Short communication: Effect of yeast cell wall supplementation on peripheral leukocyte populations and mRNA expression of cytokines in lactating dairy cows

M. Aung,¹ H. Ohtsuka,² and K. Izumi^{3*}

¹Department of Animal Nutrition, University of Veterinary Science, Nay Pyi Taw, 15013, Myanmar

²Large Animal Internal Medicine, Rakuno Gakuen University, Ebetsu, 069-8501, Japan

³College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu, 069-8501, Japan

ABSTRACT

This study was designed to examine the effect of yeast cell wall (YCW) supplementation on peripheral leukocyte populations and mRNA expression of cytokines in lactating dairy cows. Fourteen Holstein lactating cows were assigned to 1 of 2 treatments; the control group ($n = 7$) were fed a total mixed ration without supplementation and cows in the YCW group ($n = 7$) were fed a total mixed ration supplemented with YCW (SafMannan; Phileo, Lesaffre Animal Care, Lille, France; 10 g/cow per day). Blood samples were collected 3 times during the experimental period [wk 0 (before any treatment), wk 4, and wk 8]. Peripheral leukocyte populations and cytokine mRNA expression of peripheral blood mononuclear cells were measured using flow cytometry and real-time PCR, respectively. Among the peripheral leukocyte populations, TcR1-N12⁺ and CD14⁺ T cells increased at wk 4, and CD4⁺ T cells and CD8⁺ T cells increased at wk 4 and wk 8 with YCW supplementation. The mRNA level of *IL8* tended to be increased in the YCW group at wk 4. Expression of *IL12A* was lower in the YCW group than in the control group before the experiment (wk 0) but no differences were observed at later time points (wk 4 and wk 8). Expression of *IL12A* decreased in the control group and increased in the YCW group. Expression of *CCR2* increased at wk 4, and *CCL2* and *CCL3* were increased at wk 8 in the YCW group. Thus, YCW supplementation increased the mRNA expression of cytokines in peripheral blood mononuclear cells of lactating dairy cows.

Key words: yeast cell wall, cytokines, leukocyte populations, dairy cow

Short Communication

Several feed additives are used to promote the health and production performance of animals used in livestock production. In recent decades, the use of feed additives has been emphasized over antibiotics to enhance animal health and immunity because of concerns regarding antibiotic resistance and drug residues. Yeast is a natural fermentation product, and its derivatives, especially yeast cell wall, contain polysaccharides such as mannan and β -glucan, which are immunomodulating compounds (Jensen et al., 2008). Yeast cell wall can act as a nonspecific modulator of the immune system (Kogan, 2000), with beneficial effects on local and systemic immune responses (Murphy et al., 2007) and innate immune function (Lavigne et al., 2007).

Feeding live yeast increases the expression of genes that enhance neutrophil function, specifically those encoding the IL-4 receptor and IL-1B (Wang et al., 2009), and increases the numbers of circulating T and B lymphocytes (Cakiroglu et al., 2010) in dairy cows. Yeast-containing additive also increased expression of the neutrophil-activating chemokine receptor IL8R and the lymphocyte adhesion molecule L-selectin in whole blood of dairy cows (Playford et al., 2014). In an experiment in transition cows, enzymatically hydrolyzed yeast enhanced the humoral and mucosal immunity and modulated uterine inflammatory signals and mammary gland health (Yuan et al., 2015).

Supplementation of milk replacer with yeast cell wall (YCW) was shown to enhance the acute phase response, antibody production, health scores, and performance in calves (Heinrichs et al., 2003; Ghosh and Mehla, 2012). Moreover, it improved the immune response to rotavirus in dry cows, enhanced the subsequent transfer of rotavirus antibodies to calves (Franklin et al., 2005), and enhanced immune status in lactating cows (Nocek et al., 2011). Although the effects of YCW on immune status of dairy cows have been described by many researchers as mentioned above, its effects on peripheral

Received September 26, 2019.

Accepted January 28, 2020.

*Corresponding author: izmken@rakuno.ac.jp

leukocyte populations and cytokine mRNA expression still need to be elucidated. Walachowski et al. (2016) studied the effect of YCW on cytokine mRNA expression in mice and found that YCW induces release of proinflammatory cytokines and chemokines (*TNFA*, *IL6*, *IL1B*, and *CCL2*) and anti-inflammatory cytokines (*IL10*). We hypothesized that YCW supplementation would enhance cellular immune function with the release of proinflammatory cytokines and chemokines. Thus, this study was designed to examine the effect of YCW supplementation on peripheral leukocyte populations and cytokine mRNA expression of lactating dairy cows.

Fourteen lactating Holstein cows were assigned into 2 treatment groups, with 3 primiparous and 4 multiparous lactating cows in each group. At the start of experiment, cows in the control and YCW groups averaged (mean $\pm$ SE) 164 ± 18 and 152 ± 25 DIM and 1.71 ± 0.29 and 1.57 ± 0.20 parity, respectively. The powder form of yeast cell wall (*Saccharomyces cerevisiae*; SafMannan; Phileo, Lesaffre Animal Care, Lille, France) was used as the dietary supplement in this experiment. Cows in the control group ($n = 7$) were fed a TMR only and the cows in the YCW group ($n = 7$) were fed the TMR with 10 g/cow per day of YCW. The experiment lasted for 8 wk. Cows were housed in a freestall barn covered with rubber chip mattresses, on which wheat straw was spread as bedding. A mineralized salt block and water were freely available at all times. This experiment was carried out at the Rakuno Gakuen Field Education and Research Center (Rakuno Gakuen University, Ebetsu, Hokkaido, Japan). This work was approved by the Animal Experiment Committee of Rakuno Gakuen University for use of animals in experiments.

Blood samples were collected 3 times during the experimental period: wk 0 (before any treatment), wk 4, and wk 8, from the tail vein before morning feeding. Each sample was collected into 2 tubes, one containing K_2 -EDTA for analysis of leukocytes and the second containing heparin for extraction of RNA before analysis of cytokine expression. Peripheral leukocyte populations and cytokine mRNA expression were analyzed by flow cytometry and real-time PCR, respectively.

Two milliliters of each K_2 -EDTA blood sample was mixed with 4 mL of 0.83% ammonium chloride (NH_4Cl) to break down the red blood cells. The blood samples were centrifuged at $1,500 \times g$ for 5 min. After centrifugation, the supernatant was discarded and the leukocytes were collected. The leukocyte samples were washed 3 times with PBS incubated with each mAb. The following antibodies were used: MMIA (mAb for CD3 antigen expressed on pan T cells; VMRD, Pullman, WA), CACT183A (mAb for CD4 antigen expressed

on T helper lymphocytes; VMRD), CACT80C (mAb for CD8 antigen expressed on cytotoxic lymphocytes; VMRD), GC6A (mAb for CD45R antigen expressed on nonactivated cells; VMRD), CACT61A (mAb for TcR1-N12 antigen expressed on $\gamma\delta$ T lymphocytes; VMRD), CAT82A (mAb for MHC class II antigen expressed on monocyte/B lymphocytes; VMRD) and MY4 (mAb for CD14 antigen expressed on monocyte/macrophages; Coulter Immunology, Miami, FL). After suspension in cold PBS, the cells (1×10^6) were incubated with primary mAb at 4°C for 1 h and then washed with PBS. To visualize cell surface markers, rat anti-mouse IgM-Alexa Fluor 488 conjugated antibody and goat anti-mouse IgG-phycoerythrin-conjugated antibody were added and incubated at 4°C for 30 min. After incubation, the cells were washed with PBS and analyzed using 2-color flow cytometry. The data were acquired by 10,000 counts per sample using a Gallios Flow Cytometer (Beckman Coulter, Fullerton, CA).

Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation. Ten milliliters of heparinized blood diluted 2-fold in PBS was placed above Ficoll-Conray solution (Immuno-Biological Laboratories, Gunma, Japan; specific gravity 1.077) and centrifuged at $400 \times g$ for 30 min at room temperature. Mononuclear cells at the interface were collected and washed twice in PBS by centrifugation at $400 \times g$ for 5 min. The isolated PBMC were treated with 0.83% ammonium chloride and rocked gently for 5 min to remove any residual red blood cells. Then, PBMC were washed with PBS and suspended in RPMI 1640 (Invitrogen, Tokyo, Japan) containing 40 U/mL penicillin G (Meiji Seika, Tokyo, Japan) and 0.2 mg/mL streptomycin (Meiji Seika). The isolated PBMC were dispensed into 48-well plates (Greiner Bio-One, Tokyo, Japan) at a density of 5×10^6 cells/well at a final volume of 1 mL/well in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS; Cansera International Inc., Rexdale, Canada). Then, RNA was extracted from PBMC and real-time PCR was performed using the StepOnePlus Real-Time PCR System (Applied Biosystems, Carlsbad, CA). For RNA isolation and cDNA synthesis, the SuperPrep cell lysis & RT kit for qPCR (Toyobo Ltd., Osaka, Japan) was used. In total, 2×10^6 cells from each sample were mixed with 50 μ L of cell lysis mixture (including genomic DNA remover) for 5 min before adding the stop solution. Then, 8 μ L each of mRNA solution extracted from 32 μ L of reaction mixture was added. The primers used to evaluate expression of *IL4*, *IL8*, *IL12A*, *IL17A*, *IFNG*, *ACTB*, *CCL2*, *CCL3*, and *CCR2* were designed as described in Table 1. We used the real-time PCR Kit to analyze RNA expression of cytokines (Toyobo

Table 1. Primers used for real-time PCR expression analysis

Gene	Accession number	Product length (bp)	Primer designation	Sequence (5'-3')
<i>IL4</i>	NM_173921	117	Forward	GCCCCAAAGAACACAACCTGA
			Reverse	GAGATTTCCTGTCAAGTCCGC
<i>IL8</i>	NM_173925.2	150	Forward	GTGAAGAGAGCTGAGAAGCAAG
			Reverse	CACCAGACCCACACAGAACA
<i>IL12A</i>	NM_174356.1	101	Forward	CACCCCGCATTCCTACTTCT
			Reverse	TGACTTTGGCTGAGGTTTGG
<i>IL17A</i>	NM_001008412.2	138	Forward	ATCTCACAGCGAGCACAAGT
			Reverse	GTGGGATGATGACTCCTGCC
<i>IFNG</i>	NM_174086	108	Forward	TCAAATTCGCGTGGATGATCT
			Reverse	CTTCTCTTCCGCTTTCTGAGG
<i>ACTB</i>	NM_173979.3	76	Forward	CCCAGATCATGTTTCGAGACC
			Reverse	GAGGCATACAGGGACAGCAC
<i>CCL2</i>	NM_174006.2	83	Forward	ACCAGCAGCAAGTGTCTCTAAA
			Reverse	CATTTCTGCTTGGGGTCTGC
<i>CCL3</i>	NM_174511.2	117	Forward	CTGACGCTCAAGCCACACT
			Reverse	CGCCAAATGGTGCCGAGAAG
<i>CCR2</i>	XM_010817920.1	109	Forward	TGAAGAACCCACCACCAGTT
			Reverse	GAAGACCAGCGAGTAGAGCG

Ltd.). The thermal cycling conditions were 3 min at 95°C followed by 15 s at 95°C (40 cycles) and 1 min at 60°C. Melting curve analysis was conducted to verify the presence of gene-specific peak and the absence of the primer dimer. The final quantification of mRNA of immune-related molecules was carried out using the comparative threshold cycle method described by Livak and Schmittgen (2001). This method was used following a validation experiment in which efficiencies of the target gene ranged from 96 to 100%, and expression of the reference gene (*ACTB*) of control and YCW groups was 17.27 ± 0.55 and 17.45 ± 0.33 ($P = 0.79$), respectively.

The data were analyzed with repeated measures for non-normal distribution by Mann-Whitney U test to determine the effect of YCW supplementation on peripheral leukocyte populations and cytokine mRNA expression of lactating dairy cows in wk 0, 4, and 8 of the experimental period. We used SPSS 2007 software (version 16.0, SPSS Inc./IBM Corp., Armonk, NY) for all statistical procedures. Differences were considered significant at $P < 0.05$ and to have a tendency at $P < 0.10$.

The effects of YCW supplementation on peripheral leukocyte populations of lactating dairy cows are shown in Figure 1, where it can be seen that the populations of CD3⁺ and MHC class-II⁺CD14⁺ were not affected ($P > 0.05$) by YCW supplementation. The population of TcR1-N12⁺ (T cell receptor) cells was increased in the YCW group at wk 4 ($P = 0.03$). Populations of CD4⁺CD45R⁺ (naïve helper T cells) and CD4⁺CD45R⁺ (helper T cell) also increased with YCW supplementation at wk 4 ($P < 0.01$) and wk 8 ($P = 0.01$), respectively. CD4⁺ is a glycoprotein found on the

surface of immune cells such as helper T cells, monocytes, macrophages, and dendritic cells; it helps to send signals to other types of immune cells, such as CD8⁺, which then destroy the infectious particle (Zhang et al., 2009). Consistent with this concept, a positive effect of YCW supplementation on CD8⁺CD45R⁺ (naïve cytotoxic T cells) was observed at wk 8 ($P < 0.01$) and on CD8⁺CD45R⁺ (cytotoxic T cells) populations at wk 4 ($P < 0.01$) and wk 8 ($P < 0.01$). The population of CD14⁺ cells was increased at wk 4 ($P = 0.02$) with YCW supplementation. This finding was supported by a previous report showing that the β -glucan of YCW can increase the functionality of macrophages and neutrophils (Williams et al., 1996). Moreover, yeast β -glucan can activate macrophages, neutrophils, natural killer cells, and B and T lymphocytes in vivo (Majtán et al., 2005) and in vitro (Jensen et al., 2008). Branson et al. (2016) stated that feeding a yeast-containing additive potentially increases recognition and responses to T-cell activation.

The effects of YCW supplementation on cytokine mRNA expression in PBMC of lactating dairy cows are shown in Figure 2; expression of *IL4*, *IL17A*, and *IFNG* was not affected ($P > 0.05$) by YCW supplementation. Expression of *IL12A* was lower in the YCW group than in the control group at wk 0 ($P = 0.01$); however, no differences were observed at wk 4 ($P = 0.98$) or wk 8 ($P = 0.41$). Decreased expression of *IL12A* expression (-3.27% and -35.23%) in the control group and increased expression (54.07% and 37.16%) in the YCW group were observed in wk 4 and wk 8, respectively. Thus, we can assume that YCW activates expression of *IL12A*, which is likely due to the β -glucans included in YCW. Dectin-1, the major receptor for fungal

Figure 1. Changes in peripheral leukocytes in lactating dairy cows supplemented with yeast cell wall (YCW; SafMannan; Phileo, Lesaffre Animal Care, Lille, France) and an unsupplemented control group. Data are shown as means $\pm$ SE. *Significant difference between groups at the same time point ($P < 0.05$).

Figure 2. Changes in cytokine mRNA expression in peripheral blood mononuclear cells of lactating dairy cows supplemented with yeast cell wall (YCW; SafMannan; Phileo, Lesaffre Animal Care, Lille, France) and an unsupplemented control group. Data are shown as means $\pm$ SE. *Significant difference ($P < 0.05$) or †tendency to differ ($P < 0.10$) between groups at the same time.

β -glucan, works with toll-like receptor (TLR) to promote responses associated with T helper (Th)1 immunity, including production of cytokines such as *IL12A* (Lin et al., 2006), which enhances the lytic activity of natural killer/lymphokine-activated killer cells and stimulates the production of *IFNG* by resting PBMC (Schoenhaut et al., 1992). The greater *IL8* expression observed in YCW group at wk 4 ($P = 0.08$) is similar to that reported by Uchiyama et al. (2012), which stated that supplementation of β -glucan tended to increase *IL8* expression in dairy cows; IL-8 serves as a chemotactic factor for neutrophils and lymphocytes (Hoffmann et al., 2002). Moreover, yeast β -glucan can activate cytokine production in vivo and in vitro (Majtán et al., 2005; Jensen et al., 2008).

An obvious effect of YCW supplementation on mRNA expression of *CCL2* and *CCL3* (encoding chemokine ligand-2 and -3) was observed at wk 8 ($P < 0.01$ and $P = 0.04$, respectively), and a similar effect on *CCR2* (receptor for *CCL2*) expression was found at wk 4 ($P = 0.01$). In an experiment conducted in mice, YCW induced release of proinflammatory cytokines and chemokines (*TNFA*, *IL6*, *IL1B*, and *CCL2*) but also anti-inflammatory cytokines (*IL10*; Walachowski et al., 2016). Zanello et al. (2011) reported that yeast modulated expression of genes involved in inflammation and recruitment and activation of immune cells in porcine intestinal epithelial cells. Chemokine ligand-2 and -3 are also known as monocyte chemoattractant protein 1 (MCP1) and macrophage inflammatory protein 1- α (MIP-1- α), respectively, which recruit monocytes, memory T cells, and dendritic cells to the sites of inflammation (Xu et al., 1996). Walachowski et al. (2016) noted that chemokine production induced by YCW recruits immune cells, such as monocytes, via *CCL2*.

In conclusion, YCW supplementation increased the leukocyte populations (T cell receptor, helper T cells, cytotoxic T cells, and $CD4^+$ cells) as well as expression of chemokines and chemokine receptor (*CCL2*, *CCL3*, and *CCR2*). Thus, YCW supplementation increased mRNA expression of cytokines in PBMCs of lactating dairy cows. This finding highlighted that dietary supplementation of YCW might enhance the cellular immune function of lactating dairy cows.

ACKNOWLEDGMENTS

The authors thank the members of Laboratory of Ruminology (Rakuno Gakuen University, Japan) for their assistance in sample collection and animal care, and the staff of the research farm of Rakuno Gakuen University for their help in animal care. The authors

are very grateful to YPTECH Co. Ltd. (Tokyo, Japan) for its funding assistance. The authors declare that they have no conflicts of interest.

REFERENCES

- Branson, J. A., D. J. McLean, N. E. Forsberg, and G. Bobe. 2016. Yeast-containing feed additive alters gene expression profiles associated with innate immunity in whole blood of a rodent model. *Innate Immun.* 22:249–256. <https://doi.org/10.1177/1753425916640326>.
- Cakiroglu, D., Y. Meral, D. Pekmezci, E. E. Onuk, and Y. B. Kabak. 2010. Effects of yeast culture (*Saccharomyces cerevisiae*) on humoral and cellular immunity of Jersey cows in early lactation. *J. Anim. Vet. Adv.* 9:1534–1538. <https://doi.org/10.3923/javaa.2010.1534.1538>.
- Franklin, S. T., M. C. Newman, K. E. Newman, and K. I. Meek. 2005. Immune parameters of dry cows fed mannan oligosaccharide and subsequent transfer of immunity to calves. *J. Dairy Sci.* 88:766–775. [https://doi.org/10.3168/jds.S0022-0302\(05\)72740-5](https://doi.org/10.3168/jds.S0022-0302(05)72740-5).
- Ghosh, S., and R. K. Mehla. 2012. Influence of dietary supplementation of prebiotics (mannan oligosaccharide) on the performance of crossbred calves. *Trop. Anim. Health Prod.* 44:617–622. <https://doi.org/10.1007/s11250-011-9944-8>.
- Heinrichs, A. J., C. M. Jones, and B. S. Heinrichs. 2003. Effects of mannan oligosaccharide or antibiotics in neonatal diets on health and growth of dairy calves. *J. Dairy Sci.* 86:4064–4069. [https://doi.org/10.3168/jds.S0022-0302\(03\)74018-1](https://doi.org/10.3168/jds.S0022-0302(03)74018-1).
- Hoffmann, E., O. Dittrich-Breiholz, H. Holtmann, and M. Kracht. 2002. Multiple control of interleukin-8 gene expression. *J. Leukoc. Biol.* 72:847–855.
- Jensen, G. S., K. M. Patterson, and I. Yoon. 2008. Yeast culture has anti-inflammatory effects and specifically activates NK cells. *Comp. Immunol. Microbiol. Infect. Dis.* 31:487–500. <https://doi.org/10.1016/j.cimid.2007.08.005>.
- Kogan, G. 2000. (1 $\rightarrow$ 3, 1 $\rightarrow$ 6)- β -D-glucans of yeast and fungi and their biological activity. Pages 107–152 in *Bioactive Natural Products (Part D): Studies in Natural Products Chemistry*. Vol. 23. A. Rahman, ed. Elsevier, Amsterdam, the Netherlands.
- Lavigne, L. M., X. M. O'Brien, M. Kim, J. W. Janowski, J. E. Albina, and J. S. Reichner. 2007. Integrin engagement mediates the human polymorphonuclear leukocyte response to a fungal pathogen-associated molecular pattern. *J. Immunol.* 178:7276–7282. <https://doi.org/10.4049/jimmunol.178.11.7276>.
- Lin, Y. L., S. S. Lee, S. M. Hou, and B. L. Chiang. 2006. Polysaccharide purified from *Ganoderma lucidum* induces gene expression changes in human dendritic cells and promotes T helper 1 immune response in BALB/c mice. *Mol. Pharmacol.* 70:637–644. <https://doi.org/10.1124/mol.106.022327>.
- Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. *Methods* 25:402–408. <https://doi.org/10.1006/meth.2001.1262>.
- Majtán, J., G. Kogan, E. Kováčová, K. Bíliková, and J. Simúth. 2005. Stimulation of TNF-alpha release by fungal cell wall polysaccharides. *Z. Naturforsch. C* 60:921–926. <https://doi.org/10.1515/znc-2005-11-1216>.
- Murphy, E. A., J. M. Davis, A. S. Brown, M. D. Carmichael, A. Ghaffar, and E. P. Mayer. 2007. Oat β -glucan effects on neutrophil respiratory burst activity following exercise. *Med. Sci. Sports Exerc.* 39:639–644. <https://doi.org/10.1249/mss.0b013e3180306309>.
- Nocek, J. E., M. G. Holt, and J. Oppy. 2011. Effects of supplementation with yeast culture and enzymatically hydrolyzed yeast on performance of early lactation dairy cattle. *J. Dairy Sci.* 94:4046–4056. <https://doi.org/10.3168/jds.2011-4277>.
- Playford, M. C., K. Dawson, S. E. Playford, A. N. Smith, S. W. Page, K. Collins, and N. Forsberg. 2014. Effect of an immunomodulatory feed additive on markers of immunity in pasture-fed dairy cows. *Aust. Vet. J.* 92:479–481. <https://doi.org/10.1111/avj.12269>.

- Schoenhaut, D. S., A. O. Chua, A. G. Wolitzky, P. M. Quinn, C. M. Dwyer, W. McComas, P. C. Familletti, M. K. Gately, and U. Gubler. 1992. Cloning and expression of murine IL-12. *J. Immunol.* 148:3433–3440.
- Uchiyama, H., A. Iwai, Y. Asada, D. Muramatsu, S. Aoki, K. Kawata, K. Kusano, K. Nagashima, D. Yasokawa, M. Okabe, and T. Miyazaki. 2012. A small scale study on the effects of oral administration of the β -glucan produced by *Aureobasidium pullulans* on milk quality and cytokine expressions of Holstein cows, and on bacterial flora in the intestines of Japanese black calves. *BMC Res. Notes* 5:189. <https://doi.org/10.1186/1756-0500-5-189>.
- Walachowski, S., G. Tabouret, and G. Foucras. 2016. Triggering dec-1-pathway alone is not sufficient to induce cytokine production by murine macrophages. *PLoS One* 11:e0148464. <https://doi.org/10.1371/journal.pone.0148464>.
- Wang, Y. Q., S. B. Puntunney, J. L. Burton, and N. E. Forsberg. 2009. Use of gene profiling to evaluate the effects of a feed additive on immune function in periparturient dairy cattle. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 93:66–75. <https://doi.org/10.1111/j.1439-0396.2007.00780.x>.
- Williams, D. L., A. Mueller, and W. Browder. 1996. Glucan-based macrophage stimulators: A review of their anti-infective potential. *Clin. Immunother.* 5:392–399. <https://doi.org/10.1007/BF03259335>.
- Xu, L. L., M. K. Warren, W. L. Rose, W. Gong, and J. M. Wang. 1996. Human recombinant monocyte chemotactic protein and other C-C chemokines bind and induce directional migration of dendritic cells *in vitro*. *J. Leukoc. Biol.* 60:365–371. <https://doi.org/10.1002/jlb.60.3.365>.
- Yuan, K., L. G. D. Mendonça, L. E. Hulbert, L. K. Mamedova, M. B. Muckey, Y. Shen, C. C. Elrod, and B. J. Bradford. 2015. Yeast product supplementation modulated humoral and mucosal immunity and uterine inflammatory signals in transition dairy cows. *J. Dairy Sci.* 98:3236–3246. <https://doi.org/10.3168/jds.2014-8469>.
- Zanello, G., M. Berri, J. Dupont, P. Y. Sizaret, R. D’Inca, H. Salmon, and F. Meurens. 2011. *Saccharomyces cerevisiae* modulates immune gene expressions and inhibits ETEC-mediated ERK1/2 and p38 signaling pathways in intestinal epithelial cells. *PLoS One* 6:e18573. <https://doi.org/10.1371/journal.pone.0018573>.
- Zhang, S., H. Zhang, and J. Zhao. 2009. The role of CD4 T cell help for CD8 CTL activation. *Biochem. Biophys. Res. Commun.* 384:405–408. <https://doi.org/10.1016/j.bbrc.2009.04.134>.

ORCIDS

- M. Aung <https://orcid.org/0000-0002-8874-4172>
 K. Izumi <https://orcid.org/0000-0002-9179-957X>