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Immunomodulatory Potential of Microencapsulated Multispecies Probiotic Consortium in Newcastle Disease Virus Vaccinated Chicken

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ABSTRACT

A study was conducted to investigate the effect of microencapsulated probiotic consortium containing *Lactobacillus plantarum, Enterococcus faecium, Enterococcus hirae, Pediococcus acidilactici* and *Weissella paramesenteroides* on immune modulation in Newcastle Disease vaccinated chicken. Humoral immune response was assessed by ELISA. Th1, Th2 cytokine response and cell mediated immune response were assessed by using Real time PCR and flow cytometry respectively. Results indicated significantly (P< 0.01) higher antibody titer and also higher IL-2, IL-12, IL-4 and IL-10 cytokine expression in NDV vaccinated multispecies probiotic fed group compared to commercial probiotic fed and control groups. It was also observed that higher proportions of Bu1A (B cell receptor) and CD3 (T cell receptor) positive cells in chicken fed with multispecies probiotic supplementation. Hence, it is concluded that multispecies probiotic played an important role in augmenting humoral and cell mediated immune response against NDV.

Key words: Immunomodulation, Multispecies probiotic, Microencapsulation, Newcastle Disease.

INTRODUCTION

In poultry industry, prevention and control of diseases have led to a substantial increase in use of antibiotics in recent years. The search for alternative to antibiotics in poultry feed and addition of probiotics has been proposed due to increasing reports of drug resistance developing microorganisms led to severe diseases and its residues presence in meat which is potential health hazard to consumers (Fuller, 2001). According to the FAO and WHO, probiotics are “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Lee and Salminen, 2009). It has been reported that certain *Lactobacilli* can induce an increase in the cellular or humoral systemic immune response (Perdigon *et al*.1988) and can also influences the cells such as macrophage involved in the inflammatory immune response (Kato *et al*. 1983; Perdigon *et al*. 1988). The mRNA expression levels of Toll like receptors (TLRs) TLR2 and TLR4 was increased (P<0.05) when graded levels of the probiotic and prebiotic were supplemented, while, TLR7 did not show any significant change (Sheoran *et al*, 2018). There are also reports about the effect of LAB (Lactic Acid Bacteria) as immunostimulator and as inducer of cytokine release (De Simone *et al*. 1995). Several studies has been carried over to assess the effect of probiotics on humoral (Landy and Kavyani, 2013; Talebi *et al*. 2015) and cell mediated immune response (Brisbin *et al*. 2011; Dalgaard *et al*. 2010) in NDV vaccinated chicken.

Timmerman *et al*. (2006) provided evidence that multispecies probiotics are more effective than monospecies probiotics and also species-specific probiotics elicit different health effects than do probiotics derived from another host species. It has been shown that probiotics stimulate different subsets of immune system cells to produce cytokines, which in turn play a role in the induction and regulation of the immune response. Ghafoor *et al*. (2005) studied effects of probiotics (Protexin) on the hemagglutination inhibition HI titer of antibodies against avian influenza virus (AIV) and post field AIV challenge. The findings were compared with the cyclophosphamide treated AIV-vaccinated; untreated and AIV-vaccinated and unvaccinated and control birds. This investigation revealed that protexin treated chicks have higher AIV-HI antibody and no AIV post challenge mortality compared to the cyclophosphamide treated and untreated chicks. The overall finding of this study clearly demonstrate that the use of this multi strain probiotics has good effect on immune response of broilers.

Khalifa *et al*. (2014) evaluated the effect of probiotic and/or *Escherichia coli* infection on antibody responses to routine vaccination against New Castle Disease Virus (NDV) and Infectious Bursal Disease Virus (IBDV) in broiler chicks. They concluded that using of probiotic routinely in ration of broiler chicks enhances immune status and improves

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humoral immune response against NDV and IBDV as well as treatment of *E. coli* infection in chicks before vaccination program improve the antibody response against NDV and IBDV vaccines. Keeping in view of the above facts, a study has been undertaken to evaluate the effect of microencapsulated multispecies probiotic supplementation on immune response against Newcastle disease vaccinated broiler chicken.

MATERIALS AND METHODS

Probiotic consortium preparation, Experimental chicken and management

Probiotic consortia containing *Lactobacillus plantarum, Enterococcus faecium, Enterococcus hirae, Pediococcus acidilactici and Weissella paramesenteroides* was made based on their immunomodulatory potential using *in vitro* cytokine expression studies and T and B cell receptors profiling (Divya *et al*, 2019) was used in this study.

Commercial day-old VenCobb broiler chicks (n=40) were wing banded, weighed individually and assigned to four groups on the basis of initial body weight. Each trial group had 10 broiler chicks. Group-1 were fed with microencapsulated probiotic formulation and vaccinated against NDV B1 and Lasota vaccine, Group-2 were fed with commercial probiotic organisms and vaccinated against NDV B1 and Lasota vaccine, Group-3 were used as Positive Control group without probiotic treatment and vaccinated against NDV B1 and Lasota vaccine, Group-4 were used as Negative Control Group – unvaccinated group without probiotic treatment Chicks were fed with chick mash during 1st two weeks of age, followed by fed with grower mash. The broilers were reared for a period of five weeks under cage system.

Microencapsulated probiotic consortium in a beads form (2×10^8 CFU/g) containing *Lactobacillus plantarum, Enterococcus faecium, Enterococcus hirae, Pediococcus acidilactici* and *Weissella paramesenteroides*. commercial probiotic consists of *Saccharomyces cerevisiae* SC-47, *Saccharomyces boulardii*, *Lactobacillus acidophilus*, *Propionibacterium freudenreichii* and Sea weed powder. All the birds were kept under uniform management conditions throughout the experimental period.

Vaccination schedule

The birds were vaccinated against Newcastle Disease according to the routine vaccination schedule. Chicks were vaccinated against B1 Strain (live, lentogenic) of NDV at 7th day of age (ocular), then by Lasota strain (live, lentogenic) at 21st day of age (oral) based on the routine vaccination schedule and dose.

Feed supplementation

Experimental birds were supplemented with 1gram of microencapsulated probiotic consortium per chick for 1-7 days age, then 3 grams per chick for 7-14 days age, followed by 6 grams per chick for 14-35 days age. The encapsulated beads containing probiotic organisms were mixed in feed and fed to chicken. The commercial probiotic powder was fed to chicken as per recommended level mentioned in product details. Commercial feed was fed to chicken as per recommended level mentioned in broiler performance goals-venCobb400 feed chart.

Assessment of humoral immune response by ELISA

Serum collection: Sera collected (n=24) from 6 birds each in all four trial groups on 7th, 14th and 28th after primary and booster vaccination against NDV vaccine.

Serum antibody titres assessment: Enzyme linked immuno sorbent assay (ELISA) was performed to assess the antibody titres against NDV. The absorbance was measured at 405nm by ELISA plate Reader. The optical density was converted into titre values based on the calculation recommended by kit manufacturer (Silva *et al*., 2009).

Assessment of chicken Th 1 and Th 2 cytokine profile by real time PCR

Preparation of peripheral blood mononuclear cells (PBMC): Blood samples (n=24) from 6 birds each in all four trial groups after 7 days of primary and booster vaccination. Peripheral blood mononuclear cells were prepared (Brisbin *et al*. 2010).

RNA extraction and quantification: RNA was extracted from peripheral mononuclear cell suspension (n=24) by using Nucleospin™ RNA extraction kit. The extracted RNA was quantified using Picodrop Spectrophotometer (Nanodrop 2000, Thermo Scientific Inc).

cDNA synthesis: cDNA was synthesized from extracted RNA using iScript cDNA synthesis Kit (Bio Rad Inc) as per the manufacturer's instructions by the addition of the following reagents of total 20 µl mix in 0.2 ml PCR tubes *viz.,* RNA (2 µg) - 10 µl, iScript Reaction Mix (5X)- 4 µl, iScript reverse transcriptase - 1 µl and nuclease free water - 5 µl.

The tubes were then incubated at 25°C for 5 minutes, subsequently at 42°C for 30 minutes and finally at 85°C for 5 minutes. The resultant synthesized cDNA was stored at -20°C until further use.

Cytokine profiling by Real time PCR: Real time quantification of cytokines expression with respective to *in vivo* in different groups were fed with microencapsulated probiotic consortium, commercial probiotic powder and control group were performed in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad) using the SyBr green dye. Further protocol was followed as per Brisbin *et al*. (2010). The lists of primers used in the real time PCR were given in the following Table 1.

Assessment of cell mediated immune response by flow cytometry

Preparation of splenic leucocytes: Spleen tissues were collected (n=24)after 21 days of 1st vaccine against NDV in sample collection containers. Further process was followed as per protocol mentioned in the Brisbin *et al.* (2010).

Table 1: List of primers used in Real time-PCR.

Primer Category	Primer name	Primer sequence	No of bases	Reference
Th1 response	IL2F	5'- TTGGCTGTATTTCGGTAGCA- 3'	20	Nang *et al*., (2011)
	IL2R	5'- GTGCACTCCTGGGTCTCAGT- 3'	20	
	IL12p40 F	5'- TTGCCGAAGAGCACCAGCCG- 3'	20	Brisbin *et al*., (2010)
	IL12p40 R	5'- CGGTGTGCTCCAGGTCTTGGG- 3'	21	
Th2 response	IL4 F	5'- GGAGAGCATCCGGATAGTGA- 3'	20	Nang *et al*., (2011)
	IL4 R	5'- TGACGCATGTTGAGGAAGAG- 3'	20	
	IL10 F	5'- AGCAGATCAAGGAGACGTTC- 3'	20	Brisbin *et al*., (2010)
	IL10R	5'- ATCAGCAGGTACTCCTCGAT- 3'	20	
Reference gene	bActin F	5'- GTACCCTGGCATTGCTGACCC- 3'	20	
	bActin R	5'- CGGATTCATCGTACTCCTGC- 3'	20	

F – Forward primer, R – Reverse primer.

Assessment of B cell receptor expression using FITC conjugated Bu1A antibody by flow cytometry: Samples were prepared for analysis of B cell expression by flow cytometry as per protocol mentioned by Dong *et al*. (2011). Bu1A FITC conjugate was used to analyze the B cell expression in this study.

Analysis of T cell receptor expression using FITC conjugated CD3 antibody by flow cytometry: The same protocol was followed (as mentioned in 2.6.2) for flow cytometry studies using B cell receptor and instead of Bu1A FITC conjugate, 0.15µg of rat anti human CD3: FITC antibody (Bio-Rad Laboratories India Pvt. Ltd) targeted against T cell was used. Phenotypical results were expressed as both the percentage of Bu1A and CD3 positive cells or mean fluorescence intensity (MFI) with in a particular cell subset. Data was analyzed by using BD FACS software (Dong *et al*. 2011).

Assessment of innate immune response through macrophage activity by immunohistochemistry method: To assess the innate immune response, immunohisto-chemistry was performed and enumerated macrophage population in spleen tissues by using CD163 receptor after 21 days of post vaccination. Macrophage count was done by electron microscopy in 6 fields at 10x magnification.

RESULTS AND DISCUSSION

Multispecies probiotics effect on innate immune response

Macrophage activity in probiotic treated chicken: The immunocytochemistry showed increase in number of macrophages in probiotc treared group which provided the evidence for involvement of probiotic towards innate immunity. Approximatly, 438, 359 and 119 macrophages were observed in microencapsulated probiotic treated group, commercial probiotic treated group and control group, respectively. Results were shown in Fig 1. Similar results were observed by Lin and Karin (2007). They concluded that macrophage activity was increased in probiotic fed group chicken and phagocytic activity of macrophages also increased due to probiotic feeding (*L. plantarum*), subsequently cytokine expression also increased upon macrophage activation. Kristeen *et al*. (2017) stated that macrophage population increased upon infection with both genotypes of NDV but NDV AF2240 resulted in a higher peak in macrophage cell number at 2.86 × 10^5 ± 16 cells compared to NDV IBS002 at 1.70 × 10^5 ± 15 cells on the fourth day after infection.

Multispecies Probiotic effect on humoral immune response: Antibody titre values to find out the humoral immune response against NDV were measured by ELISA

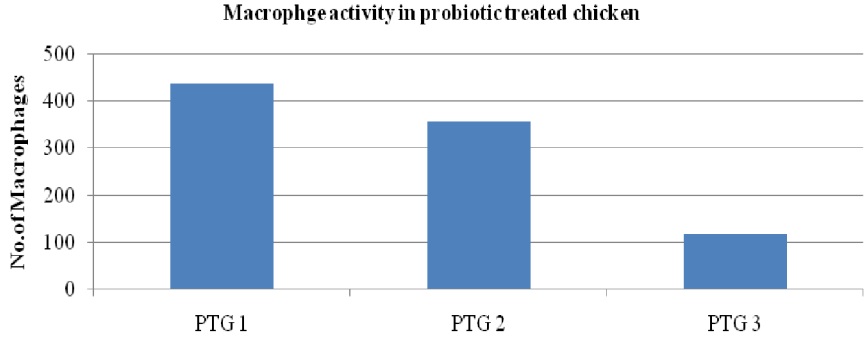

Fig 1: Macrophge activity in probiotic treated chicken.

PTG-1: microencapsulated probiotic treated group, PTG-2: commercial probiotic treated group, PTG-3: vaccinated and without probiotic treatment group.

and the results presented in Fig 2. Microencapsulated probiotic treated group has shown higher NDV antibody titres compared to commercial probiotic and control groups. These results were in agreement with the findings of Landy and Kavyani (2013), Nikpiran *et al*. (2013) and Khammas and Saigh (2014) where multi-strain probiotic (primalac®) supplementation had shown significantly higher ELISA antibody titres against NDV vaccine compared to control groups. Similarly, Khaksefidi and Ghoorchi (2006) also reported that antibody production against NDV in broiler chicks treated with probiotic was significantly higher at 10 days post immunization than that in untreated group. Talebi *et al*. (2015) reported that supplementation of single species of probiotic bacterium (*Enterococcus faecium*) had the highest antibody titre against NDV and significantly (p=0.0049) differ when compared with those of only vaccinated chickens. The health benefits and immunomodulatory properties offered by probiotic bacteria are strain specific and there is no universal strain or species that can provide all proposed benefits. Hence, multispecies probiotic is comparatively better than single species probiotic bacteria to exhibit beneficial effects in chicken (Izquierdo *et al*., 2009).

Multispecies Probiotic effect on cell mediated immunity: Microencapsulated probiotic fed group chicken had shown higher IL- 2 and IL-12 cytokine expression which are responsible for driving the Th1 immune response and also higher IL-4 and IL-10 cytokine expression which are responsible for driving the Th2 immune response compared to commercial probiotic fed group chicken (Figs 3 and 4). Similar results were found in study of Brisbin *et al*. (2011) who reported that chickens fed with mixture of *Lactobacillus* cultures (*lactobacillus salivaris, L. reuteri, L. acidophillus*) had shown higher IL-12p40 cytokine expression compared to chicken fed with only PBS. The chicken IL 2 is an active growth factor for different immune cell types including T cells. The chicken IL4 stimulates the Th2 cells which is responsible for humoral immunity. Avian IL -12 is a pro-inflammatory cytokine produced by phagocytic cells and antigen-presenting cells (APC) within a few hours after bacteria or intracellular parasites infection and it also activates NK cells, and stimulate the production of Interferon-g (IFN-gamma). The role of IL-10 in chicken on many cell types such as non killer cell and also T and B lymphocytes has been observed (Giansanti *et al*., 2006). There was also increased lymphocyte count due to the effect of supplementing probiotics to feed (Hanamanta *et al*., 2010).

Multispecies probiotics effect on T-cell and B-cell mediated immune responses: Flow cytometry analysis was carried out for all groups chicken and staining percentages were determined from 10^4 cells per sample by analysis on a fluorescence activated cell sorter (FACScan;

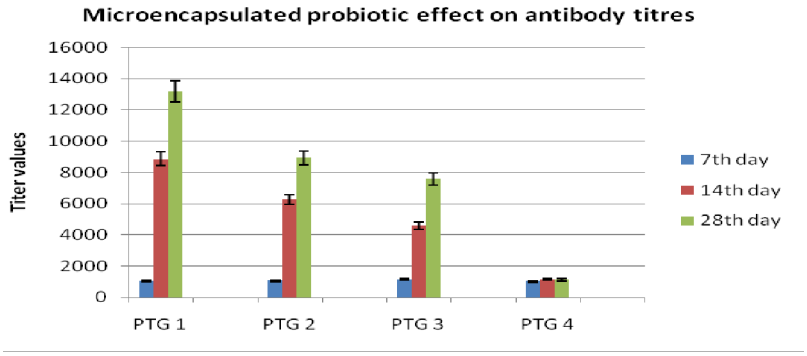

Fig 2: ELISA antibody titre levels in different groups of chicken.

Legend: PTG (probiotic trial group), PTG-1: NDV vaccinated microencapsulated probiotic treated group, PTG-2: NDV vaccinated commercial probiotic treated group, PTG-3: vaccinated and without probiotic treated group.

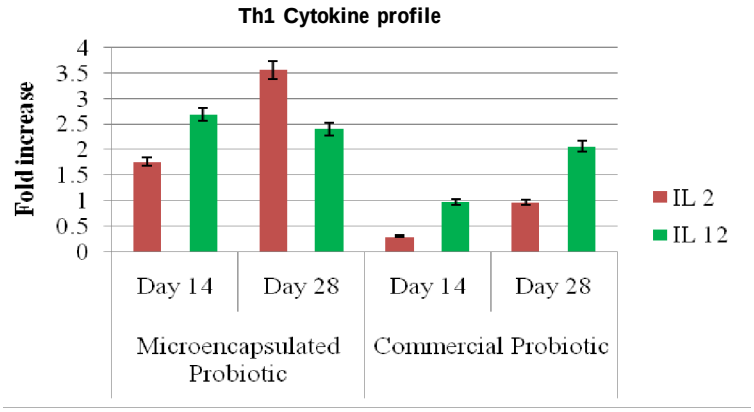

Fig 3: Th1 Cytokine profile of probiotic treated chicken.

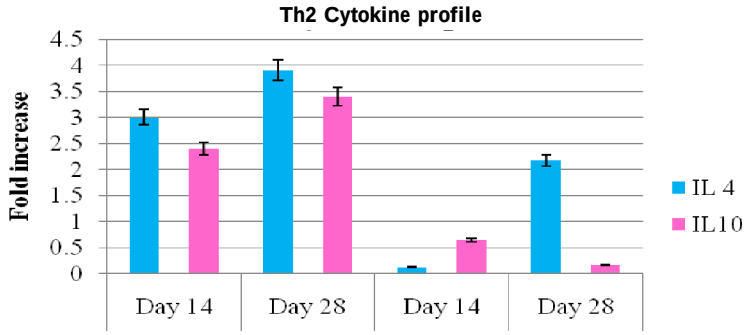

Fig 4: Th2 cytokine expression of probiotic treated chicken.

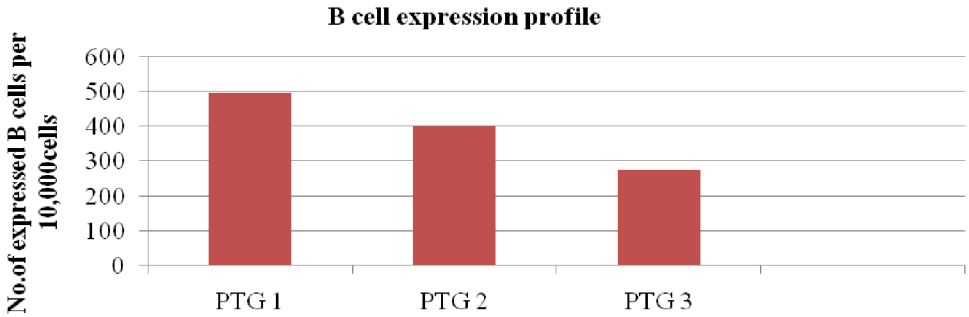

Fig 5: B cell expression profile of probiotic treated chicken.

Legend: PTG-1: microencapsulated probiotic treated group, PTG-2: commercial probiotic treated group, PTG-3: vaccinated and without probiotic treatment group.

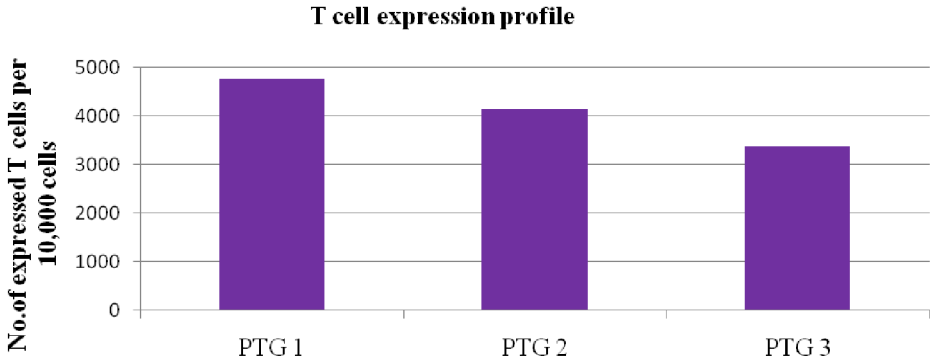

Fig 6: T cell expression profile of probiotic treated chicken.

Legend: PTG-1: microencapsulated probiotic treated group, PTG-2: commercial probiotic treated group, PTG-3: vaccinated and without probiotic treatment group.

Becton Dickinson). The gated stained and unstained populations of cells were determined from a mean value for the negative control samples of each group of chicken. Higher proportions of Bu1A and CD3 positive cells were observed in chicken fed with probiotic when compared to control group chicken.(Figs 5 and 6). Huang *et al*. (2013) reported that probiotics enhanced the formation of pool of $CD8^+$ T cells in the intestinal mucosa and also correlated with the results of Haghighi *et al*. (2008) who reported that the elevation of $CD8^+$ T cells density by probiotics treatment. Similarly, in study of Dalgaard *et al*. (2010) reported that the proliferative capacity of peripheral $CD4^+$ and $CD8^+$ cells specific for NDV was addressed 3 weeks after vaccination and found to be significantly higher in L133 bred line than in L130 bred line chicken.

CONCLUSION

Microencapsulated Probiotic consortium containing *Lactobacillus plantarum, Enterococcus faecium, Enterococcus hirae, Pediococcus acidilactici* and *Weissella paramesenteroides* used in this study has improved the immune response in terms of both innate and adaptive immunity as exemplified in *in vivo* trial conducted in commercial chicken.

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REFERENCES

Brisbin, J.T., Gong, J., Parvizi, P. and Sharif, S., (2010). Effects of lactobacilli on cytokine expression by chicken spleen and cecal tonsil cells. Clinical and Vaccine Immunology. 17(9):1337-1343.

Brisbin, J.T., Gong, J., Orouji, S., Esufali, J., Mallick, A.I., Parvizi, P., Shewen, P.E. and Sharif, S., (2011). Oral treatment of chickens with lactobacilli influences elicitation of immune responses. Clinical and Vaccine Immunology. 18(9): 1447-1455.

Dalgaard, T.S., Norup, L.R., Pedersen, A.R., Handberg, K.J., Jørgensen, P.H. and Juul-Madsen, H.R., (2010). Flow cytometric assessment of chicken T cell-mediated immune responses after Newcastle disease virus vaccination and challenge. Vaccine. 28(28):4506-4514.

De Simone D.C, (1995). Influence of a diet additioned with yogurt on the immune system. EOS J. Immunol. Immunopharmacol. 1: 30-35.

Divya Manjari K, Divya G, Saravanan TS and Parthiban M (2019). *In vitro* selection of potential immunomodulatory multispecies lactic acid bacteria in broilers. Indian J.Anim. Res. 53(5): 655-660.

Dong, H., I. Rowland and P. Yaqoob, (2011). Comparatives effects of six probiotic strains on immune function *in vitro*. Br. J. Nutr. 108: 459-470.

Fuller, R., (2001). The chicken gut microflora and probiotic supplements. The Journal of Poultry Science. 38(3): 189-196.

Ghafoor, A., Naseem, S., Younus, M. and Nazir, J., (2005). Immunomodulatory effects of multistrain probiotics (Protexin™) on broiler chicken vaccinated against avian influenza virus (H9). International Journal of Poultry Science. 4(10): 777-780.

Giansanti, F., Giardi, M.F. and Botti, D., (2006). Avian cytokines-an overview. Current Pharmaceutical Design. 12(24): 3083-3099.

Haghighi HR, Abdul-Careem MF, Dara RA, Chambers JR and Sharif S, (2008). Cytokine gene expression in chicken cecal tonsils following treatment with probiotics and Salmonella infection. Vet. Microbiology. 126: 225-233.

Hanamanta, N., and Narayana, S. M. (2010). Immune responses in broiler chickens supplemented with prebiotic, probiotic, their combination and g-probiotic spl. Indian J. Anim. Res. 44 (2): 150-152

Huang, A., Shibata, E., Nishimura, H., Igarashi, Y., Isobe, N. and Yoshimura, Y., (2013). Effects of probiotics on the localization of T cell subsets in the intestine of broiler chicks. J. Poultry Sci. 50(3): 275-281.

Izquierdo, M., Ibañez, J., Calbet, J.A., Navarro-Amezqueta, I., González-Izal, M., Idoate, F., *et al* (2009). Cytokine and hormone responses to resistance training. European Journal of Applied Physiology. 107(4):397.

Kato, I., Yokokura, T. and Mutai, M. (1983). Macrophage activation by Lactobacillus casei in mice, Microbiology and Immunology. 27: 611-618.

Khaksefidi, A. and Ghoorchi, T. (2006). Effect of probiotic on performance and immunocompetence in broiler chicks. J. Poult. Sci. 43: 296-300.

Khalifa, A., (2014). Effect of probiotics and Escherichia coli infection on performance of some viral vaccination in broiler chicks. Animal Health Research Journal. 2(4). 69-78.

Khammas, E.J. and AL-saigh, H.A., (2014). Study the Effect of Protexin® as a Probiotic and levamisole on the Immunity of Broilers. Kufa Journal For Veterinary Medical Sciences. 5(2): pp.55-63.

Kristeen-Teo, Y.W., Yeap, S.K., Tan, S.W., Omar, A.R., Ideris, A., Tan, S.G. and Alitheen, N.B., (2017). The effects of different velogenic NDV infections on the chicken bursa of Fabricius. BMC Veterinary Research. 13(1):151.

Landy, N and A. Kavyani, (2013). Effects of using a multi-strain p robiotic on Performance, immune responses and caecal microflora composition in broiler chickens reared under cyclic heat stress condition. Iranian J. Appl. Anim. Sci. 3(4): 703-708.

Lee, Y.K. and Salminen, S., (2009). *Handbook of Probiotics and Prebiotics*. John Wiley and Sons.

Lin, W.W. and Karin, M., (2007). A cytokine-mediated link between innate immunity, inflammation, and cancer. Journal of Clinical Investigation. 117(5):1175-1183.

Nang, N.T., J.S. Lee, B.M. Song, Y.M. Kang, H.S. Kim and S.H. Seo, (2011). Induction of inflammatory cytokines and toll – like receptors in chicken infected with avian H9N2 influenza virus. Vet Res. 42: 64.

Nikpiran H, Taghavi M, Khodadadi A and Athari SS., (2013). Influence of probiotic and prebiotic on broiler chickens performance and immune status. J. Novel Applied Sci. 2(8):256-259.

Perdigón G., Macias M.E.N., Alvarez S., Oliver G., De Holgado R.A.P. (1988). Systemic augmentation of the immune response in mice by feeding fermented milks with Lactobacillus casei and Lactobacillus acidophlilus. Immunology. 63:17-23.

Schultz, M., Linde, H.J., Lehn, N., Zimmermann, K., Grossmann, J., Falk, W. and Schölmerich, J., (2003). Immunomodulatory consequences of oral administration of Lactobacillus rhamnosus strain GG in healthy volunteers. J.Dairy research. 70(2):165-173.

Sheoran N, Maan S, Kumar A, Batra K, Chaudhary D, Sihag S, Kumar V, Maan NS (2018). Probiotic and prebiotic supplementation improving the production performance and immune characteristics of laying hens. Indian Journal of Animal Research. 1:52(10).

Silva, V.K., da Silva, J.D.T., Torres, K.A.A., de Faria Filho, D.E., Hada, F.H. and De Moraes, V.B., (2009). Humoral immune response of broilers fed diets containing yeast extract and prebiotics in the prestarter phase and raised at different temperatures. J.Appl.Poult. Res. 18(3):530-540.

Talebi, A., A. Amani, M. Pourmahmod, P.Saghaei and R. Rezaie. (2015). Synbiotic enhances immune responses against infectious bronchitis, infectious bursal disease, Newcastle disease and avian influenza in broiler chickens, Vet. Research Forum. 6 (3):191-197.

Timmerman, H.M., Veldman, A., Van den Elsen, E., Rombouts, F.M. and Beynen, A.C., (2006). Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. Poult. Sci. 85(8):1383-1388.