Biological Functions and Applications of Chitosan in Dairy Cows: Postprint

Authors: Sun Mingwei, Tong Jinjin, Zhang Hua, Yang Delian, Zhang Jie, Jiang Linshu, Xiong Benhai

Date: 2018-12-24T00:00:00+00:00

Abstract

Chitosan is a natural active product obtained from the deacetylation of chitin, characterized by mucoadhesive properties and positive charge, which can effectively promote intestinal nutrient absorption and the proliferation and differentiation of mammary epithelial cells, thereby exerting protective effects on intestinal and mammary tissues. Dietary supplementation of chitosan can alter rumen fermentation patterns and microbial community structure in dairy cows, reduce ruminal methane production, and improve production performance and immune function. Furthermore, chitosan offers advantages such as no drug resistance, safety, and no residues; it not only inhibits the growth of major pathogens causing mastitis in dairy cows but also effectively enhances the antioxidant capacity of mastitic cows and promotes inflammatory recovery, conferring broad application prospects in dairy production practice. This review summarizes the biological functions of chitosan in dairy cows and research progress on its application in production, providing theoretical support for the further application of chitosan in dairy production practice.

Full Text

Chitosan: Biological Functions and Application in Dairy Cows

SUN Mingwei[1], TONG Jinjin[1], ZHANG Hua[1], YANG Delian[1], ZHANG Jie[1], JIANG Linshu[1]*, XIONG Benhai[2]

[1]Key Laboratory for Dairy Cow Nutrition, College of Animal Science and Technology, Beijing University of Agriculture, Beijing 102206, China
[2]Institute of Animal Science and Veterinary Medicine, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Abstract: Chitosan is a natural bioactive product derived from the deacetylation of chitin. Due to its mucoadhesive properties and positive charge, chitosan can effectively promote intestinal nutrient absorption and stimulate the proliferation and differentiation of mammary epithelial cells, thereby exerting protective effects on intestinal and mammary tissues. Dietary supplementation with chitosan can alter rumen fermentation patterns and microbial community structure, reduce methane production, and improve both productive performance and immune function in dairy cows. Moreover, chitosan offers advantages such as no drug resistance, safety, and absence of residues. It not only inhibits the growth of major pathogens causing mastitis but also effectively enhances the antioxidant capacity of affected cows and promotes inflammatory recovery, demonstrating broad application prospects in dairy production. This review synthesizes current research progress on the biological functions of chitosan in dairy cows and its practical applications, providing theoretical support for further utilization in dairy production.

Keywords: chitosan; biological function; mammary epithelial cell; mastitis; dairy cows

---

Chitosan is obtained through the deacetylation of chitin, chemically known as -(1,4)-2-amino-2-deoxy-D-glucose. Chitin, the primary component of exoskeletons in arthropods and fungi, consists of N-acetyl-D-glucosamine monomer units linked by -1,4-glycosidic bonds. At room temperature, chitosan appears as a white, translucent solid with a pearly luster. It is insoluble in alkaline solutions and strong acids but dissolves readily in dilute hydrochloric acid and most organic acids. Chitosan exhibits excellent adsorption, hygroscopicity, film-forming ability, permeability, biocompatibility, and biodegradability, making it a non-toxic, environmentally friendly material widely used in textiles, medicine, food, and cosmetics. While chitin monomers are acetylglucosamine with minimal solubility, chitosan monomers are glucosamine containing hydroxyl and amino groups, resulting in significantly greater chemical activity and solubility than chitin, which expands its practical applications considerably.

The degree of deacetylation substantially influences chitosan' s physicochemical properties and its ability to regulate various signals and factors in vivo, including solubility, ion-binding capacity, flocculation power, and N-selective acylation ability. Research indicates that lower molecular weight chitosan exhibits more pronounced antibacterial effects against *E. coli.* Protonated chitosan can bind to negatively charged molecules such as growth factors, nucleic acids, and cytokines both in vivo and in vitro, interacting with various bioactive factors to enhance specific cell proliferation or induce stem cell differentiation into functional cells. Arkoun et al. demonstrated that chitosan disrupts negatively charged bacterial cell membranes, causing the release of intracellular proteins and DNA, establishing its role as a bacterial membrane disruptor and perforator.

1 Effects of Chitosan on Bovine Intestinal and Mammary Epithelial Cells

The regulatory effects of chitosan on epithelial cells have been extensively studied. Due to its mucoadhesive properties and positive charge, chitosan effectively promotes intestinal nutrient absorption and improves intestinal health by activating the AMPK signaling pathway in intestinal epithelial cells. Chitosan also stimulates proliferation of various embryonic and adult mammary epithelial cells. Chitosan-based formulations can modify cell adhesion peptides and other conjugates, significantly enhancing their capacity to support cell growth and maintain homeostasis. Studies have shown that chitosan stimulates cell surface receptors, acts as a ligand binding integrin complexes, and initiates downstream signaling cascades that exert morphogenetic effects on mammary epithelial cells while regulating their growth, polarity, morphology, and differentiation.

However, stem cell transplantation research often faces challenges from damaged microenvironments that ultimately lead to rejection or immunogenic responses. To create a more suitable microenvironment for transplanted cells, chitosan can interact with extracellular matrix (ECM) components in mammary tissue, including collagen IV, laminin, tenascin, fibrin, and proteoglycans, thereby providing protective effects. Nelson et al. reported that mammary epithelial cells suspended in micro-collagen chambers can assemble basement membranes and polarize into epithelial cells within 24 hours in vitro. Nowak et al. found that culturing mammary epithelial cells in chitosan-containing matrices activates hemidesmosomal 6 1 integrin heterodimers, influencing mammary epithelial cell survival and differentiation. Yang et al. demonstrated that under serum-free conditions, chitosan biomaterials exhibit broad application potential in mammary tissue engineering. The interaction between chitosan and various growth factors has been attributed to chitosan' s cationic nature, which allows it to interact with various anionic molecules and growth factors. Nevertheless, the effects and mechanisms of chitosan and its derivatives on bovine mammary epithelial cells require further investigation.

2.1 Effects on Rumen Fermentation

Research has shown that chitosan can alter rumen fermentation patterns by shifting volatile fatty acid (VFA) profiles and increasing propionate concentration, thereby affecting acetate and butyrate production, reducing ruminal methane output, and decreasing energy loss during feed intake. Chitosan reportedly acts on the cell walls of Gram-positive hydrogen-producing bacteria, interfering with ion flux and reducing the acetate-to-propionate ratio in the rumen, which consequently lowers intestinal methane emissions. Studies have demonstrated that chitosan promotes a shift in rumen fermentation patterns, increasing propionate concentration while decreasing acetate concentration and methane production. It also enhances microbial protein synthesis and fiber degradation, modifies rumen microbial community structure, improves dairy cow performance, immune function, and antioxidant capacity, and positively influences hindgut microbial structure.

Goiri et al. reported that in vitro experiments showed increased propionate and decreased acetate concentrations when chitosan was applied to starch-based but not cellulose-based matrices. Mingoti et al. also observed a linear increase in propionate concentration with increasing dietary chitosan levels in dairy cows. Chitosan has been shown to reduce amino acid deamination in the rumen, as evidenced by decreased concentrations of isobutyric and isovaleric acids derived from isoleucine and valine deamination. Reduced ruminal amino acid deamination allows more amino acids to reach the duodenum, thereby improving amino acid utilization. Additionally, chitosan can increase milk nitrogen excretion without altering nitrogen intake, thereby improving nitrogen utilization efficiency.

2.2 Effects on Immune Regulation

As a safe, non-toxic feed additive, chitosan has been extensively studied and applied in monogastric animals, where it has been shown to improve growth performance and immunity in pigs, and enhance growth performance, nutrient metabolism, gastrointestinal environment, and antioxidant capacity in poultry. In contrast, research on chitosan's effects in ruminants remains relatively limited. Existing studies have demonstrated that chitosan exerts immunomodulatory effects on bovine mastitis. Dietary supplementation with 0.1% chitosan has been reported to increase serum immunoglobulin G (IgG) and immunoglobulin M (IgM) levels, total blood lymphocyte count, lymphocyte percentage, and lymphocyte transformation rate, thereby enhancing humoral immune function in dairy cows.

Feeding chitosan to mastitic cows has been shown to significantly increase the number of active T and B lymphocytes while decreasing neutrophil phagocytic rate and index, indicating that chitosan can resist inflammation, clear circulating immune complexes, regulate neutrophil phagocytic activity, and enhance both humoral and cellular immune functions. The mechanism may involve chitosan' s amino groups accepting protons in the body, elevating bodily fluid pH and creating an environment that activates lymphocytes, thereby enhancing cellular immunity, humoral immunity, and natural killer cell function while strengthening immune surveillance. Additionally, chitosan can indirectly modulate the immune system by promoting the growth and reproduction of *Bifidobacterium*, as it serves as a growth factor for these bacteria. *Bifidobacterium* can activate the intestinal mucosal immune system by stimulating Paneth cells, promoting immunoglobulin A (IgA) secretion. Simultaneously, *Bifidobacterium* can enhance the cytotoxic activity of intraepithelial lymphocytes in the small intestine, strengthening immune surveillance in the intestinal tract and thereby improving overall immune function.

3 Antibacterial Effects of Chitosan and Its Therapeutic Role in Bovine Mastitis

Chitosan exhibits multiple biological functions, including inhibiting bacterial growth and activity, enhancing immunity, antiviral effects, and lipid reduction, demonstrating antibiotic-like characteristics. With its safety, lack of residues, and low potential for resistance development, chitosan has become a research focus for mastitis prevention and treatment. Currently, antibiotics remain the primary approach for preventing and treating mastitis, but their long-term, high-dose clinical use has led to enhanced microbial resistance, reduced antibiotic efficacy, and serious problems with antibiotic residues in dairy products. Therefore, effective control of bovine mastitis is crucial for the healthy and rapid development of the dairy industry.

3.1 Antibacterial Effects

Chitosan' s antimicrobial properties are particularly prominent, demonstrating strong antibacterial activity. This is primarily attributed to its protonated ammonium groups that ionize in slightly acidic solutions, conferring a positive charge that interacts with negatively charged substances on bacterial cell membranes, disrupting membrane stability and inhibiting normal microbial metabolism. Reportedly, chitosan exerts its effects through three levels of interaction: with outer cellular components, with cell membranes, and with cytoplasmic components. Electron microscopic examination of various microorganisms treated with chitosan indicates that its action occurs at the microbial cell surface. The properties and structure of bacterial cell membranes play a crucial role, with Gram-positive bacteria being more sensitive to chitosan than Gram-negative bacteria. This difference primarily stems from distinct cell wall and membrane architectures. Gram-positive bacterial cell walls consist of a thick, dense layer of peptidoglycan and teichoic acid, with peptide chains cross-linked by 5-75 glycine residues, whereas Gram-negative bacterial cell walls are multilayered structures comprising the cell wall, cytoplasmic membrane, and a semi-permeable bilayer lipid outer membrane. Consequently, differences in negative charge accumulation on cell walls determine microbial sensitivity to chitosan.

Generally, chitosan' s polycationic nature, delivered by positively charged glucosamine, induces multifaceted changes on the cell surface, causing intracellular material leakage and ultimately lethal damage to bacterial activity. The initial contact between polycationic chitosan macromolecules and bacterial cells most likely occurs through electrostatic reactions mediated by negatively charged teichoic acids on Gram-positive bacteria. This aligns with findings from Şenel et al. and Lanctôt et al., which demonstrated strong antibacterial effects of chitosan against Gram-positive bacteria. Additionally, some studies have indicated that when environmental pH exceeds 6.3, chitosan' s hydrophobic and chelating effects influence antibacterial activity; however, De Paiva et al. showed that chitosan is unaffected by ruminal pH, yielding slightly different results between studies. Positively charged chitosan may be absorbed by cells and interact with fungal and bacterial DNA, thereby inhibiting DNA and RNA transcription and protein synthesis. Previous reports have suggested that chitosan can penetrate plant cells, based on detection of chitosan in plant cytoplasm and nuclei within 15 minutes of application to plant tissue surfaces. Therefore, the mechanisms involve: (1) electrostatic reactions between chitosan and negatively charged cell membrane components (phospholipids and proteins); (2) reactions with amino acids in Gram-positive bacterial cell walls; and (3) reactions with lipopolysaccharides in Gram-negative bacterial outer membranes, thereby affecting cell membrane integrity and permeability. In summary, cell membrane charge contributes to chitosan' s antibacterial activity.

Guan Ming et al. developed a dairy cow hoof care solution containing water-soluble chitosan that effectively kills infectious pathogens causing hoof diseases, promotes wound healing, prevents and treats various hoof diseases, and enhances cows' autoimmunity and disease resistance. Despite chitosan' s strong antibacterial activity, feeding chitosan does not alter microbial protein synthesis, and Del Valle et al. reported no impairment of milk quality or yield in dairy cows. According to Tian Feng et al., supplementation with 15 g/d of chitosan increased milk production by an average of 1.8 kg per cow per day compared to the control group without chitosan.

3.2 Therapeutic Effects on Bovine Mastitis

Research has shown that chitosan produces no side effects when used to treat clinical mastitis in dairy cows. In vitro antibacterial tests have demonstrated that water-soluble chitosan exhibits varying degrees of inhibition against *Streptococcus agalactiae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli*, *Salmonella*, and *Streptococcus* species. Freter et al. confirmed that low-concentration chitosan exhibits the strongest antibacterial activity, as it can neutralize the negative charges on bacterial cell surfaces, causing bacterial cells to aggregate. In contrast, high concentrations may impart positive charges to bacterial surfaces, keeping cells in suspension rather than aggregating them, thereby reducing antibacterial efficacy.

For cows with subclinical mastitis, dietary supplementation with different concentrations of chitosan has been shown to significantly reduce somatic cell counts in milk at a dosage of 5 g/d. Asli et al. found that intramammary injection of 2.6 kDa molecular weight chitosan inhibited *Staphylococcus aureus* activity in a dose-dependent manner. Antioxidant capacity plays a crucial role when mastitis occurs. Under normal conditions, the production, utilization, and clearance of free radicals maintain dynamic equilibrium. During mastitis, specific immune cells introduce substantial numbers of free radicals to aid in pathogen elimination. However, excessive free radical production coupled with weakened clearance capacity causes lipid peroxidation, destroys biological membrane structures, and exacerbates mammary infection severity. Studies on feeding chitosan to mastitic cows have demonstrated its antioxidant function in regulating free radical levels and promoting mammary inflammation recovery.

Research by Shang Changfa et al. and Ren Haijun has shown that dietary chitosan supplementation stimulates antioxidant function, improves health status, reduces milk somatic cell counts, and decreases the likelihood of mammary tissue infection. Guo Xiaoping et al. similarly reported that chitosan oligosaccharides improved milk performance and reduced somatic cell counts in cows with subclinical mastitis.

5 Summary

Chitosan holds broad application prospects in dairy production. It can modify rumen fermentation patterns, reduce methane production, decrease energy loss during feed intake, and improve feed conversion efficiency. Due to its safety, non-toxicity, and antibacterial properties, chitosan has become a research focus for mastitis treatment. However, the mechanisms underlying chitosan' s effects on bovine mammary epithelial cells, its regulation of immune responses, and its signaling pathway modulation require further investigation. Additionally, safety studies on chitosan application in dairy production necessitate deeper exploration.

References

[1] DEEPTHI S, VENKATESAN J, KIM S K, et al. An overview of chitin or chitosan/nano ceramic composite scaffolds for bone tissue engineering[J]. International Journal of Biological Macromolecules, 2016, 93: 1338-1353.

[2] ANITHA A, SOWMYA S, KUMAR P T S, et al. Chitin and chitosan in selected biomedical applications[J]. Progress in Polymer Science, 2014, 39(9): 1644-1667.

[3] 孙晓婷, 郭亚. 壳聚糖的应用及发展 [J]. 成都纺织高等专科学校学报, 2016, 33(2): 165-169.

[4] LI X S, MIN M, DU N, et al. Chitin, chitosan, and glycated chitosan regulate immune responses: the novel adjuvants cancer vaccine[J]. Clinical and Developmental Immunology, 2013, 2013: 387023.

[5] BALÁZS N, SIPOS P. Limitations of pH-potentiometric titration for the determination of the degree of deacetylation of chitosan[J]. Carbohydrate Research, 2007, 342(1): 124-130.

[6] RODRÍGUEZ-VÁZQUEZ M, VEGA-RUIZ B, RAMOS-ZÚÑIGA R, et al. Chitosan and its potential use as a scaffold for tissue engineering in regenerative medicine[J]. BioMed Research International, 2015, 2015: 821279.

[7] ARKOUN M, DAIGLE F, HEUZEY M C, et al. Antibacterial electrospun chitosan-based nanofibers: A bacterial membrane perforator[J]. Food Science & Nutrition, 2017, 5(4): 865-874.

[8] 孙露银, 闫彩凤. 壳聚糖促进肠道上皮细胞吸收的机制 [J]. 医学综述, 2015, 21(20): 3686–3688.

[9] MUANPRASAT C, WONGKRASANT P, SATITSRI S, et al. Activation of AMPK by chitosan oligosaccharide in intestinal epithelial cells: mechanism of action and potential applications in intestinal disorders[J]. Biochemical Pharmacology, 2015, 96(3): 225–236.

[10] GANDRA J R, OLIVEIRA E R, TAKYIA C S, et al. Chitosan improves the chemical composition, microbiological quality, and aerobic stability of sugarcane silage[J]. Animal Feed Science and Technology, 2016, 214: 44–52.

[11] LAUTO A, MAWAD D. Chitosan-ECM bandages for photochemical tissue repair[C]//Quantum electronics conference & lasers and electro-optics. Sydney, NSW, Australia: IEEE, 2011.

[12] TADDEI I, FARALDO M M, TEULIÈRE J, et al. Integrins in mammary gland development and differentiation of mammary epithelium[J]. Journal of Mammary Gland Biology and Neoplasia, 2003, 8(4): 383–394.

[13] PATIL S V, NANDURI L S Y. Interaction of chitin/chitosan with salivary and other epithelial cells—an overview[J]. International Journal of Biological Macromolecules, 2017, 104: 1398–1406.

[14] NELSON C M, KHAUV D, BISSELL M J, et al. Change in cell shape is required for matrix metalloproteinase-induced epithelial-mesenchymal transition of mammary epithelial cells[J]. Journal of Cellular Biochemistry, 2008, 105(1): 25–33.

[15] NOWAK M, FREUDENBERG U, TSURKAN M V, et al. Modular GAG-matrices to promote mammary epithelial morphogenesis in vitro[J]. Biomaterials, 2016, 112: 20–30.

[16] YANG T L, LIN L, HSIAO Y C, et al. Chitosan biomaterials induce branching morphogenesis in a model of tissue-engineered glandular organs in serum-free conditions[J]. Tissue Engineering Part A, 2012, 18(21/22): 2220–2230.

[17] BELANCHE A, PINLOCHE E, PRESKETT D, et al. Effects and mode of action of chitosan and ivy fruit saponins on the microbiome, fermentation and methanogenesis in the rumen simulation technique[J]. FEMS Microbiology Ecology, 2016, 92(1): fiv160.

[18] POULSEN M, SCHWAB C, BORG J B, et al. Methylotrophic methanogenic Thermoplasmata implicated in reduced methane emissions from bovine rumen[J]. Nature Communications, 2013, 4: 1428.

[19] DANIELSSON R, DICKSVED J, SUN L, et al. Methane production in dairy cows correlates with rumen methanogenic and bacterial community structure[J]. Frontiers in Microbiology, 2017, 8: 226.

[20] 张静. 壳聚糖对奶牛产奶性能、营养物质消化及相关血清生化指标的影响 [D]. 硕士学位论文. 呼和浩特: 内蒙古农业大学, 2015.

[21] KITTELMANN S, PINARES-PATIÑO C S, SEEDORF H, et al. Two different bacterial community types are linked with the low-methane emission trait in sheep[J]. PLoS One, 2014, 9(7): e103171.

[22] 田雨佳, 闫素梅, 任海军, 等. 壳聚糖在奶牛瘤胃中的降解率及其对瘤胃发酵的影响 [J]. 中国畜牧杂志, 2011, 47(19): 53-56.

[23] GOIRI I, GARCIA-RODRIGUEZ A, OREGUI L M. Effect of chitosans on in vitro rumen digestion and fermentation of maize silage[J]. Animal Feed Science and Technology, 2009, 148(2/3/4): 276-287.

[24] MINGOTI R D, FREITAS J E, GANDRA J R, et al. Dose response of chitosan on nutrient digestibility, blood metabolites and lactation performance in Holstein dairy cows[J]. Livestock Science, 2016, 187: 35-39.

[25] OKAMOTO Y, WATANABE M, MIYATAKE K, et al. Effects of chitin/chitosan and their oligomers/monomers on migrations of fibroblasts and vascular endothelium[J]. Biomaterials, 2002, 23(9): 1975-1979.

[26] LIU P, PIAO X S, KIM S W, et al. Effects of chito-oligosaccharide supplementation on the growth performance, nutrient digestibility, intestinal morphology, and fecal shedding of *Escherichia coli* and *Lactobacillus* in weaning pigs[J]. Journal of Animal Science, 2008, 86(10): 2609-2618.

[27] 田丰, 任海军, 史彬林, 等. 壳聚糖对奶牛产奶性能、乳体细胞数及血清免疫球蛋白的影响 [J]. 饲料工业, 2009, 30(5): 31-33.

[28] 商常发, 陈会良, 顾有方, 等. 壳聚糖对乳房炎奶牛免疫功能的影响 [J]. 中国牛业科学, 2006, 32(6): 45-49.

[29] TOKURA S, TAMURA H, AZUMA I. Immunological aspects of chitin and chitin derivatives administered to animals[J]. EXS, 1999, 87: 279-292.

[30] 陈文学, 史俊华. 双歧杆菌的研究进展 [J]. 中国微生态学杂志, 2000, 12(5): 298-300.

[31] 周小洁, 车向荣, 于霏. 壳聚糖的生物学功能及其在家禽生产中的应用 [J]. 畜禽业, 2005(1): 12-14.

[32] 章廊, 胡正华, 徐强, 等. 壳聚糖对奶牛乳腺炎的疗效及血清铜镁的影响 [J]. 上海畜牧兽医通讯, 2012(6): 18-19.

[33] KONG M, CHEN X G, XING K, et al. Antimicrobial properties of chitosan and mode of action: a state of the art review[J]. International Journal of Food Microbiology, 2010, 144(1): 51-53.

[34] PRODHAN M Y, ISSAK M, NAKAMURA T, et al. Chitosan signaling in guard cells requires endogenous salicylic acid[J]. Bioscience, Biotechnology, and Biochemistry, 2017, 81(8): 1536-1541.

[35] SAVARD T, BEAULIEU C, BOUCHER I, et al. Antimicrobial action of hydrolyzed chitosan against spoilage yeasts and lactic acid bacteria of fermented vegetables[J]. Journal of Food Protection, 2002, 65(5): 828-833.

[36] RAAFAT D, SAHL H G. Chitosan and its antimicrobial potential—a critical literature survey[J]. Microbial Biotechnology, 2009, 2(2): 186-201.

[37] ZAKRZEWSKA A, BOORSMA A, BRUL S, et al. Transcriptional response of *Saccharomyces cerevisiae* to the plasma membrane-perturbing compound chitosan[J]. Eukaryotic Cell, 2005, 4(4): 703-715.

[38] ŞENEL S, MCCLURE S J. Potential applications of chitosan in veterinary medicine[J]. Advanced Drug Delivery Reviews, 2004, 56(10): 1467-1480.

[39] LANCTÔT S, FUSTIER P, TAHERIAN A R, et al. Effect of intramammary infusion of chitosan hydrogels at drying-off on bovine mammary gland involution[J]. Journal of Dairy Science, 2017, 100(3): 2269-2281.

[40] DE PAIVA P P G, DE JESUS E F, DEL VALLE T A, et al. Effects of chitosan on ruminal fermentation, nutrient digestibility, and milk yield and composition of dairy cows[J]. Animal Production Science, 2016, 57(2): 301-307.

[41] RABEA E I, BADAWY E T, STEVENS C V, et al. Chitosan as antimicrobial agent: applications and mode of action[J]. Biomacromolecules, 2003, 4(6): 1457-1465.

[42] HADWIGER L A, BECKMAN J M, ADAMS M J. Localization of fungal components in the pea-*Fusarium* interaction detected immunochemically with anti-chitosan and anti-fungal cell wall antisera[J]. Plant Physiology, 1981, 67(1): 170-175.

[43] 关鸣, 叶锋, 张一楠, 等. 一种奶牛护蹄液及其制备方法: CN201510947303.9[P]. 2016-04-06.

[44] DEL VALLE T A, DE PAIVA P G, DE JESUS E F, et al. Dietary chitosan improves nitrogen use and feed conversion in diets for mid-lactation dairy cows[J]. Livestock Science, 2017, 201: 22-29.

[45] 孙先明, 刘思当, 季云峰. 壳聚糖对奶牛免疫功能及生产性能的影响 [J]. 中国兽医杂志, 2008, 44(1): 40-41.

[46] FRETER R, STAUFFER E, CLEVEN D, et al. Continuous-flow cultures as in vitro models of the ecology of large intestinal flora[J]. Infection & Immunity, 1983, 39(2): 666-675.

[47] ASLI A, BROUILLETTE E, STER C, et al. Antibiofilm and antibacterial effects of specific chitosan molecules on *Staphylococcus aureus* isolates associated with bovine mastitis[J]. PLoS One, 2017, 12(5): e0176988.

[48] 商常发, 陈会良, 金光明, 等. 壳聚糖对乳房炎奶牛血清自由基代谢的影响 [J]. 中国草食动物科学, 2006, 26(3): 44-46.

[49] 任海军. 壳聚糖对奶牛产奶性能和免疫功能影响的研究 [D]. 硕士学位论文. 呼和浩特: 内蒙古农业大学, 2008.

[50] 郭小萍, 范守民, 刘宜勇, 等. 低聚壳聚糖对隐性乳房炎奶牛产奶性能和体细胞数的影响 [J]. 草食家畜, 2016(4): 32-35, 49.

Corresponding author: Professor, E-mail: kjxnb@vip.sina.com
(Responsible editor: WANG Zhihang)