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Atractylodis macrocephalae *Koidz*

Wei Xu, Ran Guan, Yisong Lu, Xiaoyan Su, Ye Xu, Aifang Du and Songhua Hu*

Abstract

Background: Mastitis is considered the most significant and persistent disease in dairy cows, bringing about large economic losses. Subclinical mastitis brings about major cost implications, for it is difficult to detect due to absence of any visible indications and can persist in the mammary tissue throughout lactation. Immunomodulators have been widely used to reduce intramammary infections by modulating bovine mammary gland. *Atractylodis macrocephalae* Koidz. polysaccharides (*RAMP*), extracted from herbal medicine, has been used widely especially for its immunomodulatory function for many years. The objective of this study was to estimate an oil emulsified *Atractylodis macrocephalae* Koidz. polysaccharides (*RAMP-O*) as a potential therapeutic agent to treat subclinical mastitis by subcutaneous injection of *RAMP-O* in the area of supramammary lymph node in lactating cows via analysis of SCC, IMIs and NAGase.

Results: Injection of *RAMP-O* in the area of supramammary lymph node significantly reduced milk SCC and NAGase activity compared with control. The quarters with bacterial infection were also progressively reduced in *RAMP-O* treated cows and only 9 quarters were found to have bacterial infection, while no obvious change was found in the control group.

Conclusions: Subcutaneous injection of *RAMP-O* in the area of supramammary lymph node had therapeutic value in the treatment of bovine subclinical mastitis by reducing SCC, NAGase and IMIs in milk. Considering both the therapeutic effect and the cost of *RAMP-O*, 32 mg per dose was found most suitable to reduce milk SCC and NAGase. Therefore, *RAMP-O* deserves further study for its use in treatment of bovine mastitis.

Keywords: *Atractylodis macrocephalae* Koidz, Polysaccharides, Mastitis, Supramammary lymph node

Background

Mastitis is a common disease in dairy cows, causing significant economic losses. The prevalence of mastitis in dairy cows is 1–3% in the United States, 4–7% in the United Kingdom, and 10–15% in the Netherlands [1]. In China, the prevalence of mastitis in dairy cows is 11–15% [2]. The most common pathogens causing mastitis are *Streptococcus aureus*, *Escherichia coli*, and *Staphylococcus aureus*. The clinical signs of mastitis include redness, swelling, and pain in the mammary gland, as well as changes in the color and consistency of the milk. The diagnosis of mastitis is based on clinical signs, laboratory tests, and imaging. The treatment of mastitis includes antibiotics, anti-inflammatories, and supportive care. The prevention of mastitis includes good hygiene, proper milking techniques, and regular udder examinations. The use of immunomodulators, such as polysaccharides, has been shown to reduce the incidence of mastitis in dairy cows. *Atractylodis macrocephalae* Koidz. polysaccharides (*RAMP*) is a natural immunomodulator that has been used in traditional Chinese medicine for centuries. It has been shown to have anti-inflammatory and immunomodulatory effects in various animal models. In this study, we evaluated the efficacy of an oil emulsified *RAMP* (*RAMP-O*) in the treatment of subclinical mastitis in dairy cows. The results showed that *RAMP-O* significantly reduced milk SCC and NAGase activity compared with control. The quarters with bacterial infection were also progressively reduced in *RAMP-O* treated cows and only 9 quarters were found to have bacterial infection, while no obvious change was found in the control group. These findings suggest that *RAMP-O* has therapeutic value in the treatment of bovine subclinical mastitis by reducing SCC, NAGase and IMIs in milk. Considering both the therapeutic effect and the cost of *RAMP-O*, 32 mg per dose was found most suitable to reduce milk SCC and NAGase. Therefore, *RAMP-O* deserves further study for its use in treatment of bovine mastitis.

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(IL-1 α , IL-1 β)

F- α)

A. ac lodi mac ocephalae Koid . Com-

po i ae

C . I (RAM) ~~2~~ -

C 2000 18 .

RAM , 19 . -

RAM -

. L (RAMP) RAM

. RAMP - -

(FMD)

20-22 .

RAM

IMI

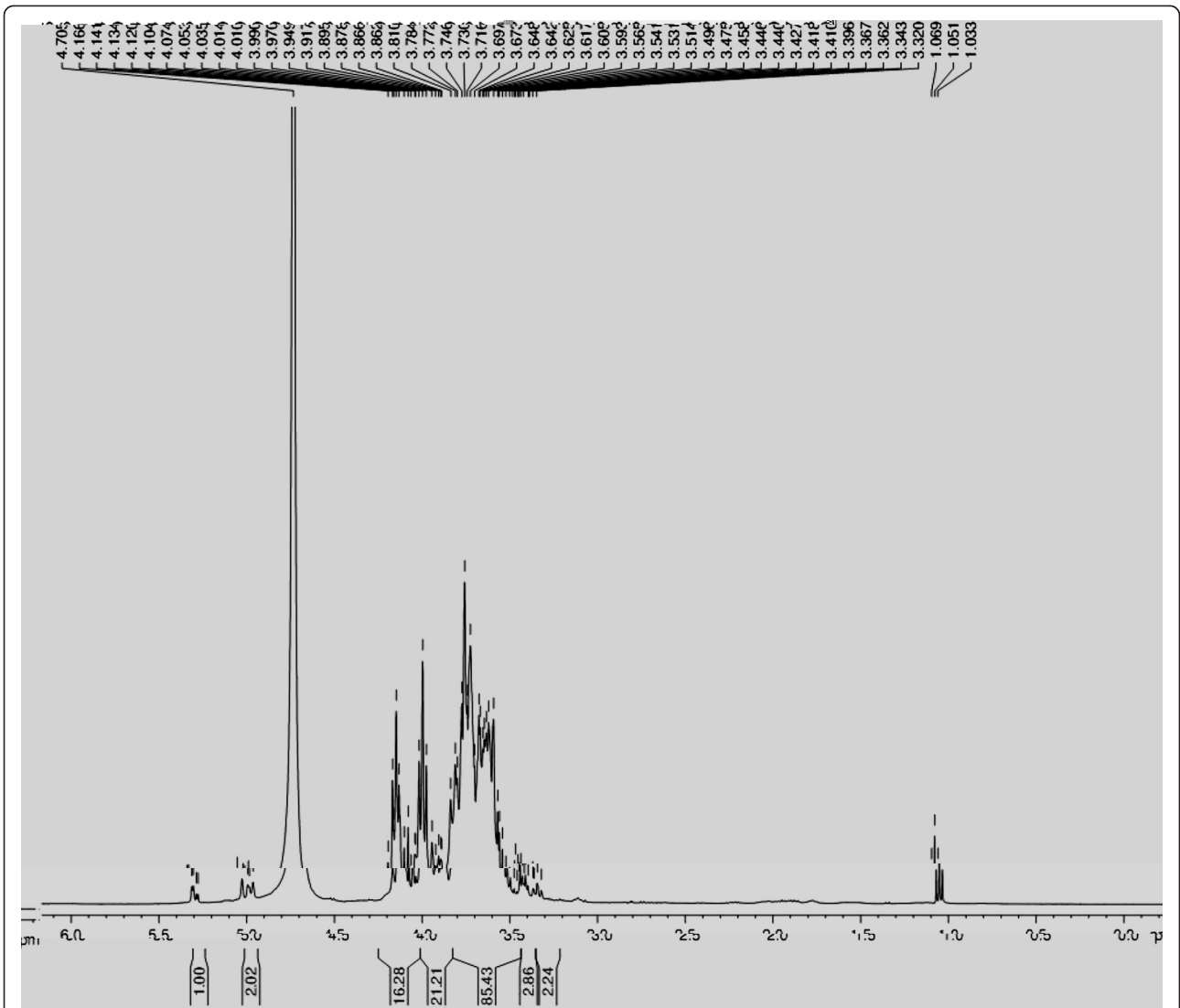


Fig. 2 ¹H NMR spectrum of RAMP. The ¹H NMR spectra showed that RAMP was a polysaccharide with both α and β configurations, while the β-configuration is dominant

4000 ; 7.8 0.4 , 7.6 0.3 8.1 Simultaneous injections of RAMP-O in both left and right
 0.4 , , 4,8 areas of the supramammary lymph node reduced SCC
 12 RAMP and NAGase activity in milk
 , RAMP-O I 1, (16), (32)
 (48) RAMP-O

Irritation induced by subcutaneous injection of RAMP-O D , 1 3.
 in the area of supramammary lymph node AG , CC
 I , 32 RAMP-O 1 3 (1 2). A
 CC 220,000/ .
 CC 2 3. C
 A 3 (F . 3). , CC 45.3 % 46.0 %
 - 55.2 % 60.3 %, 2 3; AG
 1 2. , 2 3.

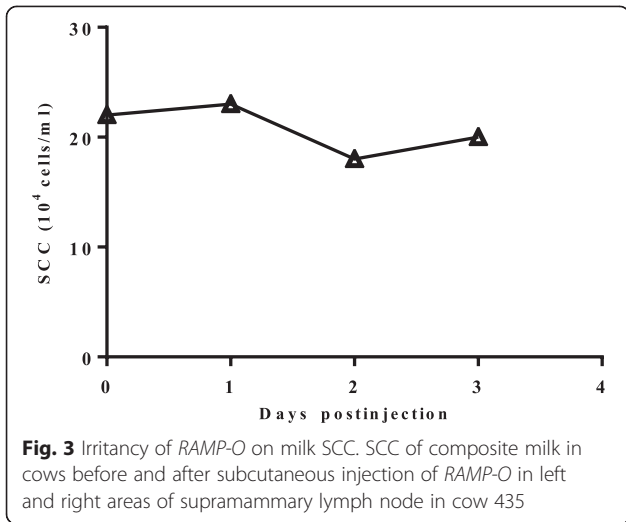


Fig. 3 Irritancy of *RAMP-O* on milk SCC. SCC of composite milk in cows before and after subcutaneous injection of *RAMP-O* in left and right areas of supramammary lymph node in cow 435

32 *RAMP-O* ...
RAMP-O (32) ...
 AG (CC) 3 4). I ...
 (5).

Changes of bacteria infected quarters before and after treatment

B ... 21 ...
RAMP-O ... 23 ...
 (6).
 2 ...
S aph lococc a e, *S ep ococc agalac iae*, *S ep ococc d galac- iae*, *S ep ococc be i*, ...
 (C), . A , ...
RAMP-O ... 9

Discussion

I ,
A ac lodi mac o- cephalae K . (*RAMP-O*)
 . F
 IL-2 *S aph lococc a e* IMI 20-30 % 23 . G (G)
 M 13-15 .
 G IL-1 α , IL-1 β F- α
 A 16, 17 . I
RAMP-O . A *RAMP-* IMI
RAMP *A ac lo- di mac ocephalae* Koid .
 24
Shen Ling Bai Zh San (, , , , ,)
 96 %.
RAMP
 FMD 21, 25 . I 2013,
 C *RAMP* . *RAMP* . C
RAMP , , , , , 1.00:
 2.49: 2.07: 4.94: 11.33: 1.35 21 . I *RAMP* F I ¹H
 M
 α - β -

Table 1 Influence of *RAMP-O* on composite milk SCC

RAMP-O (mg)	No. of cows	Weeks post treatment			
		0 [†]	1	2	3
16	6	73.01 ± 10.05	65.79 ± 5.96	63.62 ± 17.94a*	54.65 ± 16.66a**
32	6	72.93 ± 18.42	62.31 ± 19.10	46.54 ± 25.14a	42.34 ± 21.30a*
48	6	71.48 ± 16.07	49.85 ± 23.49	45.01 ± 17.57A**	41.83 ± 14.81A*
Control	6	76.39 ± 14.38	77.20 ± 13.81	85.17 ± 12.15	77.47 ± 14.24

[†] P - P -
 *P - **P -
 † -
RAMP-O (-)

Table 2 Influence of RAMP-O on composite milk NAGase

RAMP-O (mg)	No. of cows	Weeks post treatment			
		0 [†]	1	2	3
16	6	52.07 ± 12.50	46.75 ± 10.09*	46.31 ± 9.79*	33.91 ± 9.27a*
32	6	52.46 ± 12.24	46.18 ± 9.49*	25.36 ± 6.77A**	23.79 ± 5.47A**
48	6	52.11 ± 11.35	27.27 ± 8.09A**	24.97 ± 6.85A**	21.09 ± 3.44A**
Control	6	52.66 ± 12.12	52.90 ± 12.07	55.09 ± 11.73	53.13 ± 11.81

RAMP-O ()

P, P, *P, †

β-

26–28 . F M

A agal memb anace . I CC CC 34 .

25, 29, 30 . RAMP I RAMP-O

RAMP-O CC 1 2 32 48

RAMP-O RAMP-O IMI CC

CC, AG IMI RAMP-O 2.

M . I AG

31 . 35 . AG M

. A 36 .

32 . M , M

AG IMI . A

AG 37 . I

AG (2 4)

12 . CC RAMP-O

IMI RAMP-O

Conclusions

A , RAMP-O

Table 3 Influence of RAMP-O on quarter milk SCC

Group	No. of quarters ^{††}	Weeks post treatment			
		0 [†]	1	2	3
RAMP-O	14	63.39 ± 21.92	48.59 ± 27.52	40.18 ± 11.79a*	37.38 ± 11.36a*
Control	12	60.32 ± 10.20	54.53 ± 53.63	70.64 ± 67.24	64.37 ± 54.85

RAMP-O ()

P, *P, †, ††

CC, AG IMI . C
RAMP-O, 32
AG . , RAMP-O CC

Methods

Extraction of polysaccharide fraction of *Atractylodis macrocephalae* Koidz. polysaccharides (RAMP)

D *A ac lodi mac ocephalae* Koid .
H C . L , H ,
C .
21 . B ,
(100)
2 .
15 . F , 95 % 3000
4 C .
M A D101,
(- M 7000 D)
(RAMP,
, 8.53).

RAMP-O 1- - *RAMP-O* 1 -

Irritancy test of *RAMP-O* 435

32 48 .G 2

1, 2, 3

. E - *RAMP-O* , CC AG

CC 500,000 / . C

RAMP-O 32 *RAMP-O* .

Estimation of somatic cell count (SCC)

F CC, 40-

42 C. A ,

F M (F E , H ,

D).

3 CC .

Selection of cows

A C C I -

I H -

J , -

C , 2,000 H , -

1 CC 2

500,000 / ,

CC 500,000 / .

Bacteriological examination

M 37 C 24 48 .A -

. A 3

A , 18-24

37 C. F

M C 39, 40 .

Experimental design

Experiment 1

A 24 H 4 6 .

. G 1 3

RAMP-O 16, 32 48 ,

. E

. G 4

. C -

1, 2,

3 *RAMP-O* AG .

CC AG .

Experiment 2

A 22 H 37 C. 1 15

2

Statistical analyses

D 20.0 -

(.E).

44 : 1 11 ; 2 11 12

. G 1 - I -

RAMP-O (16) *RAMP-O*

RAMP-O 32 . C -

RAMP-O

. P 0.05

Abbreviations

RAMP: *Atractylodis macrocephalae* Koidz. polysaccharides; RAMP-O: Oil emulsified *Atractylodis macrocephalae* Koidz. polysaccharides; SCC: Somatic cell count; IMIs: Intramammary infections; NAGase: N-Acetyl-β-D-glucosaminidase; CNS: Coagulase-negative staphylococci; GS: *Ginseng saponins*; FTIR: Transmission Fourier transform infrared spectroscopy; NMR: Nuclear magnetic resonance; PMN: Polymorphonuclear leukocyte.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors participated in the draft of the manuscript. Moreover, WX, RG, YSL, XYS and XY carried out the sampling collection, microbiological isolation and biochemical identification; WX and YSL conducted the preparation of RAMP-O; WX and XYS performed statistical analysis; and SHH and AFD conceived, designed, and coordinated the study, and wrote the final manuscript. All authors read and approved the final manuscript.

Acknowledgements

We gratefully acknowledge the participation of the owners and veterinarians of Yi Kang Dairy Farm. The authors also acknowledge the Zhejiang Province Science and Technology Correspondent Special Team (2012T2T2109) for the financial support.

Published online: 25 July 2015

References

1. Bannerman DD. Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows. *J Anim Sci.* 2009;87(13 suppl):10–25.
2. Gonen E, Vallon Eberhard A, Elazar S, Harmelin A, Brenner O, Rosenshine I, et al. Toll-like receptor 4 is needed to restrict the invasion of *Escherichia coli* P4 into mammary gland epithelial cells in a murine model of acute mastitis. *Cell Microbiol.* 2007;9(12):2826–38.
3. Hawari AD, Al-Dabbas F. Prevalence and distribution of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Jordan. *Am J Anim Vet Sci.* 2008;3(1):36.
4. Dohoo IR, Meek AH. Somatic cell counts in bovine milk. *Can Vet J.* 1982;23(4):119.
5. Gröhn YT, Wilson DJ, González RN, Hertl JA, Schulte H, Bennett G, et al. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. *J Dairy Sci.* 2004;87(10):3358–74.
6. Pilla R, Malvisi M, Snel GM, Schwarz D, König S, Czerny C, et al. Differential cell count as an alternative method to diagnose dairy cow mastitis. *J Dairy Sci.* 2013;96(3):1653–60.
7. Schukken YH, Lam TJ, Nielsen M, Hogeveen H, Barkema HW, Grommers FJ. Subclinical and clinical mastitis on dairy farms in The Netherlands: epidemiological developments. *Tijdschr Diergeneesk.* 1995;120(7):208–13.
8. Memon J, Kashif J, Yaqoob M, Liping W, Yang Y, Hongjie F. Molecular characterization and antimicrobial sensitivity of pathogens from sub-clinical and clinical mastitis in Eastern China. *Pak Vet J.* 2003;23(2):170–174.
9. Cao LT, Wu JQ, Xie F, Hu SH, Mo Y. Efficacy of nisin in treatment of clinical mastitis in lactating dairy cows. *J Dairy Sci.* 2007;90(8):3980–5.
10. Persson Y, Nyman AJ, Grönlund-Andersson U. Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. *Acta Vet Scand.* 2011;53(1):36.
11. Sordillo L, Daley M. Role of cytokines in the prevention and treatment of bovine mastitis (1995). In: Myers MJ, Murtaugh MP, editors. *Cytokines in animal health and disease.* New York: Marcel Dekker Publ; 1995.
12. Pyörälä S. New strategies to prevent mastitis. *Reprod Domest Anim.* 2002;37(4):211–6.
13. Concha C, Hu S, Holmberg O. The proliferative responses of cow stripping milk and blood lymphocytes to pokeweed mitogen and ginseng in vitro. *Vet Res.* 1996;27(2):107–15.

14. Hu S, Concha C, Cooray R, Holmberg O. Ginseng-enhanced oxidative and phagocytic activities of polymorphonuclear leucocytes from bovine peripheral blood and stripping milk. *Vet Res.* 1995;26(3):155–61.
15. Hu S, Concha C, Johannisson A, Meglia G, Waller KP. Effect of subcutaneous injection of ginseng on cows with subclinical *Staphylococcus aureus* mastitis. *J Vet Med B.* 2001;48(7):519–28.
16. Baravalle C, Dallard BE, Cadoche MC, Pereyra E, Neder VE, Ortega HH, et al. Proinflammatory cytokines and CD14 expression in mammary tissue of cows following intramammary inoculation of *Panax ginseng* at drying off. *Vet Immunol Immunop.* 2011;144(1):52–60.
17. Baravalle C, Dallard BE, Ortega HH, Neder VE, Canavesio VR, Calvino LF. Effect of *Panax ginseng* on cytokine expression in bovine mammary glands at drying off. *Vet Immunol Immunop.* 2010;138(3):224–30.
18. Chen W, He GF, Jiang MH, Qiu XM. Advances of the rhizome of *Atractylodis macrocephalae* Koidz, during the recent decades. *Lishizhen Med Met Medica Res.* 2007;18(2):338–40.
19. Duan Q, Xu DJ, Liu CX, Li CL. Advances of the rhizome of *Atractylodis macrocephalae* Koidz. *Chinese Tradit Herbal Drugs.* 2008;39(5):4–6.
20. Li R, Sakwivatkul K, Yutao L, Hu S. Enhancement of the immune responses to vaccination against foot-and-mouth disease in mice by oral administration of an extract made from *Rhizoma Atractylodis Macrocephalae* (RAM). *Vaccine.* 2009;27(15):2094–8.
21. Xie F, Li Y, Su F, Hu S. Adjuvant effect of *Atractylodis macrocephalae* Koidz. polysaccharides on the immune response to foot-and-mouth disease vaccine. *Carbohydr Polym.* 2012;87(2):1713–9.
22. Xie F, Sakwivatkul K, Zhang C, Wang Y, Zhai L, Hu S. *Atractylodis macrocephalae* Koidz. polysaccharides enhance both serum IgG response and gut mucosal immunity. *Carbohydr Polym.* 2013;91(1):68–73.
23. Daley MJ, Furda G, Dougherty R, Coyle PA, Williams TJ, Johnston P. Potentiation of antibiotic therapy for bovine mastitis by recombinant bovine interleukin-2. *J Dairy Sci.* 1992;75(12):3330–8.
24. Zhou MY. Shen Ling Bai Zhu San in treatment of 125 cases with infections in the upper respiratory tract. *Sci Technol Chin Mater Medica.* 2001;8(2):68.
25. Li R, Chen W, Wang W, Tian W, Zhang X. Extraction, characterization of *Astragalus* polysaccharides and its immune modulating activities in rats with gastric cancer. *Carbohydr Polym.* 2009;78(4):738–42.
26. Jin M, Huang Q, Zhao K, Shang P. Biological activities and potential health benefit effects of polysaccharides isolated from *Lycium barbarum* L. *Int J Biol Macromol.* 2013;54:16–23.
27. Schepetkin IA, Quinn MT. Botanical polysaccharides: macrophage immunomodulation and therapeutic potential. *Int Immunopharmacol.* 2006;6(3):317–33.
28. Sun Y. Structure and biological activities of the polysaccharides from the leaves, roots and fruits of *Panax ginseng* CA Meyer: An overview. *Carbohydr Polym.* 2011;85(3):490–9.
29. Hong-quan LI. Analysis on chemical components and structure of *Astragalus* polysaccharides [J]. *J Tradit Chinese Vet Med.* 2008;5:2.
30. Zhang N, Li J, Hu Y, Cheng G, Zhu X, Liu F, et al. Effects of astragalus polysaccharide on the immune response to foot-and-mouth disease vaccine in mice. *Carbohydr Polym.* 2010;82(3):680–6.
31. Schalm OW, Carroll EJ, Jain NC. Bovine mastitis. In: *Bovine mastitis. a symposium.* Philadelphia, USA: Lea & Febiger; 1971.
32. Sun H, Xue F, Qian K, Fang H, Qiu H, Zhang X, et al. Intramammary expression and therapeutic effect of a human lysozyme-expressing vector for treating bovine mastitis. *J Zhejiang Univ Sci B.* 2006;7(4):324–30.
33. Rainard P, Riollet C. Innate immunity of the bovine mammary gland. *Vet Res.* 2006;37(3):369–400.
34. Moroni P, Sgoifo Rossi C, Pisoni G, Bronzo V, Castiglioni B, Boettcher PJ. Relationships between somatic cell count and intramammary infection in buffaloes. *J Dairy Sci.* 2006;89(3):998–1003.
35. Kitchen BJ, Kwee WS, Middleton G, Andrews RJ. Relationship between the level of N-acetyl-β-D-glucosaminidase (NAGase) in bovine milk and the presence of mastitis pathogens. *J Dairy Res.* 1984;51(01):11–6.
36. Pyörälä S. Indicators of inflammation in the diagnosis of mastitis. *Vet Res.* 2003;34(5):565–78.
37. Pyörälä S, Pyörälä E. Accuracy of Methods Using Somatic Cell Count and N-Acetyl-β-D-Glucosaminidase Activity in Milk to Assess the Bacteriological Cure of Bovine Clinical Mastitis. *J Dairy Sci.* 1997;80(11):2820–5.

38. Bitter T, Muir HM. A modified uronic acid carbazole reaction. *Anal Biochem.* 1962;4(4):330–4.
39. Barnes-Pallesen FD, Blackmer P, Britten A, Bushnell RB, Van Damme DM, Welcome F. *Laboratory and field handbook on bovine mastitis.* Arlington, VA: Natl. Mastitis Council; 1987.
40. Brown RW, Barnum DA, Jasper DE, McDonald JS, Schultze WD. *Microbiological procedures for use in the diagnosis of bovine mastitis.* Natl. Arlington, VA: Mastitis Council. Inc; 1981. p. 16.

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