect the improvement of growth performance.

The reactive oxygen species (ROS) were usually produced spontaneously through metabolism of living cells (Cui et al. 2010). The overproduction of ROS caused oxidative stress which had a negative impact on organisms, including homeostatic disorders, immunocompromise and aging-associated diseases (Afonso et al. 2007).

Antioxidative enzymes played an important role in scavenging effectively the free radicals to protect the body against damage from peroxides. Over the years, polysaccharides, such as CHI, were widely used as antioxidants to prevent oxidative damage of animals (Tomida et al. 2009).

T-SOD and CAT enzymes constituted the main component of enzymatic antioxidant defense system. T-SOD could change efficiently oxygen free

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radicals into hydrogen peroxide which was then deoxidized to water by CAT. MDA is the end product of lipid peroxidation induced by excessive ROS, which reflected the degree of lipid peroxidation, and also was one of the indices of cellular injury. Our results showed that 500 mg/kg CHI could elevate the level of T-SOD, and decreased MDA content of serum to enhance anti-oxidative function in beef cattle, which was beneficial particularly for fattener, because the meat quality was related to antioxidative capacity of organism (Ouali et al. 2006). In addition, it is worth mentioning that the oxidative injury in animals caused by stress (e.g. early weaning) can recover with the development of the antioxidation system via feedback regulation of some proteins (Yin et al. 2014), therefore further research should be conducted to investigate the mechanism of regulating antioxidation system by CHI.

In this study, the effects of CHI on immune and antioxidative function were weakened with increasing the dose of CHI, and the mechanism may be postulated as follows: firstly, nitric oxide (NO) was shown to be an important factor in modulating immune responses and inflammation. CHI enhanced the NO content and inducible nitric oxide synthase (iNOS) activity in serum in a quadratic dose-dependent manner (Li et al. 2009). Secondly, nutrients were necessary to maintain the immune and antioxidative function; it has been generally speculated that the high-dose CHI was prone to emulsify nutrients and form a gel and finally affected the absorption of nutrients in the gastrointestinal tract. Xu et al. (2014) showed that CHI improved digestibility of major nutrients (DM, crude protein, Ca, and P) in weaned pigs in a quadratic dose-dependent manner.

CONCLUSION

In the current study, dietary supplementation with 500 mg/kg CHI enhanced the levels of IgA, IgM, and IL-1 and the activity of T-SOD, and decreased the contents of sCD4 and MDA, whereas 1000 mg/kg CHI had no obvious effects. It was suggested that 500 mg/kg CHI probably improved the immune function and antioxidative function of beef cattle; in addition, the conclusion might reflect the improvement of growth performance in our previous trials. However, further studies are needed to expound the effects of CHI on immune and antioxidative functions in beef cattle.

REFERENCES

Afonso V., Champy R., Mitrovic D., Collin P., Lomri A. (2007): Reactive oxygen species and superoxide dismutases: role in joint diseases. Joint Bone Spine, 74, 324–329.

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

Cui J.J., Yuan J.F., Zhang Z.Q. (2010): Anti-oxidation activity of the crude polysaccharides isolated from *Polygonum cillinerve* (Nakai) Ohwi in immunosuppressed mice. Journal of Ethnopharmacology, 132, 512–517.

David A.Z., Connie J.R., Kenneth W.H., Jeffrey S., John W.G. (2007): Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous vaccination. Vaccine, 11, 2085–2094.

Esumi K., Takei N., Yoshimura T. (2003): Antioxidant-potentiality of gold–chitosan nanocomposites. Colloids and Surfaces B: Biointerfaces, 32, 117–123.

Frey A., Di J.C., Zurakowski D. (1998): A statistically defined endpoint titer determination method for immunoassays. Journal of Immunological Methods, 221, 35–41.

Guo Z.Y., Xing R.E., Liu S., Zhong Z.M., Li P.C. (2008): Synthesis and hydroxyl radicals scavenging activity of quaternized carboxymethyl chitosan. Carbohydrate Polymers, 73, 173–177.

Johnson R.W. (1997): Inhibition of growth by pro-inflammatory cytokines: an integrated view. Journal of Animal Science, 75, 1244–1255.

Kong X.F., Zhou X.L., Lian G.Q., Blachier F., Liu G., Tan B.E., Nyachoti C.M., Yin Y.L. (2014): Dietary supplementation with chitooligosaccharides alters gut microbiota and modifies intestinal luminal metabolites in weaned Huanjiang mini-piglets. Livestock Science, 160, 97–101.

Li H.Y. (2009): Effects of chitosan on immune function in broilers and the underlying mechanisms. Ph.D. Thesis. Hohhot, Inner Mongolia Agricultural University. (in Chinese)

Li H.Y., Yan S.M., Shi B.L. (2009): Effect of chitosan on nitric oxide content and inducible nitric oxide synthase activity in serum and expression of inducible nitric oxide synthase mRNA in small intestine of broiler chickens. Asian-Australasian Journal of Animal Sciences, 22, 1048–1053.

Li J.L., Shi B.L., Yan S.M., Jin L., Guo Y.W., Xu Y.Q., Li T.Y., Guo X.Y. (2013): Effects of dietary supplementation of chitosan on humoral and cellular immune function in weaned piglets. Animal Feed Science and Technology, 186, 204–208.

Lim B.O., Yamada K., Nonaka M., Kuramoto Y., Hung P., Sugano M. (1997): Dietary fibers modulate indices of intestinal immune function in rats. Journal of Nutrition, 127, 663–667.

doi: 10.17221/7910-CJAS

Liu M., Shi T., Liu J.Q. (2011): Effects of chitosan on antioxidative function and immune organ index in broilers. Journal of Chinese Feed, 15, 22–25. (in Chinese)

Liu Q.H., Liang X.W., Wang G.L. (2007): Effects of dietary supplementation of chitosan on dry matter intake and blood parameters for dry dairy cows. Journal of Animal and Feed Sciences, 16, 430–435.

McKee A.S., Munks M.W., Marrack P. (2007): How do adjuvants work? Important considerations for new generation adjuvants. Immunity, 27, 687–690.

Metcalfe D.D. (1991): Food allergy. Current Opinion in Immunology, 3, 881–886.

NRC (2000): Nutrient Requirements of Beef Cattle. 7th Ed. The National Academies Press, Washington, USA.

Ouali A., Herrera-Mendez C.H., Coulis G., Becila S., Boudjellal A., Aubry L., Sentandreu M.A. (2006): Revisiting the conversion of muscle into meat and the underlying mechanisms. Meat Science, 74, 44–58.

Ren H.J. (2008): Effects of chitosan on production performance and immune function in dairy cattle. Ph.D. Thesis. Hohhot, Inner Mongolia Agricultural University. (in Chinese)

Renckens R., Roelofs J.J., Knapp S., de Vos A.F., Florquin S., van der Poll T. (2006): The acute-phase response and serum amyloid A inhibit the inflammatory response to *Acinetobacter baumannii* Pneumonia. Journal of Infectious Diseases, 193, 187–195.

Schley P.D., Field C.J. (2002): The immune-enhancing effects of dietary fibres and prebiotics. British Journal of Nutrition, 87, S221–S230.

Siwicki A.K., Anderson D.P., Rumsey G.L. (1994): Dietary intake of immunostimulants by rainbow trout affects nonspecific immunity and protection against furunculosis. Veterinary Immunology and Immunopathology, 41, 125–139.

Song C.Y., Jiang J.Y., Han X.J., Shi J.S., Wei S.D. (2010): Effects of immunological stress on interleukin-1β, cortisol and growth hormone in different breed piglets. Reproduction and Physiology, 46, 19–22. (in Chinese)

Sun T., Yao Q., Zhou D., Mao F. (2008): Antioxidant activity of N-carboxymethyl chitosan oligosaccharides. Bioorganic & Medicinal Chemistry Letters, 18, 5774–5776.

Tokura S., Tamura H., Azuma I. (1999): Immunological aspects of chitin and chitin derivatives administered to animals. EXS, 87, 279–292.

Tomida H., Fujii T., Furutani N., Michihara A., Yasufuku T., Akasaki K., Maruyama T., Otagiri M., Gebicki J.M., Anraku M. (2009): Antioxidant properties of some different molecular weight chitosans. Carbohydrate Research, 344, 1690–1696.

Uehara S., Gothoh K., Handa H., Tomita H., Tomita Y. (2003): Immune function in patients with acute pancreatitis. Journal of Gastroenterology and Hepatology, 18, 363–370.

Xiao D.F., Tang Z.R., Yin Y.L., Zhang B., Hu X.G., Feng Z.M., Wang J.Q. (2013): Effects of dietary administering chitosan on growth performance, jejunal morphology, jejunal mucosal sIgA, occludin, claudin-1 and TLR4 expression in weaned piglets challenged by enterotoxigenic *Escherichia coli*. International Immunopharmacology, 17, 670–676.

Xu Y., Shi B., Yan S., Li J., Li T., Guo Y., Guo X. (2014): Effects of chitosan supplementation on the growth performance, nutrient digestibility, and digestive enzyme activity in weaned pigs. Czech Journal of Animal Science, 59, 156–163.

Yin J., Wu M.M., Xiao H., Ren W.K., Duan J.L., Yang G., Li T.J., Yin Y.L. (2014): Development of an antioxidant system after early weaning in piglets. Journal of Animal Science, 92, 612–619.

Yin Y.L., Tang Z.R., Sun Z.H., Liu Z.Q., Li T.J., Huang R.L., Ruan Z., Deng Z.Y., Gao B., Chen L.X., Wu G.Y., Kim S.W. (2008): Effect of galacto-mannan-oligosaccharides or chitosan supplementation on cytoimmunity and humoral immunity in early-weaned piglets. Asian-Australasian Journal of Animal Sciences, 21, 723–731.

Yoon H.J., Moon M.E., Park H.S., Kim H.W., Im S.Y., Lee J.H., Kim Y.H. (2008): Effects of chitosan oligosaccharide (COS) on the glycerol-induced acute renal failure *in vitro* and *in vivo*. Food and Chemical Toxicology, 46, 710–716.

Yu P., Zhao Y.G., Shi B.L., Yan S.M., Hong L., Xu Y.Q., Guo Y.W., Tian L.X. (2012): Effects of dietary supplementation of chitosan on growth performance and nutrient digestibility in beef cattle. Journal of Feed Industry, 33, 44–47. (in Chinese)

Zhang J. (2012): Effects of dietary immunostimulants on growth, non-specific immune response and stress resistance of juvenile pacific white shrimp, *Litopenaeus vannamei*. Ph.D. Thesis. Guang Zhou, Sun Yat-sen University. (in Chinese)

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