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Research Article
Salmonella Colonization of Production Hens Fed a Parietal Yeast Fraction with High Levels of Mannan and Beta-Glucan

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Abstract

Background and Objective: *Salmonella* is a gram-negative rod-shaped pathogen responsible for approximately 1 million foodborne illnesses per year in the U.S. Previous studies with highly concentrated levels of mannans (>20%) in yeast cell wall have shown to reduce *Salmonella* counts in broiler ceca when added to feed. This study was conducted to understand the effects of concentrated mannans on *Salmonella* in egg producing hens. **Materials and Methods:** A total of 24 Hy-Line W36 layers were challenged with *Salmonella* Typhimurium, 12 birds fed basal diet only (Control) and 12 birds fed the basal diet plus treatment of 500 ppm cell wall. At one-week post challenge, all birds were humanely euthanized and cecal prevalence and enumeration were recorded. **Results:** Cecal counts on birds challenged with *S.* Typhimurium showed a final count of 4.71 $\log_{10}$ CFU mL^{-1}, while yeast cell wall sample counts were 3.71 $\log_{10}$ CFU mL^{-1} ($p = 0.015$). **Conclusion:** A 1 log reduction of cecal *Salmonella* is a biologically important result indicating there may be some potential for this yeast cell wall to impact levels of *Salmonella* Typhimurium in the ceca.

Key words: Foodborne illness, laying hens, parietal yeast fraction, *Salmonella* Typhimurium, yeast cell wall

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INTRODUCTION

Salmonella infections and illnesses across the globe are a persistent concern, as the World Health Organization (WHO) ranks it as 1 of the 4 key global causes of diarrheal illness[1]. These organisms are most commonly associated as ubiquitous in the gastrointestinal tract of poultry, though pathogenicity is far more common in humans. In 2017/2018, outbreak rates have shown no signs of slowing down, as there was an outbreak of *Salmonella* Infantis in 29 states from raw chicken products, an outbreak in 48 states of multiple *Salmonella* serovars linked to live poultry and two of the largest recalls in egg industry history from *Salmonella* Enteritidis and *Salmonella* Braenderup[2]. While *Salmonella* Enteritidis remains the top disease causing serovar in the United States, *Salmonella* Typhimurium remains number 2 and together these serovars have been responsible for over half of all human foodborne *Salmonella* infections[2]. Much attention has been paid to infection from poultry products as a source, as proliferation of the organism is common in many flocks. Surveys have reported prevalence as high as 49% on some layer farms[3] and prevalence as high as 76% in broiler flocks in Canada[4]. With colonization often not causing any clinical infections in many flocks, *Salmonella* prevalence in poultry meat becomes a concern in the processing plant. In the United States the Department of Agriculture (USDA) performance standards are divided into 3 categories, with Category 3 representing those plants which more than 50% of the time fail the standard to have *Salmonella* positives less than 7 out of 52 samples. As of November 2018, 13% of all US chicken plants were in Category 3[5].

In the European Union (EU), the incidence for *Salmonellosis* attributed to eggs and egg products was 65% in 2011, despite poultry meat having a higher prevalence than eggs tested, indicating the increased possibility of consuming raw egg products[6]. Eggs are widely considered to be one of the most nutritious and cost-effective sources of complete protein and this has led to their expanded production in developing nations around the world. Per capita consumption of eggs has reached 179 globally and 264 in the U.S.[7]. This consumption has grown by more than twelve eggs per capita over the last 5 years and the U.S. layer flock exists today at an estimated 319 million layers in production. *Salmonella* Enteritidis is of particular concern in layers, as its virulence factors make it capable of reaching internal organs, particularly ovaries and oviduct[8-10]. The estimated *Salmonella* Enteritidis prevalence rate based on environmental sampling in U.S. layer flocks was 7.1% in 1999 but only 1.7% in a 2013 sampling survey[11]. This could be the result of increased vaccination for the serovar or overall improved biosecurity and monitoring of flocks. Despite this low prevalence rate, over 80% of all *Salmonella* Enteritidis infections are traced back to eggs or egg-containing foods. This is estimated to be about 174,000 illnesses per year[12]. An FDA farm to table assessment concluded that of the 47 billion shell eggs laid each year, as many as 2.3 million were contaminated with *S.* Enteritidis[13]. Hens that are colonized with *Salmonella* Enteritidis can shed the bacteria in feces up to 18 days and the population models of modern poultry production make cross contamination a major issue. Normal hen activities involve pecking and often coprophagy and this increases the risk of fecal oral transmission in a layer house. This route of infection is concerning, as a controlled challenge showed oral inoculation resulted in a bacteremia with seeding of the liver, spleen, peritoneum, ovule and oviduct. However, the birds remained clinically normal with normal egg production[14]. As the egg industry has progressed, about 15% of the birds in the U.S. have moved to cage free systems, further increasing the risk of birds coming in contact with feces and egg shells becoming contaminated[7]. To curb the prevalence of the organism in poultry production systems, multiple intervention strategies have been employed in commercial production systems. Part of heightened oversight through the Food Safety Modernization Act and the Egg Safety Rule, these efforts are expected to prevent the 30 deaths per year that are caused by *Salmonella* Enteritidis from contaminated eggs[1]. This comprehensive approach looks at many areas such as rodent control and monitoring, as this has proven to be a high-risk correlation for *Salmonella* Enteritidis prevalence. Sampling of commercial layer farms rated with low rodent densities in 2012 came back negative for *Salmonella*, while commercial farms with high rodent densities cultured 8% positive for *Salmonella* Enteritidis and *Salmonella* Infantis. For this concern, the egg rule states producers must use a program to control rodents, flies and other pests that includes monitoring for pest activity and removing debris and vegetation that may provide harborage for pests[15]. Cleaning and disinfecting to remove visible manure, feathers and feed after a removing a flock that was positive for *Salmonella* Enteritidis is also part of the rule, as is sourcing pullets from flocks that are NPIP monitored as *Salmonella* Enteritidis clean. Egg producers are required to test for *Salmonella* Enteritidis when hens are 40-45 weeks of age and must test eggs when an environmental test turns up an *Salmonella* Enteritidis positive. Other measures required are controlled temperature, control of eggs throughout the logistics process, maintenance of a

biosecurity program and an approved *Salmonella* Enteritidis monitoring plan on every farm. Not every farm is identical and thus there are a variety of other measures employed in the industry to curb *Salmonella* Enteritidis prevalence. Vaccinating flocks, sanitizing feed and including feed additives in the diet can all contribute to lower risk of *Salmonella*. Vaccination has shown promising yet mixed results, in some cases live vaccines only offer a shortened protection vs killed vaccines offering longer protection[16]. *Salmonella* Enteritidis vaccines in particular when administered 3 times have shown no detection in intestine, internal organs, or eggs of production hens[17]. Feed additives are a category of microbial intervention that has recently gained more attention from producers given the consumer push for eggs and poultry administered no antibiotics. This has led to an increased body of research on probiotics and prebiotics, focusing on efficacy against *Salmonella* colonization. Yeast has been a common part of poultry diets, especially layers, for well over a decade. It is known for its immunomodulatory properties, helping contribute to heightened immune response, as well as intestinal integrity[18]. The microbial profile of the poultry intestine is very important in determining digestibility of nutrients and overall health, as 70% of immune tissue is located in the intestine. Yeast has been shown to alter the microbial profile of the intestine, particularly increasing the number of lactic acid producing bacteria and Bifidobacteria[19]. This in turn leads to improved gut barrier function, which is unfavorable to pathogens and provides efficient utilization of feedstuffs[20]. There is also evidence of yeast influencing the histomorphology of the small intestine as well as improving the humoral immune response, both of which can result in improved growth performance[21]. In looking at specific pathogens, much has been shown in vitro to prove that mannose from hydrolyzed yeast cell wall can agglutinate and bind many gram-negative pathogens[22,23]. *Salmonella* in particular with type I fimbriae have shown particular affinity for mannose *in vitro*[24]. Mannans and β-1, 3 -1, 6 Glucans in hydrolyzed yeast cell wall have also shown to significantly reduce pathogens in combination, such as *Salmonella* in broiler ceca[25]. Line *et al.*[26] showed that dried yeast can influence colonization of ceca, reducing the number of positives in broilers by 92%. While this research seems to indicate a trend toward influencing *Salmonella* populations in young chickens, more proven in vivo studies are needed in laying hens to improve egg safety. The study aimed to evaluate the efficacy of a commercially available yeast cell wall on the reduction of *Salmonella* Typhimurium in the ceca of laying hens.

MATERIALS AND METHODS

A total of 90 replacement pullets were received from a commercial facility and divided into 2 groups of 45 birds each and housed in large floor pens equipped with hanging feeders and nipple drinkers. A basal pullet grower diet was formulated following nutritional recommendations for W-36 Hy-Line pullets[27] (table 1). The basal diet was divided into 2 batches. The first batch remained as a basal diet to serve as the control group. The second batch was supplemented with a proprietary yeast fraction derived from the cell wall of *Saccharomyces cerevisiae* at 500 ppm. The grower diet was fed for approximately 3 weeks. During this period the lighting system was set to provide minimum light exposure to prevent stimulation of ovary function. At 17 weeks of age, both treatments were provided a layer diet formulated following nutritional recommendations for W-36 Hy-Line pullets (table 2). Treatments were maintained in the same fashion as the grower treatments. At 17 weeks of age, the birds were light stimulated with a lighting program that followed the recommendations of the W-36 Hy-Line Management Guide[27]. Lighting was increased on a weekly basis until 16 h of daily light was reached. This lighting plan was maintained throughout the remainder of the study. At 20 weeks of age, 24 pullets were transported to the USDA Southern Plains Facilities. Birds were randomly divided into treatment and control group (12 birds in treatment and 12 birds in control) and allocated into cages (1 bird per cage). Birds were housed in an environmentally controlled rearing room equipped with feeders and nipple drinkers. A second batch of layer feed was made to replicate the layer feed provided at 17 weeks of age, with the same supplementation of treatments. After a 2-week acclimation period (22 weeks of age) the

Table 1: Pullet diet

Ingredients	Percentage
Corn	75.67
Soybean meal	18.97
DL-methionine	0.11
Lysine HCl	0.05
L-threonine (98.5%)	0.02
Limestone	0.83
Biofos	3.58
Salt	0.21
Sodium bicarb	0.26
Trace mineral[1]	0.05
Trace vitamins[2]	0.25

[1]Per pound of premix; Cu: Copper minimum 1.40%; I: Iodine minimum 800.0 ppm, Fe: Iron minimum 12.00%, Mn: Manganese minimum 12.00%, Zn: Zinc minimum 12.00%, [2]Per pound of premix; Vitamin A: 4,000,000 IU, Vitamin D3: 1,400,000 IU, Vitamin E: 16,666 IU, Vitamin B12: 6 mg, Riboflavin (B2): 2166 mg, Niacin (B3): 16,666mg, d-Pantothenic acid (B5): 7334 mg, Choline: 47,383 mg, Menadione: 534 mg, Folic acid (B9): 634 mg, Pyridoxine (B6): 2,600 mg, Thiamine (B1): 1,066 mg, d-Biotin: (B7) 200 mg

pullets were orally gavaged with 8.7×10^9 CFU mL^{-1} of *Salmonella* Typhimurium. (3 mL gavage per bird). One week after the *Salmonella* challenge, the layers were humanely euthanized and samples were taken from the ceca and ovary for further analysis.

For the first phase of the trial (14-19 week), birds were allocated in 2 floor pens equipped with handing feeders and nipple drinkers located at the Texas A&M Poultry Research Center. For the second phase of the trial (Challenge: 20-23 week), birds were allocated into an environmentally controlled rearing rooms equipped with 2 stainless steel A-frame layer cages. No antibiotics or coccidiostats were used in this experiment. The pullets received standard vaccinations as part of the vaccination program up to 8 weeks of age. Industry type pullet and layer diets were formulated to meet the birds requirements according to the Hy-Line W36 management guide. Birds were kept as closely to this feeding program as possible. The only difference between the control and treatment diets was the inclusion of yeast cell wall (YCW) feed additive at 500 ppm in the treatment group. Birds were observed daily with regard to general flock condition, temperature, water, feed and egg production. Egg production factors were recorded daily at the end of the day. *Salmonella* prevalence in ceca samples were compared between treatment groups using Fisher's exact test. *Salmonella* counts in culture-positive ceca were compared between treatments using a two-sample t-test. A Tobit regression model was also used to compare treatments with respect to *Salmonella* counts in ceca while considering culture-negative samples to be censored at a lower limit of 2.5 $\log_{10}$ CFU mL^{-1}. For the comparison of *Salmonella* counts, samples with a negative culture result by the direct plating method but a positive result by enrichment were arbitrarily assigned a count of 500 CFU mL^{-1}, which was equal to one-half the minimum detection limit of the direct plating assay. Counts were log-transformed prior to statistical analysis. All statistical testing assumed a two-sided alternative hypothesis and $p<0.05$ was considered significant. Analyses were performed using commercially available statistical software (Stata version 15.1, StataCorp LLC, College Station, TX).

Samples of ceca and ovaries were taken from the birds at termination and divided in half. For ceca samples, half of each ceca was placed into a conical tube containing Rappaport Vassiliadis (RV) broth to use as an enrichment method for determination of prevalence. For ovary samples, the ovaries were weighed and divided in half. Half was placed into a conical tube containing RV broth. After 24 h of incubation, samples were homogenized by shaking and a sterile loop was used to plate a sample onto Xylose-Lysine-Tergitol 4 (XLT-4) Agar. Samples were deemed positive after 24 h of incubation through the visual identification of colonies on the plates.

For ceca and ovary counts, the other halves of the ceca and ovaries were diluted using a 10x dilution series and plated onto XLT-4 Agar treated with Novobiocin and Nalidixic acid for use as a selective growth media. Counts were determined by visual inspection of colonies after 48 h of incubation.

Table 2: Layer diet

Ingredients	Percentage
Corn	50.22
Soybean meal	29.88
DL-methionine	0.29
L-threonine (98.5%)	0.05
Soybean oil	4.88
Limestone	11.68
Biofos	2.23
Salt	0.48
Trace mineral[1]	0.05
Trace vitamins[2]	0.25

[1]Per pound of premix; Cu: Copper minimum 1.40%, I: Iodine minimum 800.0 ppm, Fe: Iron minimum 12.00%, Mn: Manganese minimum 12.00%, Zn: Zinc minimum 12.00%, [2]Per pound of premix; Vitamin A: 4,000,000 IU, Vitamin D3: 1,400,000 IU, Vitamin E: 16,666 IU, Vitamin B12: 6 mg, Riboflavin (B2): 2166 mg, Niacin (B3): 16,666 mg, d-Pantothenic acid (B5): 7334 mg, Choline: 47,383 mg, Menadione: 534 mg, Folic acid (B9): 634 mg, Pyridoxine (B6): 2,600 mg, Thiamine (B1): 1,066 mg, d-Biotin (B7): 200 mg

RESULTS

Results are summarized in Table 3-5. Table 3 shows the prevalence of *S*T in the ceca samples and Table 4 the ovary samples. Control samples were 100% positive (12 of 12) when cultured for *S*T, while YCW samples were 83.33% positive (10 of 12). Control ovaries were 33.33% positive (4 of 12) vs 25% positive for YCW (3 of 12). Table 5 shows mean ceca counts and after enumeration control sample counts were 4.71 $\log_{10}$ CFU mL^{-1}, while YCW sample counts were 3.41 $\log_{10}$ CFU mL^{-1} ($p = 0.015$). Figure I shows the levels of *Salmonella* Typhimurium in the positive ceca as a dot plot.

Table 3: *Salmonella* Typhimurium prevalence (%) in ceca samples by treatment group

Treatments	No. samples	No. positive (%)	p-value
Control	12	12 (100.0)[a]	0.478
Safmannan	12	10 (83.3)[a]	
Total	24	22 (91.7)	

Percentages with a superscript in common do not differ with a level of significance of 5%

Table 4: *Salmonella* Typhimurium prevalence (%) in ovary samples by treatment group

Treatments	No. samples	No. positive (%)
Control	12	4 (33.3)
Safmannan	12	3 (25.0)
Total	24	7 (31.8)

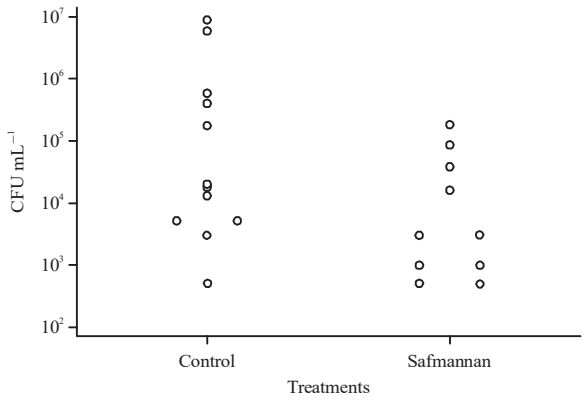

Fig. 1: Dot plot of *Salmonella* Typhimurium counts in culture-positive ceca samples by treatment

Table 5: Estimated marginal means (SE) for *Salmonella* $\log_{10}$ CFU mL^{-1} in ceca samples by treatment based on a Tobit censored regression model. There were 2 left-censored (culture-negative) observations and 22 uncensored (culture-positive) observations

Treatments	No.	Mean (SE)	p-value
Control	12	4.71[b] (0.35)	0.015
Yeast cell wall (500 ppm)	12	3.41[a] (0.35)	

Marginal means with a superscript in common do not differ with a level of significance of 5%

DISCUSSION

There was a low number of *Salmonella* Typhimurium positive ovaries (Table 4) in both groups compared to the number of positive ceca (Table 3). This is consistent with previous studies demonstrating that *Salmonella* Typhimurium is less commonly isolated from the interior of eggs than *Salmonella* Enteritidis[28]. There were no statistical differences between treatments in the number of ovaries or ceca colonized by ST. The YCW treatment significantly reduced the level of ST in the ceca by 1 log resulting in less ST being shed into the environment. *Salmonella* spp. can bind to mannose via the type-1 binding fimbriae. The cell wall fraction of *Saccharomyces cerevisiae* has been shown to bind a variety of gram negative organisms[29]. Reduction of the level of *Salmonella* in the ceca will reduce the overall load in the environment leading to reduced risk of egg shell contamination and transmission of foodborne illness. Yeast cell wall significantly reduced the load of *Salmonella* Typhimurium in the ceca of the layer type hens. The use of YCW as a prebiotic in layer diets can decrease the cecal load of *Salmonella* Typhimurium leading to lower contamination of the environment effectively reducing the risk of the zoonotic transmission of *Salmonella* Typhimurium.

CONCLUSION

The yeast cell wall product in this trial reduced the level of *Salmonella* Typhimurium in the ceca by 1 log. A 1 log reduction of cecal *Salmonella* is a biologically important result indicating there may be some potential for this yeast cell wall to impact levels of *Salmonella* Typhimurium in the ceca. A reduction of Salmonella in the ceca will reduce the amount shed into the environment reducing the risk of fecal contamination of eggs that enter the food supply.

SIGNIFICANCE STATEMENT

This study discovered that yeast cell wall with high levels of mannan and beta-glucan can reduce the level of *Salmonella* Typhimurium in the ceca of laying hens. The reduction of cecal *Salmonella* load benefits poultry producers by reducing risk of *Salmonella* contamination of the food supply. This study can help researchers to uncover new ways to reduce the risk of *Salmonella* contamination of food using on farm interventions.

REFERENCES

1. WHO., 2018. World Health Statistics 2018: Monitoring Health for the SDGs, Sustainable Development Goals. World Health Organization, Geneva, Switzerland, ISBN-13: 9789241565585, Pages: 103.
2. CDC., 2013. An atlas of *Salmonella* in the United States, 1968-2011: Laboratory-based enteric disease surveillance. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA., USA.
3. Hulaj, B., P. Cabeli, I. Goga, N. Taylor, C. Hess and M.L. Hess, 2016. Survey of the prevalence of *Salmonella* species on laying hen farms in Kosovo. Poult. Sci., 95: 2030-2037.
4. Poppe, C., R.J. Irwin, S. Messier, G.G. Finley and J. Oggel, 1991. The prevalence of *Salmonella enteritidis* and other *Salmonella* spp. among Canadian registered commercial chicken broiler flocks. Epidemiol. Infect., 107: 201-211.
5. FSIS., 2019. Pathogen reduction-*Salmonella* and *Campylobacter* performance standards verification testing. United States Department of Agriculture, Food Safety and Inspection Service, Maryland, USA., February 25, 2019.
6. EFSA Panel on Biological Hazards, 2011. Scientific opinion on a quantitative estimation of the public health impact of setting a new target for the reduction of *Salmonella* in broilers. EFSA J., Vol. 9, No. 7. 10.2903/j.efsa.2011.2106
7. UEP., 2019. Facts and stats. United Egg Producers (UEP), Johns Creek, GA., USA. https://unitedegg.com/facts-stats/.

8. Guan, J., C. Grenier and B.W. Brooks, 2006. *In vitro* study of *Salmonella enteritidis* and *Salmonella typhimurium* definitive type 104: Survival in egg albumen and penetration through the vitelline membrane. Poult. Sci., 85: 1678-1681.
9. Humphrey, T.J., A. Baskerville, S. Mawer, B. Rowe and S. Hopper, 1989. *Salmonella enteritidis* phage type 4 from the contents of intact eggs: A study involving naturally infected hens. Epidemiol. Infect., 103: 415-423.
10. Poppe, C., 1999. Epidemiology of *Salmonella enterica* Serovar Enteritidis. In: *Salmonella enterica* Serovar Enteritidis in Humans and Animals: Epidemiology, Pathogenesis and Control, Saeed, A.M., R.K. Gast, M.E. Potter and P.G. Wall (Eds.). Blackwell, Ames, IA., USA., ISBN: 0813827078, pp: 3-18.
11. USDA., 2014. Layers 2013, part II: Control and prevention of *Salmonella* enteritidis on table-egg farms in the United States, 2013. United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Animal Health Monitoring System, Fort Collins, CO., USA., August 2014.
12. CDC., 2017. Foodborne diseases active surveillance network (FoodNet): FoodNet 2015 surveillance report (final data). U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA., USA.
13. Gale, S.F., 2006. Radlo foods hatches a high-tech egg safety plan. https://www.foodsafetymagazine.com/magazine-archive1/december-2005january-2006/radlo-foods-hatches-a-high-tech-egg-safety-plan/.
14. Shivaprasad, H.L., J.F. Timoney, S. Morales, B. Lucio and R.C. Baker, 1990. Pathogenesis of *Salmonella* enteritidis infection in laying chickens. I. Studies on egg transmission, clinical signs, fecal shedding and serologic responses. Avian Dis., 34: 548-557.
15. Food and Drug Administration, 2009. Prevention of *Salmonella* enteritidis in shell eggs during production, storage and transportation. Federal Register, 74: 33030-33101.
16. Groves, P.J., S.M. Sharpe, W.I. Muir, A. Pavic and J.M. Cox, 2016. Live and inactivated vaccine regimens against caecal *Salmonella* Typhimurium colonisation in laying hens. Aust. Vet. J., 94: 387-393.
17. Kilroy, S., R. Raspoet, F. Haesebrouck, R. Ducatelle and F. van Immerseel, 2016. Prevention of egg contamination by *Salmonella* Enteritidis after oral vaccination of laying hens with *Salmonella* Enteritidis Δ*tolC* and Δ*acrABacrEFmdtABC* mutants. Vet. Res., Vol. 47. 10.1186/s13567-016-0369-2
18. Swiatkiewicz, S., A. Arczewska-Wlosek and D. Jozefiak, 2014. Immunomodulatory efficacy of yeast cell products in poultry: A current review. World's Poult. Sci. J., 70: 57-68.
19. Roto, S.M., P.M. Rubinelli and S.C. Ricke, 2015. An introduction to the avian gut microbiota and the effects of yeast-based prebiotic-type compounds as potential feed additives. Front. Vet. Sci., Vol. 2. 10.3389/fvets.2015.00028
20. Chen, C.Y., S.W. Chen and H.T. Wang, 2017. Effect of supplementation of yeast with bacteriocin and *Lactobacillus* culture on growth performance, cecal fermentation, microbiota composition and blood characteristics in broiler chickens. Asian-Australas. J. Anim. Sci., 30: 211-220.
21. Muthusamy, N., S. Haldar, T.K. Ghosh and M.R. Bedford, 2011. Effects of hydrolysed *Saccharomyces cerevisiae* yeast and yeast cell wall components on live performance, intestinal histo-morphology and humoral immune response of broilers. Br. Poult. Sci., 52: 694-703.
22. Perez-Sotelo, L.S., M. Talavera-Rojas, H.G. Monroy-Salazar, S. Lagunas-Bernabe, J.A. Cuaron-Ibarguengoytia, R.M. de Oca Jimenez and J.C. Vazquez-Chagoyan, 2005. *In vitro* evaluation of the binding capacity of *Saccharomyces cerevisiae* Sc47 to adhere to the wall of *Salmonella* spp. Rev. Latinoam. Microbiol., 47: 70-75.
23. Tiago, F.C.P., F.S. Martins, E.L.S. Souza, P.F.P. Pimenta and H.R.C. Araujo *et al*., 2012. Adhesion to the yeast cell surface as a mechanism for trapping pathogenic bacteria by *Saccharomyces probiotics*. J. Med. Microbiol., 61: 1194-1207.
24. Ganner, A. and G. Schatzmayr, 2012. Capability of yeast derivatives to adhere enteropathogenic bacteria and to modulate cells of the innate immune system. Applied Microbiol. Biotechnol., 95: 289-297.
25. Kiros, T.G., T. Gaydos, J. Corley, R. Raspoet, R. Berghaus and C. Hofacre, 2018. Effect of *Saccharomyces cerevisiae* yeast products in reducing direct colonization and horizontal transmission of *Salmonella* Heidelberg in broilers. J. Applied Poult. Res., 28: 23-30.
26. Line, E.J., S.J. Bailey, N.A. Cox, N.J. Stern and T. Tompkins, 1998. Effect of yeast-supplemented feed on *Salmonella* and *Campylobacter* populations in broilers. Poult. Sci., 77: 405-410.
27. Hy-Line International, 2016. Hy-Line W-36 commercial layers: Management guide. Hy-Line International, West Des Moines, IA., USA., January 2016. https://www.hyline.com/UserDocs/Pages/36_COM_ENG.pdf.
28. Wales, A.D. and R.H. Davies, 2011. A critical review of *Salmonella* Typhimurium infection in laying hens. Avian Pathol., 40: 429-436.
29. Posadas, G.A., P.R. Broadway, J.A. Thornton, J.A. Carroll and A. Lawrence *et al*., 2017. Yeast pro- and paraprobiotics have the capability to bind pathogenic bacteria associated with animal disease. Transl. Anim. Sci., 1: 60-68.