Acta Scientiarum. Animal Sciences
ISSN: 1807-8672
Editora da Universidade Estadual de Maringá - EDUEM

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Acta Scientiarum. Animal Sciences, vol. 42, e48425, 2020
Editora da Universidade Estadual de Maringá - EDUEM

DOI: https://doi.org/10.4025/actascianimsci.v42i1.48425

Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v42i1.48425

Auto-lysed yeast and yeast extract effects on dry matter intake, blood cells counts, IgG titer and gene expression of IL-2 in lactating dairy cows under heat stress

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ABSTRACT. The objective of this study was to assess the effects of auto-lysed yeast and yeast extract on performance and immune responses of cows in hot climate in the early lactation period. Twenty five lactating dairy cows randomly assigned to 5 groups and 5 replicates. Cows received basal diet with or without auto-lysed yeast (20 or 40 g/d/head) or yeast extract (20 or 40 g/d/head) as on top-dressed. There were no differences for daily dry matter intake, milk production milk fat and the counts of red blood cells and white blood cells among treatments ($p > 0.05$). There were significant differences among treatments for immunoglobulin G (IgG) level, lymphocyte and neutrophil percentages. Yeast extract had no effect on IgG level, but auto-lysed yeast increased IgG level and neutrophil percentage and decreased lymphocyte percentage ($p < 0.05$). The highest relative interleukin-2 gene expression was for cows received auto-lysed yeast at the level of 40 g/d/head. Yeast extract had no significant effect on interleukin-2 gene expression as compared to the control group. It was concluded that auto-lysed yeast at the level of 40 g/d/head had no effect on performance, but it could positively influence on immune response of lactating dairy cows in hot climate during early period of lactation.

Keywords: immunoglobulin G; immune response; interleukin-2; lymphocyte; neutrophil.

Received on June 21, 2019.
Accepted on December 2, 2019.

Introduction

In the late gestation and early lactation periods, dairy cows encounter with a stressful condition, as they rapidly increase milk production, while losing body condition. Feed management during this period is important as dry matter intake is low and nutrients demand is very high (Nocek, Holt, & Oppy, 2011). Many farmers fed their cows high concentrate ration during this period. A low fiber and high starch ration leads to ruminal subclinical acidosis, which finally resulting in loss of appetite and lower feed intake and milk production, lower fertility rate and weaker immune responses to pathogens (Wankhade et al., 2017). In the summer and during heat stress periods, this condition became more complex. There are some options to overcome this problem, of which offering probiotics such as live yeast or prebiotics such as auto-lysed yeast or yeast extract. Auto-lysed yeast is used as appetite enhancer and immune modulator in non-ruminant animals (Silva et al., 2009; Yalçin, Yalçin, Cakin, Eltan, & Dağaşan, 2010). Two important components in auto-lysed yeast could impact on immune system responses, i.e., mananooligosaccharide and b-glucans (Volman, Ramakers, & Plat, 2008). The β-glucans are known as immune system modulators or stimulants. They are natural and effective stimulants of the innate immune system, and when they come in contact with the phagocytic cells, which recognize the β-1,3 and 1,6 bindings (Petravić-Tominac et al., 2010), these cells are stimulated and will produce cytokines that start a chain reaction inducing an immunomodulation and improving the response capacity of the innate immune system. It has been claimed that β-glucan of live and hydrolyzed yeast could enhance the immune response in dairy cows (Broadway, Carroll, & Burdick Sanchez, 2015; Nocek et al., 2011; Liu et al., 2014), sheep (Khalkhane, Abbasi, Zadeh, & Arian, 2013; Zabek et al., 2013), horse (Jacobs, Gordon, Felippe, & Raub, 2017) and cell cultures (Raa, 2015; Wojcik, 2014).

Yeast extract is a concentrate of aqueous extract of auto-lysed yeast and extensively applied as a media component and nutritional resource for a variety of microorganisms in bacterial culture trials. It is rich in peptides and amino acids, B vitamins, nucleotides, and inositol (Silva et al., 2009).

In the literature, there is limited information concerning the effects of, and comparison between auto-lysed yeast and yeast extract addition on performance and immune responses of ruminants under heat stress. The main purposes of this study was to assess auto-lysed yeast and yeast extract effects on performance and immune responses of dairy cows under heat stress in the early lactation period.

Material and methods

Animals and feeding

This study was done in an industrial dairy farm (3000 heads) located in Alborz province (Karaj, Iran) from the late of May and the early of July 2018 (Temp. 35°C). Experiment was repeated again during Aug and Sep (Temp. 42°C), and as the obtained results were close, the results of first experiment was presented in this study. In the peak of parturition, this study was carried out, as selection of cows with close traits and characteristic is possible. Twenty five lactating dairy cows (5 years old, 3 parities, body weight 650 ± 18, and days in milk 12-15) were selected and randomly housed in a free-stall barn and then assigned to 5 groups and 5 replicates. Cows were fed transition ration in the first week and adapted to places and in the last two days milk production was recorded. Cows were moved or replace to have the same characteristics and minimum variance in milk production for each treatment as needed (three cows were replaced). During the second week, experimental rations were fed for adaptation and followed for three weeks (Table 1). On the last day of the fourth week of experiment, cows were immunized against foot-and-mouth disease virus based on farm program and as a challenge. For each cow, daily dry matter intake and milk production were measured in the fifth week of experiment.

Table1. Ingredients and chemical composition of ration.

Ingredients	% dry matter	Chemical compositions*	Value
Alfalfa hay	23.65	NEl (Mcal kg^{-1})	1.63
Corn silage	17.29	Crude protein (%)	16.1
Wheat bran	5.59	UDP (% of CP)	33.66
Grounded corn grain	6.51	RUP (% of CP)	66.34
Rolled barley grain	28.22	ADF %	19.56
Soybean meal 44%	3.42	NDF %	33.58
Cottonseed meal	6.25	Ca %	0.87
Cottonseed hulls	6.19	P %	0.44
Salt	0.42	Mg %	0.32
Calcium Carbonate	1.07	Na %	0.26
Mineral Mix. **	0.30	K %	1.14
Zeolite	1.32	DCAB meq 100 g^{-1} DM	19

*NEl: net energy for lactation, RUP: rumen undegradable protein, RDP: rumen degradable protein, ADF, acid detergent fiber, NDF: neutral detergent fiber.
** Trace mineral salt contained: CoSO4, 0.068%; CuSO4, 1.04%; FeSO4, 3.57%; ZnO, 0.75%; MnSO4, 1.07%; KI, 0.052%; NaCl, 93.4%.

Dietary treatments

Auto-lysed yeast and yeast extract were obtained from Behan Kimia Enzyme Co. (Tehran, Iran). In the second week of experiment, cows received basal diet with or without auto-lysed yeast or yeast extract as on top-dressed for four weeks as follows: Control group: received basal diet without auto-lysed yeast or yeast extract supplementation; Auto-lysed yeast 1: received 20 g/d/head auto-lysed yeast; Auto-lysed yeast 1: received 40 g/d/head auto-lysed yeast; Yeast extract 1: received 20 g/d/head yeast extract and Yeast extract 2: received 40 g/d/head yeast extract. Cows were fed twice per day and in each meal zero, 10 or 20 g auto-lysed yeast or yeast extract was top dressed for each cow.

Blood and milk sampling and analysis

The blood samples (10 ml) were taken from tail vein three days after vaccination and divided to three parts. A part (3 mL) was poured in sterile glass tube for IgG measurement, a part (3 mL) was poured in sterile tube containing sodium citrate for blood cells counting and a part (4 ml) was poured in EDTA gel containing

tube and kept in liquid nitrogen for analysis of interleukine-2 (IL-2) gene expression. The former samples were centrifuged at 1500 × *g* for 15 min and the serum samples were stored at -70°C until measurement of the serum IgG level using ELIZA kits (Thermo Life Sciences, Basingstoke, UK).

Milk samples were collected in each milking during three consecutive days in the fifth week of experiment. Milk fat was measured using Milkoscan (Foss Electric, Belgium) apparatus. Milk yield was corrected to a 3.5% fat content where 3.5% fat corrected milk (FCM) = kg milk × (0.4255 + (16.425 × fat%/100)).

IL-2 gene expression analysis

Gene expression analysis was done according to the method described by Sweeney, Jones, Habecker, and Scott (1998) in Kharazmi Laboratory (Tehran, Iran). Blood samples for total mRNA extraction were placed in liquid nitrogen and then stored at -80°C. RNA samples were extracted and cDNA was synthesized according to the method described by BioNeer Company (Seoul, South Korea). IL-2 and β-ACTIN sequences were prepared using gene data bases of NCBI (National Center for Biotechnology Information). The β-ACTIN gene was used as an internal control. After preparing the sequences of β-ACTIN and IL-2 on NCBI, the gene-specific primers were designed by primer express software and synthesized by BioNeer Company (Seoul, South Korea). Generation analysis and melting curve was done using a Real-Time PCR System (Applied Bio systems, Foster City, CA).

Statistical Analysis

Statistical analyses were done using GLM procedure of SAS for Windows version 9.1 (Statistical Analysis System [SAS], 2002). The test of Kolmogorov-Smirnov was applied to evaluate the data normality before analysis of variance was performed. Average milk yield of last two days in the first week of experiment was applied as covariate effect in the model for analyzing milk yield. As it was not differ, thus removed. Tukey test was used to compare the means. Statistical differences were declared at $p < 0.05$.

Results and discussion

There are two main differences between auto-lysed yeast and yeast extract. Auto-lysed yeast had shorter autolysis stage of the production process compared to yeast extract, resulting in a partial hydrolysis of the yeast constituents. Also, auto-lysed yeast contains total of cell walls, but major part of cell walls are removed from yeast extract. Yeast cell wall in auto-lysed yeast and biogenic amines in yeast extract have immunogenic effects in animals (Rodríguez-Limas et al., 2014; Broadway et al., 2015). The effects of and comparison between auto-lysed yeast and yeast extract on immune responses of ruminants was not evaluated. Therefore the main objective in this study was to evaluate auto-lysed yeast and yeast extract effects on immune responses of dairy cows under heat stress.

There was no significant difference ($p < 0.05$) for dry matter intake, milk production and milk fat of cows among treatments (Table 2).

Table 2. Daily dry matter intake, milk fat and milk production of cows.

Treatment	Daily dry matter intake (kg day^{-1})	Average milk production (kg day^{-1})	Milk Fat %	3.5% Fat corrected milk (kg day^{-1})
Control	23.3	36.98	3.32	35.90
Auto-lysed yeast 1	24.5	38.01	3.27	36.58
Auto-lysed yeast 2	24.9	38.72	3.18	36.69
Yeast extract 1	24.6	38.70	3.29	37.37
Yeast extract 2	24.8	38.84	3.24	37.19
SEM	1.73	2.16	0.18	1.61

Auto-lysed yeast 1: received 20 g/d/head auto-lysed yeast; Auto-lysed yeast 2: received 40 g/d/head auto-lysed yeast; Yeast extract 1: received 20 g/d/head yeast extract and Yeast extract 2: received 40 g/d/head yeast extract. Means within a column without superscripts are not significantly different ($p > 0.05$).

This finding is disagreement with report of Jouany (2006) and Nocek et al. (2011), who demonstrated that auto-lysed yeast or yeast culture addition to dairy ration resulted in an increase in the dry matter intake and milk production. In the present study, vaccination may be affected energy partitioning in the body of cows and finally on dry matter intake and milk production.

Significant differences ($p < 0.05$) were found for blood immunoglobulin G concentration in cows received auto-lysed yeast compared to the control group (Figure 1). Yeast extract had no effect on IgG level, but auto-lysed yeast increased ($p < 0.05$) serum level of immunoglobulin G compared to the control group. The result of the present study is in line with finding of Yalçin et al. (2010). They reported that supplementation of auto-lysed yeast increased significantly the antibody levels against sheep red blood cells. Antibody responses have been used as measures of the humoral immune status of animals.

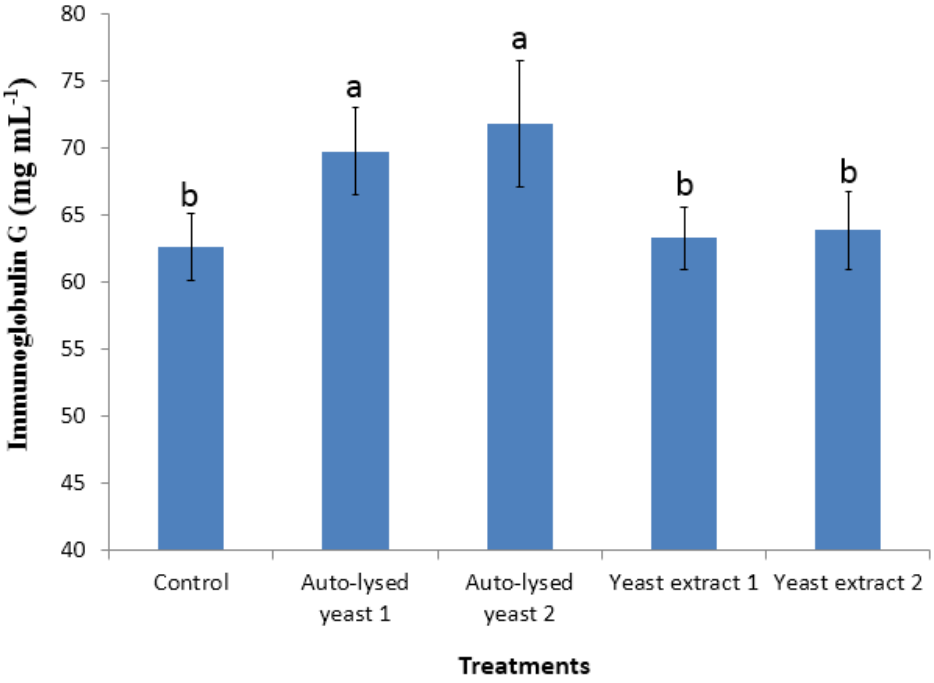

Figure 1. Serum immunoglobulin G concentration. Auto-lysed yeast 1: received 20 g/d/head auto-lysed yeast; Auto-lysed yeast 2: received 40 g/d/head auto-lysed yeast; Yeast extract 1: received 20 g/d/head yeast extract and Yeast extract 2: received 40 g/d/head yeast extract.

The higher antibody level in cows supplemented with auto-lysed yeast might be due to the combined effect of the nucleotides and the glucans and the mannans present in the auto-lysed yeast on the immune system. Oligosaccharides increase the protective antibody response and improve resistance to diseases. By direct action, it can be assumed that oligosaccharides in auto-lysed yeast would bind to macrophage reception sites by recognizing specific sugars found in glucoproteins of the epithelial surface, triggering a cascading reaction that would eventually activate macrophages and release cytokines, thereby activating the acquired immune response (Silva et al., 2009). In addition, Lei, Dong, Jin, Zhang, and Zhou (2013) observed that yeast cell wall can effectively bind lipopolysaccharides within the intestine, preventing translocation into the circulation. Lipopolysaccharides could initiate the inflammatory responses, which finally reduce the immune system activities and immunoglobulin production.

There were no differences among treatments for the counts of red blood cells and white blood cells, but differences exit for lymphocyte and neutrophil percentage (Table 3). Auto-lysed yeast at dose of 40 g/d/head significantly increased the neutrophil percentage and decreased the lymphocyte percentage. Lymphocytes is responsible for release cytokines and growth factors that regulate other immune cells and secretion of antibodies. The increase in IgG level observed in the present work for cows received auto-lysed yeast may be related to increase in lymphocytes activity. Auto-lysed yeast at level of 40 g/d/head increased significantly the neutrophil percentage compared to the control group. This means that auto-lysed yeast could reduce the inflammtatory factors, especially bioactive amines as previously reported by Humer et al. (2018).

Neutrophils are the first cells to migrate to the site of the infection to begin killing the invading microbes. Inconsistence with our finding, results from a study (Van Der Peet-Schwering, Jansman, & Smidt, 2007) showed that yeasts products can lowered the levels of neutrophils in the blood of piglets. Our finding is agreement with the report of Ahiwe et al. (2019) who reported dietary supplementation of chickens with whole yeast, yeast cell wall, yeast glucan, and yeast mannan led to decrease ($p < 0.05$) in lymphocyte and

increase in neutrophil. The lack of consistent results among different papers about immune function may be related to factors such as environmental stress, immune challenge levels, and the complexity of the diets used in these studies. Immune challenge levels was high in the present study, as cows were vaccinated three days before blood sampling. The mechanism of action of yeast products on immune function has not been fully understood.

Table 3. The blood cells counts and lymphocyte and neutrophil ratio.

Treatment	RBC* ($\times 10^6$ µL^{-1})	WBC ($\times 10^6$ µL^{-1})	Neutrophil (%)	Lymphocyte (%)
Control	6.55	12.91	18.13 ^{b}	81.87 ^{a}
Auto-lysed yeast 1**	6.20	14.09	24.07 ^{ab}	75.93 ^{ab}
Auto-lysed yeast 2	6.47	14.14	26.22 ^{a}	73.78 ^{b}
Yeast extract 1	6.28	13.01	21.40 ^{b}	78.60 ^{ab}
Yeast extract 2	6.43	12.80	22.17 ^{b}	77.83 ^{ab}
SEM	0.36	0.96	1.72	1.54

*RBC: red blood cells, WBC: white blood cells. **Auto-lysed yeast 1: received 20 g/d/head auto-lysed yeast; Auto-lysed yeast 2: received 40 g/d/head auto-lysed yeast; Yeast extract 1: received 20 g/d/head yeast extract and Yeast extract 2: received 40 g/d/head yeast extract a, b Means within a column with different superscripts are significantly different ($p < 0.05$).

There were differences among treatments for relative IL-2 gene expression (Figure 2). The highest mean was for cows received auto-lysed yeast at the level of 40 g/d/head. Yeast extract had no significant effect on the relative IL-2 gene expression as compared with the control group. Our finding is consistent with observations of some authors (Majtan, Kogan, Kovacova, Balikova, & Simuth, 2005; Brown, 2006) who reported that yeast components could promote cytokines release in macrophages such as IL-1, IL-2, and IL-6, and could modulate the immune cells (Medzhitov & Janeway, 2000).

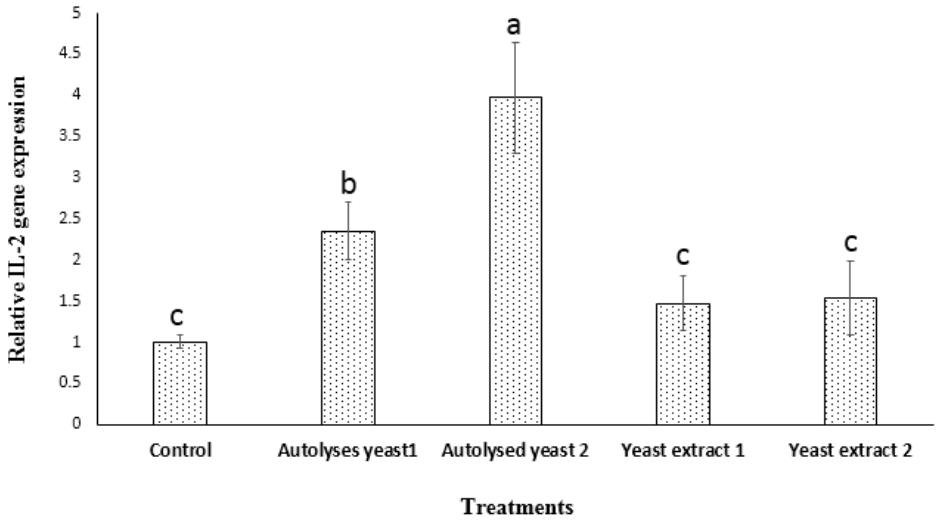

Figure 2. The relative gene expression of interleukine-2 (unit: fold change compared to the control) Auto-lysed yeast 1: received 20 g/d/head auto-lysed yeast; Auto-lysed yeast 2: received 40 g/d/head auto-lysed yeast; Yeast extract 1: received 20 g/d/head yeast extract and Yeast extract 2: received 40 g/d/head yeast extract.

Humer et al. (2018) reported that auto-lysed yeast could modulates ruminal biogenic amines production in dry cows especially those experiencing subacute ruminal acidosis. Cows in this study were in the early of lactation and received high concentrate to forage ratio and experience subacute ruminal acidosis. Biogenic amines could initiate inflammatory responses and reduce the immune function. Also, auto-lysed yeast attenuated the decreasing effect of the high-concentrate feeding on plasma tryptophan. An increased tryptophan catabolism due to the inflammation has been previously associated with suppressed immune responses (Harden et al., 2016). Therefore, auto-lysed yeast may be counteracted inflammatory conditions, through which increased the gene expression of interleukin-2. Besides this effect, auto-lysed yeast contains B-glucans which could initiate cytokine secretion. Kogan and Kocher (2007) reported that α-D-glucan and β-D-glucan are the major components of yeast cell wall and act as immunomodulating compounds. The α-D-glucan and β-D-glucan could bind pathogenic bacteria to prevent colonization in the digestive tract and able to interact with immune cells directly (Kogan & Kocher, 2007).

Conclusion

The yeast extract at two levels (20 and 40 g/d/head) used in this study had no effect, but auto-lysed yeast at level of 40 g/d/head influenced on serum IgG level, differential white blood cells and IL-2 gene expression. It was concluded that auto-lysed yeast at the level of 40 g/d/head had no effect on performance, but it could positively influence on immune response of lactating dairy cows under heat stress during early period of lactation.

Acknowledgements

The authors would like to thanks Dr. Mohsen Nikkhah and Behan Kimia Enzyme Company (Tehran, Iran) for providing hydrolyzed yeast and yeast extract samples.

References

Ahiwe, E. U., Abdallh, M. E., Chang'a, E. P., Al-Qahtani, M., Omede, A. A., Graham, H., & Iji. P. A. (2019). Influence of autolyzed whole yeast and yeast components on broiler chickens challenged with salmonella lipopolysaccharide. *Poultry Science, 98*(12), 7129–7138. doi: 10.3382/ps/pez452

Broadway, P. R., Carroll, J. A., & Burdick Sanchez, N. C. (2015). Live yeast and yeast cell wall supplements enhance immune function and performance in food-producing livestock: a review. *Microorganisms, 3*(1), 417-427. doi: 10.3390/microorganisms3030417

Brown, G. D. (2006). Dectin-1: A signalling non-TLR pattern-recognition receptor. *National Review in Immunology, 6*(1), 33-43. doi:10.1038/nri1745

Harden, J. L., Lewis, S. M., Lish, S. R., Suárez-Fariñas, M., Gareau, D., Lentini, T., ... Lowes M. A. (2016). The Tryptophan Metabolism Enzyme, L-Kynureninase, Is A Novel Inflammatory Factor In Psoriasis And Other Inflammatory Diseases. *Journal of Allergy Clinical and Immunology, 137*(6), 1830–1840. doi: 10.1016/j.jaci.2015.09.055

Humer, E., Kröger, I., Neubauer, V., Schedle, K., Reisinger, N., & Zebeli, Q. (2018). Supplementing phytogenic compounds or autolyzed yeast modulates ruminal biogenic amines and plasma metabolome in dry cows experiencing subacute ruminal acidosis. *Journal of Dairy Science, 101*(10), 9559-9574. doi: 10.3168/jds.2018-14744

Jacobs, R. D., Gordon, M. E., Felippe, M. J., & Raub, R. H. (2017). Beta 1-3,1-6 glucan supplementation modulates the immune response of horses undergoing a glucocorticoid challenge. *Journal of Animal Science, 95*(4), 165. doi: 10.2527/asasann.2017.333

Jouany, J. P. (2006). Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. *Animal Reproduction Science, 96*(1), 250–264. doi: 10.1016/j.anireprosci.2006.08.005

Khalkhane, A. S., Abbasi, K., Zadeh, F. S., & Arian A. H. (2013). Effect of dietary beta-glucan supplementation on humoral and cellular immunologic factors in lambs. *Global Veterinaria, 11*(1), 38-43. doi: 10.5829/idosi.gv.2013.11.1.73188

Kogan, G., & Kocher, A. (2007). Role of yeast cell wall polysaccharides in pig nutrition and health protection. *Livestock Science, 109*(1–3), 161-165. doi: 10.1016/j.livsci.2007.01.134

Lei, C. L., Dong, G. Z., Jin, L., Zhang, S., & Zhou, J. (2013). Effects of dietary supplementation of montmorillonite and yeast cell wall on lipopolysaccharide adsorption, nutrient digestibility and growth performance in beef cattle. *Livestock Science, 158*(1), 57-63. doi: 10.1016/j.livsci.2013.08.019

Liu, J., Ye, G., Zhou, Y., Liu, Y., Zhao, L., Liu, Y., ... Huang, K. (2014). Feeding glycerol-enriched yeast culture improves performance, energy status, and heat shock protein gene expression of lactating Holstein cows under heat stress. *Journal of Animal Science, 92*(1), 2494–2502. doi: 10.2527/jas.2013-7152

Majtan, J., Kogan, G., Kovacova, E., Balikova, K., & Simuth, J. (2005). Stimulation of TNF-alpha release by fungal cell wall polysaccharides. *Zeitschrift fur Naturforschung. C, Journal of Biosciences, 60*(1), 921–926. doi:10.1515/znc-2005-11-1216

Medzhitov, R., & Janeway, C. (2000). Innate immunity. *New England Journal of Medicine, 343*(1), 338–344. doi: 10.1056/NEJM200008033430506

Nocek, J. E., Holt, M. G., & Oppy, J. (2011). Effects of supplementation with yeast culture and enzymatically hydrolyzed yeast on performance of early lactation dairy cattle. *Journal of Dairy Science, 94*(8), 4046-4056. doi: 10.3168/jds.2011-4277

Petravić-Tominac, V., Zechner-Krpan, V., Grba, S., Srečec, S., Panjkota-Krbavčić, I., & Vidović L. (2010). Biological effects of yeast β-glucans. *Agriculturae Conspectus Scientificus, 75*(1), 149-158.

Raa, J. (2015). Immune modulation by non-digestible and non-absorbable beta-13,/1,6-glucan. *Microbial Ecology and Health Disease, 26*(1), 27824. doi: 10.3402/mehd.v26.27824

Rodríguez-Limas, W. A., Pastor, A. R., Esquivel-Soto, E., Esquivel-Guadarrama, F., Ramírez, O. T., & Palomares, L. A. (2014). Immunogenicity and protective efficacy of yeast extracts containing rotavirus-like particles: a potential veterinary vaccine. *Vaccine, 32*(24), 2794-2798. doi: 10.1016/j.vaccine.2014.02.037

Silva, V. K., Silva, J. D. T., Torres, K. A. A., Faria Filho, D. E., Hada, F. H., & Moraes, V. M. B. (2009). Humoral immune response of broilers fed diets containing yeast extract and prebiotics in the prestarter phase and raised at different temperatures. *Journal of Applied Poultry Research, 18*(1), 530–540. doi: 10.3382/japr.2009-00004

Statistical Analysis System [SAS]. (2002). *SAS/STAT User's guide, Version 9.1.* Cary, NC: SAS Institute Inc.

Sweeney, R. W., Jones, D. E., Habecker, P., & Scott, P. (1998). Interferon-gamma and interleukin gene expression in cows infected with Mycobacterium paratuberculosis. *American Journal of Veterinary Research, 59*(1), 842-847.

Van Der Peet-Schwering, C. M. C., Jansman, A. J. M., & Smidt, H. (2007). Effects of yeast culture on performance, gut integrity and blood cell composition of weanling pigs. *Journal of Animal Science, 85*(1), 3099-3109. doi: 10.2527/jas.2007-0110

Volman, J. J., Ramakers, J. D., & Plat, J. (2008). Dietary modulation of immune function by beta-glucans. *Physiology Behavior, 94*(2), 276-284. doi: 10.1016/j.physbeh.2007.11.045

Wankhade, P. R., Manimaran, A., Kumaresan, A., Jeyakumar, S., Ramesha, K. P., Sejian, V., ... Varghese, M. R. (2017). Metabolic and immunological changes in transition dairy cows: A review. *Veterinary World, 10*(11), 1367–1377. doi: 10.14202/vetworld.2017.1367-1377

Wojcik, R. (2014). The effect of Leiber Beta-S (1,3-1,6-β-D-glucan) on the phagocytic activity and oxidative metabolism of peripheral blood granulocytes and monocytes in calves. *Acta Veterinaries Brno, 83*(1), 347-354. doi: doi: 10.2754/avb201483040347

Yalçin, S., Yalçin, S., Cakin, K., Eltan, O., & Dağaşan, L. (2010). Effects of dietary yeast autolysate (*Saccharomyces cerevisiae*) on performance, egg traits, egg cholesterol content, egg yolk fatty acid composition and humoral immune response of laying hens. *Journal of Science, Food and Agriculture, 90*(10), 1695-701. doi: 10.1002/jsfa.4004

Zabek, K., Milewski, S., Wojcik, R., & Siwicki, A. K. (2013). Effect of β-1,3/1,6-D-glucan in diet on productivity and humoral and cellular defense mechanisms in sheep. *Acta Veterinaries BRNO, 82*(1), 141-146. doi: 10.2754/avb201382020141