# EFFECT OF SUPPLEMENTATION OF POSTBIOTICS AND PARAROBIOTICS ON IMMUNE RESPONSE OF BROILER CHICKENS

**Danladi Yohanna[1], Teck Chwen Loh[2,5], Hooi Ling Foo[3,4]**
[1]Department of Animal Science, Faculty of Agriculture, Taraba State University, Jalingo, Nigeria.
[2]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia Institute
[3]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[4]Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[5]Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
*Corresponding author's email: avyohanna@gmail.com*

**ABSTRACT**
*This study was conducted to investigate the effects of feeding postbiotics and paraprobiotics on the immune response of broiler chickens. A total of 336 COBB 500 one-day-old chicks were randomly divided into eight treatment groups (T1, T2, T3, T4, T5, T6, T7, and T8). The treatments were as follows: T1(Negative control)=Basal diet, T2 (Positive control)=Basal diet + 0.01% oxytetracycline, T3= Basal diet + 0.2% postbiotic TL1, T4=Basal diet + 0.2% postbiotic RS5, T5=Basal diet + 0.2% paraprobiotic RG11, T6=Basal diet + 0.2% postbiotic RI11, T7=Basal diet + 0.2% paraprobiotic RG14 and T8=Basal diet + 0.2% paraprobiotic RI11. The ileal cytokines expression using Real-Time polymerase chain reaction (RT-PCR) showed that pro-inflammatory cytokines (IL-1β, IL-6, IL-8, and IFN-α) were ($p<0.05$) downregulated by postbiotics, paraprobiotics, and the positive control. While the mRNA expression of the anti-inflammatory cytokine IL-10 was increased. In conclusion, postbiotics and paraprobiotics were found to be effective in modulating and improving the immunity of broiler chickens against infections that are due to intestinal inflammation thereby creating a healthy gut for better nutrient absorption and overall improvement of the growth of broiler chickens.*
**Keywords: Postbiotics, paraprobiotics, Immune Response, Boiler Chickens**

## INTRODUCTION

Gut microbiota exerts some influence on antibody triggered responses and immune components of the cells. Significant effects of complexity of intestinal microbiota on the gut T cell repertoire were reported in chicken (Mwangi *et al*., 2010). Brisbin *et al*. (2012) reported that *Lactobacillus* species have proven potential to induce differential cytokine expression in T cells of chicken caecal tonsils that could contribute to intestinal balance. Furthermore, treatment with a blend of probiotic bacteria (*L. acidophilus, B. bifidum*, and *S. faecalis*) in *Salmonella typhimurium* challenged broiler chicken resulted in to decrease in the expression of some important pro-inflammatory cytokines (IL-2 and IFN-γ) (Haghighi et al., 2008). Postbiotics are known as non-viable bacterial products or metabolic by-products from probiotic microorganisms with biological activity in the host (Tsilingiri et al., 2012; Tsilingiri and Rescign, 2013). While paraprobiotics was coined to indicate the use of inactivated probiotic cells (non-viable) or probiotic cell fractions to confer health benefits to the host (Taverniti and Guglielmetti, 2011). Therefore, this study was conducted to evaluate the potential effects of different strains of postbiotics and paraprobiotics supplementation on the immune response in broiler chickens.

## MATERIALS AND METHODS

### *Productions of Postbiotics and Paraprobiotics*

The production of postbiotics and paraprobiotics was done from the active culture of *L. plantarum* strains. The active culture was washed once with sterile 0.85% (w/v) NaCl (Merk, Darmstadt, Germany) solution and adjusted to $10^9$ CFU/mL to be used as a 10% (v/v) inoculum according to the method described by Mohamad *et al*. (2020), and the actual production was done according to the method described by Ooi *et al*. (2015) using MRS medium and incubated at 30 °C for 24 hours under anaerobic conditions. The cell-free supernatant collected after centrifugation at 10, 000 x g for 15 min

at 4° C was the postbiotics, while paraprobiotics was prepared by freezing the cell suspension culture strains of *L. plantarum* for 7 days at -30 °C. Postbiotic TL1, postbiotic RS5, paraprobiotic RG11, postbiotic RI11, paraprobiotic RG14, and paraprobiotic RI11 were the strains used in this study.

***Management of Birds and Experimental Design***

The birds used for this study were broiler chicken of the COBB 500 breed. The day-old chicks were obtained from a commercial hatchery. The DOCs of a total number of 336 were randomly distributed to eight treatment diets and arrange in a completely randomized design (CRD). The experiment was conducted in a closed house system. The temperature of the house was set at 33 ± 1 °C on day 1, was gradually reduced to about 25 ± 1 °C by day 15. Average relative humidity was between the range of 60 and 75%. Every treatment group had six replications with seven birds per replicate, and were managed in a 120 x 120 cm (length x width) pen cage. Treatment diets were as follows: T1(Negative control)=Basal diet, T2 (Positive control)=Basal diet + 0.01% oxytetracycline, T3= Basal diet + 0.2% postbiotic TL1, T4=Basal diet + 0.2% postbiotic RS5, T5=Basal diet + 0.2% paraprobiotic RG11, T6=Basal diet + 0.2% postbiotic RI11, T7=Basal diet + 0.2% paraprobiotic RG11 and T8=Basal diet + 0.2% paraprobiotic RI11. Vaccination and management procedures were strictly followed. The provision of water and feed for the 5 weeks were done ad libitum. The starter feeds were offered from days 0 to 2(**Tables 1**), while finisher diets were offered from days 22 until 35 of ag (**Table 2**).

**Table 1 Nutrient composition of starter diets (day 1-21)**

| | Treatment diets | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ingredients | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_7$ | $T_8$ |
| Corn | 47.50 | 47.49 | 47.20 | 47.20 | 47.20 | 47.20 | 47.20 | 47.20 |
| Soybean Meal | 40.10 | 40.10 | 40.20 | 40.20 | 40.20 | 40.20 | 40.20 | 40.20 |
| Wheat pollard | 1.50 | 1.50 | 1.60 | 1.60 | 1.60 | 1.60 | 1.60 | 1.60 |
| CPO | 6.00 | 6.00 | 5.90 | 5.90 | 5.90 | 5.90 | 5.90 | 5.90 |
| L-Lysine | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| DL-Methionine | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Dicalcium Phosphate | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Calcium carbonate | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 |
| Choline chloride | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Common salt | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Mineral Mix | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Vitamin Mix | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Antioxidant | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Toxin binder | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Antibiotics | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Postbiotic TL1 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Postbiotic RS5 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 |
| Paraprobiotic RG11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 |
| Postbiotic RI11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 |
| Paraprobiotic RG14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 |
| Paraprobiotic RI11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 |
| **Total** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** |
| **Calculated analysis** | | | | | | | | |
| ME (Kcal/Kg) | 3215.70 | 3215.70 | 3201.40 | 3201.40 | 3201.40 | 3201.40 | 3201.40 | 3201.40 |
| Protein (%) | 22.00 | 22.00 | 22.03 | 22.03 | 22.03 | 22.03 | 22.03 | 22.03 |
| Fat (%) | 7.99 | 7.99 | 7.88 | 7.88 | 7.88 | 7.88 | 7.88 | 7.88 |
| Fibre (%) | 4.11 | 4.11 | 4.12 | 4.12 | 4.12 | 4.12 | 4.12 | 4.12 |
| Calcium (%) | 1.08 | 1.08 | 1.08 | 1.08 | 1.08 | 1.08 | 1.08 | 1.08 |
| Total Phos(%) | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 |
| Avail. P (%) | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 |

T1 = negative control (Basal diet), T2 = positive control (Basal diet + 0.01% (*w*/*w*) Oxytetracycline), T3 = Basal diet + 0.2% (*v*/*w*) postbiotic TL1, T4 = Basal diet + 0.2% (*v*/*w*) postbiotic RS5, T5 = Basal diet + 0.2% (*v*/*w*) paraprobiotic RG11, T6 = Basal diet + 0.2% (*v*/*w*) postbiotic RI11, T7 = Basal diet + 0.2% (*v*/*w*) paraprobiotic RG14, T8 = Basal diet + 0.2% (*v*/*w*) paraprobiotic RI11. CPO = Crude palm oil. Dicalcium phosphate 18%; Vitamin premix provided per kilogram of diet: Vitamin A 35 MIU; vitamin D3 9 MIU; vitamin E 90 g; vitamin K3 6 g; vitamin B1 7 g; vitamin B2 22 g; vitamin B6 12 g; vitamin B12 0.070 g; biotin 300 mg; pantothenic acid 35 g; nicotinic acid, 120 g; folic acid 3 g; Phytase 25000 FTU. Mineral mix provided per kilogram of diet: Se 0.2 g, Cu 15 g, Fe 80 g, I 1 g, Mn 100 g, Na 1.5 g, Zn 80 g, K 4 g, Co 0.25 g. Antioxidant contains butylated hydroxyanisole (BHA). Toxin binder contains natural hydrated sodium calcium aluminum silicates to reduce the exposure of feed to mycotoxins. Oxytetracycline (200 mg/kg, purity ≥ 64.7%, Y.S.P. Industries (M) SDN BHD). The diets were formulated using FeedLIVE International software (Nonthaburi, Thailand).

**Table 2 Nutrient composition of finisher diets (day 22-35)**

| | Treatment Diets | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Ingredients** | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_7$ | $T_8$ |
| Corn | 51.60 | 51.59 | 51.60 | 51.60 | 51.60 | 51.60 | 51.60 | 51.60 |
| Soybean Meal | 33.50 | 33.50 | 33.50 | 33.50 | 33.50 | 33.50 | 33.50 | 33.50 |
| Wheat pollard | 4.80 | 4.80 | 4.60 | 4.60 | 4.60 | 4.60 | 4.60 | 4.60 |
| CPO | 5.20 | 5.20 | 5.20 | 5.20 | 5.20 | 5.20 | 5.20 | 5.20 |
| L-Lysine | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| DL-Methionine | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Dicalcium Phosphate | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Calcium carbonate | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 |
| Choline chloride | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Common salt | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Mineral Mix | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Vitamin Mix | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Antioxidant | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Toxin binder | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Antibiotics | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Postbiotic TL1 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Postbiotic RS5 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 |
| Paraprobiotic RG11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 |
| Postbiotic RI11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 |
| Paraprobiotic RG14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 |
| Paraprobiotic RI11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 |
| **Total** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** |
| | **Calculated analysis** | | | | | | | |
| ME (Kcal/Kg) | 3180.83 | 3180.83 | 3176.68 | 3176.68 | 3176.68 | 3176.68 | 3176.68 | 3176.68 |
| Protein (%) | 19.92 | 19.92 | 19.89 | 19.89 | 19.89 | 19.89 | 19.89 | 19.89 |
| Fat (%) | 7.29 | 7.29 | 7.29 | 7.29 | 7.29 | 7.29 | 7.29 | 7.29 |
| Fibre (%) | 4.01 | 4.01 | 3.99 | 3.99 | 3.99 | 3.99 | 3.99 | 3.99 |
| Calcium (%) | 1.06 | 1.06 | 1.06 | 1.06 | 1.06 | 1.06 | 1.06 | 1.06 |
| Total P (%) | 0.89 | 0.89 | 0.88 | 0.88 | 0.88 | 0.88 | 0.88 | 0.88 |
| Avail. P (%) | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 |

T1 = negative control (Basal diet), T2 = positive control (Basal diet + 0.01% (*w/w*) Oxytetracycline), T3 = Basal diet + 0.2% (*v/w*) postbiotic TL1, T4 = Basal diet + 0.2% (*v/w*) postbiotic RS5, T5 = Basal diet + 0.2% (*v/w*) paraprobiotic RG11, T6 = Basal diet + 0.2% (*v/w*) postbiotic RI11, T7 = Basal diet + 0.2% (*v/w*) paraprobiotic RG14, T8 = Basal diet + 0.2% (*v/w*) paraprobiotic RI11. CPO = Crude palm oil. Dicalcium phosphate 18%; Vitamin premix provided per kilogram of diet: Vitamin A 35 MIU; vitamin D3 9 MIU; vitamin E 90 g; vitamin K3 6 g; vitamin B1 7 g; vitamin B2 22 g; vitamin B6 12 g; vitamin B12 0.070 g; biotin 300 mg; pantothenic acid 35 g; nicotinic acid, 120 g; folic acid 3 g; Phytase 25000 FTU. Mineral mix provided per kilogram of diet: Se 0.2 g, Cu 15 g, Fe 80 g, I 1 g, Mn 100 g, Na 1.5 g, Zn 80 g, K 4 g, Co 0.25 g. Antioxidant contains butylated hydroxyanisole (BHA). Toxin binder contains natural hydrated sodium calcium aluminum silicates to reduce the exposure of feed to mycotoxins. Oxytetracycline (200 mg/kg, purity ≥ 64.7%, Y.S.P. Industries (M) SDN BHD). The diets were formulated using FeedLIVE International software (Nonthaburi, Thailand).

### *Ileal RNA Isolation and Real Time RT-PCR*

RNA was extracted from Ileum samples using Macherey Nagel NucleoSpin RNA plus (Macherey- Nagel GmbH & Co. KG, Germany). Approximately 30mg of samples were mixed with 350 µL of buffer LBP and homogenized. RNA purification and collection were achieved using NucleoSpin gDNA removal column, NucleoSpin RNA plus column, with wash buffer WB1, WB2, and finally, RNase-free water to elute the RNA. The purity and concentration (260/280 nm ratio absorbance) of extracted RNA were measured by a nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Purified RNA was converted to complimentary DNA (cDNA) using Biotechrabbit cDNA kit (Biotechrabbit GmbH Neuendorfstr. 24a 16761 Hennigsdorf Germany).

Real-time PCR was performed using LightCycler®480 Roche (Roche Diagnostics GmbH Roche Applied Science 68298 Mannheim, Germany). The housekeeping gene (GAPDH) was used to standardise the target genes. The qPCR master mix (20 µL) was made for every sample using a CAPITAL ™ qPCR Green Mix, $4^X$ (Biotechrabbit GmbH Neuendorfstr. 24a 16761 Hennigsdorf Germany), which comprises of 5 µL of CAPITAL ™ qPCR Green Mix, $4^X$, 1 µL of each of 4 µM forward and reverse primers, 1 µL of template cDNA and 12 µL of Nuclease free water. The targeted gene primers are presented in **Table 3.**

**Table 3. The primer sequences of cytokines used for RT-qPCR**

| Target gene | Primer sequence 5' -3' | Product size (bp) | Accession no |
|---|---|---|---|
| IL-1β | F-TGCTTCGTGCTGGAGTCACCC<br>R-GGCCGGTACAGCGCAATGTT | 98 | NM_204524.1 |
| IL-4 | F-ACCCAGGGCATCCAGAAG<br>R-CAGTGCCGGCAAGAAGTT | 114 | NM_001007079.1 |
| IL-5 | FGGAACGGCACTGTTGAAAAATAA<br>R-TTCTCCCTCTCCTGTCAGTTGTG | 111 | NM_001007084.1 |
| IL-6 | F-GCTCGCCGGCTTCGA<br>R-TGGCACCGCAGCTCATT | 71 | NM_204628.1 |
| IL-8 | F-GCCCTCCTCCTGGTTTCA<br>R-CTTGTGGATGGCATGATCT | 200 | NM_205498.1 |
| IL-10 | F-TAACATCCAACTGCTCAGCTC<br>R-CCGTAGGGCTTACAGAAAGG | 135 | NM_001004414.2 |
| IFN-α | F-ACTTCAGCTGCCTCCACACCTT<br>R-CAGGAACCAGGCACGAGCTT | 92 | NM_205427.1 |
| TGF-β | F-CGGGACGGATGAGAAGAAC<br>R-CGGCCCACGTAGTAAATGAT | 258 | NM_001318456.1 |
| GADPH | F- CTGGCAAAGTCCAAGTGGTG<br>R- AGCACCACCCTTCAGATGAG | 275 | NM_204305.1 |

**bp=base pair (product size), F=Forward, R-Reverse**

***Statistical Analysis***

Data for cytokine expression were analysed in a completely randomized design (CRD) using the General Linear Models procedures of Statistical Analysis System SAS, 2003). Duncan's Multiple Range Test was used to compare the means of the treatments. Data were presented as the mean± standard error of the mean (SEM).

## RESULTS AND DISCUSSION

**Table 4. Cytokines expression in the ileal tissue of broiler chickens fed with postbiotics and paraprobiotics**

| Parameters (mRNA Fold change) | Dietary Treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | SEM | P-value |
| IL-1β | $1^{a}$ | $0.66^{ab}$ | $0.85^{a}$ | $0.86^{a}$ | $0.91^{a}$ | $0.48^{b}$ | $0.11^{c}$ | $0.75^{ab}$ | 0.088 | 0.0004 |
| IL-4 | $1^{b}$ | $0.38^{c}$ | $0.33^{cd}$ | $1.34^{a}$ | $0.04^{f}$ | $0.23^{de}$ | $0.2^{0e}$ | $0.32^{cd}$ | 0.023 | <.0001 |
| IL-5 | $1^{b}$ | $1.02^{b}$ | $0.65^{c}$ | $1.31^{a}$ | $0.25^{d}$ | 0.15d | $0.13^{d}$ | $0.22^{d}$ | 0.030 | <.0001 |
| IL-6 | $1^{a}$ | $0.66^{abc}$ | $0.58^{b}$ | $0.82^{ab}$ | $0.48^{bc}$ | $0.77^{ab}$ | $0.40^{c}$ | $0.34^{c}$ | 0.102 | 0.0078 |
| IL-8 | $1^{a}$ | $0.77^{b}$ | $0.34^{d}$ | $0.96^{a}$ | $0.63^{bc}$ | $0.14^{e}$ | $0.12^{e}$ | $0.50^{cd}$ | 0.043 | <.0001 |
| IL-10 | $1^{d}$ | $2.34^{b}$ | $0.09^{f}$ | $3.44^{a}$ | $0.04^{e}$ | $1.17^{c}$ | $0.34^{e}$ | 1.03d | 0.034 | <.0001 |
| IFN-α | $1^{a}$ | $0.50^{c}$ | $0.22^{d}$ | $0.80^{b}$ | $0.12^{d}$ | $0.18^{d}$ | $0.13^{d}$ | $0.12^{d}$ | 0.036 | <.0001 |
| TGF-β | $1^{d}$ | $1.73^{bc}$ | $0.42^{e}$ | $0.42^{e}$ | $2.15^{b}$ | $3.18^{a}$ | $1.58^{c}$ | $1.83^{bc}$ | 0.118 | <.0001 |

$^{a-f}$ Means with different letters are significantly different ($p<0.05$). Data are shown as means and standard error, (n=6)

***Effect of Postbiotics and Paraprobiotics on Cytokine Expression***

Cytokines are small signaling molecules that coordinate the functional properties of the cells involved in immune response (Gibson, 2012). Cytokines play major roles in immune defense and development, as well as tissue regeneration and repair (including wound-healing processes) (Wigley et al., 2003; Eming et al., 2009). The pro-inflammatory cytokines (IL-1β, IL-6, and IL-8) in this study were all downregulated in the positive control, postbiotics, and paraprobiotics groups. Similar results were reported with postbiotic RI11 (Humam et al., 2020). Also, Kareem *et al.* (2017) reported a decrease of proinflammatory cytokines IL-6 with postbiotics RG14 and inulin in broiler chickens. Lower expression of proinflammatory cytokines could be an indication of lower pathogen load in postbiotics RI11 (Humam *et al.*, 2020). This can be attributed to the increase in the population of *Lactobacillus* which produces lactic acid as a product of fermentation which acts as antimicrobials inhibiting the growth of pH-sensitive gram-positive and gram-negative pathogens as reported in an *in vitro* study (Danladi, 2022). Th2 cytokines (IL-4, IL-5, and IL-10) in our current study were upregulated in the ileal tissue. The increase of IL-10 which is well known anti-inflammatory cytokine is consistent with previous findings with postbiotics (Kareem *et al*., 2017). and probiotics (Wang *et al*., 2017). The upregulation of IL-10

was a good indicator because this cytokine was highly regarded as an immunosuppressive cytokine and plays a role as the mediator of tumor regression (Kim *et al*., 2005). Another report has it that IL-10 plays an important role in regulating immune responses and inhibiting the synthesis of inflammatory and pro-inflammatory cytokines (such as IL-1β), thus downregulating inflammatory Th1 responses (de Waal Malefyt *et al.*, 1991). Postbiotics RI11 had the highest expression of TGF. However, both the positive control and paraprobiotics RG11 and RI11 upregulated TGF-β in the ileal tissue. To now, this is the first time TGF-β is being studied in chicken concerning the effect of postbiotics and paraprobiotics produced *from L. planatrum*. IFN-α expression was downregulated, however, there were no viral infections recorded during the feeding trial.

## CONCLUSION

The expression of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, and IFN-α) was downregulated, while anti-inflammatory cytokine IL-10 expression was increased with the supplementation of postbiotics and paraprobiotics. Therefore, postbiotics and paraprobiotics were found to be effective in modulating and improving the immunity of broiler chickens against infections that are due to intestinal inflammation thereby creating a healthy gut for better nutrient absorption and overall improvement in the growth of the chickens.

## REFERENCES

Brisbin, J.T.; Parvizi, P.; Sharif, S. Differential cytokine expression in T-cell subsets of chicken caecal tonsils co-cultured with three species of Lactobacillus. Benef Microbes. 2012;3(3):205-210. doi:10.3920/BM2012.0014.

Danladi, Y. Effects Feeding Postbiotics and Paraprobiotics produced from Lactiplantibacillus plantarum on growth Performance, Lipid metabolism and Gut Health in Broiler Chickens. PhD Thesis, Universiti Putra, Malaysia, .2022. Unpublished.

de Waal Malefyt, R.; Haanen, J.; Spits, H.; Koncarolo, M.G., te Velde, A.; Figdor, C., Johnson, K.; Kastelein, R.; H Yssel, H.; de Vries, J.E. Interleukin 10 (IL-10) and Viral IL-10 Strongly Reduce Antigen-specific Human T Cell Proliferation by Diminishing the Antigen-presenting Capacity of Monocytes via Dowm'egulation of Class II Major Histocompatibility Complex Expression. Journal of Experimental Medicine,1991: 174(4), 915–924. doi:10.1084/jem.174.4.915.

Eming, S.A.; Hammerschmidt, M.; Krieg, T.; Roers, A. Interrelation of immunity and tissue repair or regeneration. Seminars in Cell and Developmental Biology. 2009; 20(5): 517–527. https://doi.org/10.1016/j.semcdb.2009.04.009.

Gibson, M.S. A genetic and functional analysis of novel chicken interleukin-1 gene family members. PhD Thesis, University College, London. 2012.

Haghighi, H.R.; Abdul-Careem, M.F.; Dara, R.A.; Chambers, J.R.; Sharif, S. Cytokine gene expression in chicken cecal tonsils following treatment with probiotics and Salmonella infection. Vet Microbiol. 2008;126(1-3):225-233. doi: 10.1016/j.vetmic.2007.06.026.

Humam, A.M.; Loh, T.C.; Foo, H.L.; Izuddin, W.I.; Zulkifli, I.; Samsudin, A.A.; Mustapha, N.M. Supplementation of postbiotic RI11 improves antioxidant enzymes activity, upregulated gut barrier genes and reduced cytokines, acute phase proteins and heat shock protein70 gene expression levels in heat-stressed broilers. Poultry Science. 2020; 1–14. https://doi.org/10.1016/j.psj.2020.12.011.

Kareem, K.Y.; Loh, T.C.; Foo, H.L, Asmara, S.A, Akit, H. Influence of postbiotic RG14 and inulin combination on caecal microbiota, organic acid concentration, and cytokine expression in broiler chickens. Poultry Science. 2017; 96(4): 966–975. https://doi.org/10.3382/ps/pew362.

Kim, R.; Emi, M.; Tanabe, K. Cancer cell immune escape and tumor progression by exploitation of anti-inflammatory and pro-inflammatory responses. Cancer Biology and Therapy. 2005; 4(9): 924–933. https://doi.org/10.4161/cbt.4.9.2101.

Mohamad, Z.N.A.; Foo, H.L.; Loh, T.C.; Mohamad, R.; Rahim, R.A. Enhancement of Versatile Extracellular Cellulolytic and Hemicellulolytic Enzyme Productions by Lactobacillus plantarum RI 11 Isolated from Malaysian Food Using Renewable Natural Polymers. Molecules. 2020; 25: 1–7.

Mwangi, W.N.; Beal, R.K.; Powers, C.; Wu, X.; Humphrey, T.; Watson, M.; Bailey, M.; Friedman, A.; Smith, A.L. Regional and global changes in TCRαβ T cell repertoires in the gut are dependent upon the complexity of the enteric microflora. Dev Comp Immunol. 2010;34(4):406-417. doi:10.1016/j.dci.2009.11.009.

Ooi, M.F.; Nurzafirah, M.; Hooi, L.F.; Teck, C.L.; Rosfarizan, M.; Raha, A.R.; Arbakariya, A. Effects of carbon and nitrogen sources on bacteriocin- inhibitory activity of postbiotic metabolites produced by Lactobacillus plantarum I-UL4. Malaysian Journal of Microbiology. 2015; 11(2): 176–184. https://doi.org/10.1017/CBO9781107415324.004.

SAS. (2003). SAS, Institute Inc. SAS/STAT User's Guide: Version 9.0.

Taverniti, V.; Guglielmetti, S. The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: Proposal of paraprobiotic concept). Genes Nutr. 2011;6(3):261-274. doi:10.1007/s12263-011-0218-x.

Tsilingiri, K.; Viale, G.; Barbosa, T.; Penna, G.; Caprioli, F.; Sonzogni, A.; Viale, G.; Rescigno, M. Probiotic and postbiotic activity in health and disease: comparison on a novel polarised ex-vivo organ culture model. Gut. 2012;61(7):1007-1015. doi:10.1136/gutjnl-2011-300971.

Tsilingiri, K.; Rescigno, M. Postbiotics: What else? Benef Microbes. 2013;4(1):101-107. doi:10.3920/BM2012.0046.

Wang, H.; Ni, X.; Qing, X.; Zeng, D.; Luo, M.; Liu, L.; Li, G.; Pan, K.; Jing, B. Live probiotic Lactobacillus johnsonii BS15 promotes growth performance and lowers fat deposition by improving lipid metabolism, intestinal development, and gut microflora in broilers. Frontiers in Microbiology. 2017; 8(JUN): 1–14. https://doi.org/10.3389/fmicb.2017.01073.

Wigley, P.; Kaiser, P. Avian Cytokines in Health and Disease. Brazilian Journal of Poultry Science. 2003; 5(1): 1–14. https://doi.org/10.2174/1996327000801010013.