

Immune Response of Ross 308 Broiler Chicken (*Gallus gallus domesticus*) Fed with Brown Seaweed (*Laminaria spp.*)

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ABSTRACT

The study documented the immune response of Ross 308 broiler chickens fed 2% brown seaweed (*Laminaria spp.*) as an alternative to antibiotic growth promoters during early post-hatch and grower-finisher stages. A completely randomized design with a 3 (post-hatch) x 2 (grower-finisher) diet in factorial arrangement was employed. A total of 792 viable embryos were utilized for the post-hatch trial, which lasted for 24 hours in the hatcher; the diet included PH1 (control/ no feed and water), PH2 (standard starter and water), and PH3 (standard starter with 2%BS and water), each with three replicates. Grower-finisher feeding used 576 chicks from hatcher and randomized in 2 dietary treatments mainly GF1 (standard diet) and GF2 (standard diet with 2%BS) that spanned for 35 days under

controlled room temperature. Post-hatch and grower-finisher feeding of 2%BS did not significantly ($p>0.05$) affect the leukocyte cell count and H/L ratio of birds. The post-hatch H/L ratio of birds fed with PH3 (0.4330) was the lowest. However, birds fed with PH3 were observed to have the highest basophil cell count (1.44%). The result showed that grower-finisher feeding of 2%BS indicated a lower H/L ratio (0.4184) in contrast with the control. GF2 feeding elevated the basophil count (1.50%) and significantly ($p=0.0286$) increased the population of monocytes. The findings suggest that 2% brown seaweed improved immune response of birds.

Keywords: *Broiler chicken, brown seaweed, immune response, antibiotic growth promoters*

INTRODUCTION

An annual growth rate of 9.4 % in 2025 was accounted for in chicken production, with an estimated volume of 613.56 thousand metric tons, live weight. Out of this, broiler chicken accounted for 85.5 % (Philippine Statistics Authority, 2026). One of the reasons for the increase in production is the use of Antibiotic Growth Promoters (AGPs). El-Fateh et al. (2024) revealed an improved FCR of birds by 2.8 % ($p<0.05$) due to the addition of AGPs.

In line with the expanding global broiler industry, in-fed antibiotics have been proven to reduce low-level inflammation and immunologic stress from direct anti-inflammatory effects (Dal Pont et al., 2021; Niewold, 2007). However, due to significant reliance on the use of antibiotics in poultry farming (73% of livestock) as growth promoters and for disease prophylaxis, it poses a global threat of antimicrobial resistance (AMR) (Abreu et al., 2023; Abou-Jaoudeh et al., 2024).

AGPs were banned in 2006 by the European Union (EU), and restrictions were implemented across other regions; thus, the potential of alternatives such as prebiotics and phytogenics was contemplated (Gadde et al., 2017). But, banning of AGPs in feeds by many countries build risk to necrotic enteritis (NE)

cause by *Clostridium perfringens* led to high economic losses (Abd El-Hack et al., 2022). To overcome the issue, safe alternative growth promoters must be tested.

Prebiotics, as feed additives, can enhance beneficial gut microbiota to improve gastrointestinal health and production performance of chickens (Suresh et al., 2017; Swiatkiewicz et al., 2015). Relevantly, Tayeri et al. (2018) reported that prebiotic have significantly increase Ross 308 broiler weight gain ($p=0.001$) and reduced population of *Escherichia coli* bacteria compared to antibiotic treatment. Based on the study of Wati et al. (2015), commercial phytogetic feed additive (PFA) improved growth of Cobb 400 broiler chicks and exhibited lower humoral immune response ($p<0.05$) and heterophil-to-lymphocyte ratio ($p<0.001$) in contrast with birds fed with AGP, which indicates a better immune response. Aside from prebiotics, Rivera-Pérez et al. (2021) proved that use of the probiotic *Bacillus subtilis* (QST 713) lowered *E. coli* and *Enterococcus* counts ($p<0.05$), and *Lactobacillus* counts were significantly ($p<0.05$) higher, but hematological profiles showed no differences relevant to AGP.

Brown seaweed (*Ascophyllum nodosum*) as a prebiotic can improve the intestinal health of animals by increasing beneficial microbes and inhibiting pathogenic populations (Wassie et al., 2021). Li et al. (2021) stated that brown algae contain polysaccharides such as alginate, laminarin, and fucoidan that help in moderating immunomodulation, antibacterial, antioxidant, and prebiotic activities. Sweeney et al. (2016) reported that the extract of *A. nodosum* is beneficial in reducing the caecal bacterial load in chickens. Similarly, Gu et al. (1988) concluded that a 2% brown seaweed (*Laminaria spp.* and *Sargassum spp.*) inclusion in the broiler feed improved performance, dressing percentage, plasma HDL, and decreased plasma total cholesterol (El-Deek et al., 2011).

Leukocyte profile of Ross 308 broiler chicken fed with 2% *Laminaria spp.* in relation to immune response was still limited. This investigation pioneers brown algae feeding during early post-hatch growth until the finisher phase. The study proved that feeding brown algae as a prebiotic improved the immune defense of birds, and its effect is comparable to AGPs in poultry feeds.

METHODS

Animal Care and Use Research Permit

This study was conducted through the research protocol ACUC File No.: 2025-020 for the conduct of the experimental trial. It was approved by the Dalhousie University Faculty of Agriculture's Animal Care and Use Committee in accordance with the Canadian Council of Animal Care Guideline.

Research Design

Complete Randomized Design (CRD) with 3×2 factorial arrangements were utilized as experimental design under controlled environment system. Feeding treatments included three post-hatch diets (3 replicates) and two grower-finisher diets (2 replicates).

Diet Formulation

Two feeding trial employed in the study mainly post-hatch and grow-finisher infused with *Laminaria spp.* (Table 1). Post-hatch feeding used three treatments: PH1 (control group that received no feed and water), PH2 (standard starter diet and water), and PH3 (standard diet supplemented with 2% brown seaweed and water), which were given as a mash ration. Under the grower-finisher phase, two diets were mixed: GF1 (a standard broiler diet) and GF2 (standard broiler diet with 2% brown seaweed). The grower-finisher ration was fed during the starter (days 0-14), grower (days 14-26), and finisher (days 26-35) stages. Diets were formulated as isonitrogenous and isocaloric to meet the nutrient needs of birds (Table 2-3). Mash diet was used for starter and grower to finisher birds later transitioned to 3-mm pelletized diet.

Table 1. *Composition and guaranteed nutrient contents of brown seaweed (Laminaria spp.) as a dietary additive to broiler chicken.*

Composition	Nutrient Vale (%)
Crude Fibre (max)	6.75%
Potassium (K) (min)	1.48%
Salt (NaCl) (max)	3.80%
Iodine (I) (max)	0.09%
Sulphate (max)	0.24%

Note: Nutrient composition was based on the available data of the manufacturer (Atlantic Gro® Dried Seaweed Meal (Canadian Label by North Atlantic Organics).

Table 2. *Diet composition and computed nutrient content of standard diet fed to broiler chickens of the trial.*

	Starter diet (0-14 d)	Grower diet (15-25 d)	Finisher diet (16-35 d)
Ingredients (%)			
Corn, Grain	40.88	47.18	48.71
Wheat	10.00	10.00	10.00
Soybean Meal	43.95	37.70	35.09
Vegetable Oil	0.53	0.56	1.93
Brown seaweed	-	-	-
Limestone	1.44	1.21	1.12
Dical. Phos.	2.01	1.62	1.48
Common Salt	0.42	0.42	0.43
MCBS5	0.50	0.50	0.50
DL-Methionine	0.15	0.18	0.19
L-Lysine HCl	0.11	0.13	0.05
Pellet binding agent	-	0.50	0.50
Total	100.00	100.00	100.00
Calculated analysis			
Metabolizable energy(kcal/g)	2.90	2.95	3.05
Crude protein(%)	22.00	20.00	19.00
Calcium(%)	0.96	0.80	0.74
Available phosphorus(%)	0.48	0.40	0.37
Sodium(%)	0.18	0.18	0.18
Lysine(%)	1.32	1.19	1.05

Table 3a. *Diet composition and computed content of standard diet with 2 % brown seaweed fed to broiler chickens of the trial.*

	Starter diet (0-14 d)	Grower diet (15-25 d)	Finisher diet (16-35 d)
Ingredients (%)			
Corn, Grain	43.43	49.74	51.26
Wheat	10.00	10.00	10.00
Soybean Meal	39.93	33.67	31.06
Vegetable Oil	0.12	0.14	1.52
Brown seaweed	2.00	2.00	2.00
Limestone	1.38	1.15	1.06
Dical. Phos.	2.04	1.65	1.51
Common Salt	0.33	0.34	0.34
MCBS5	0.50	0.50	0.50

DL-Methionine	0.14	0.17	0.19
L-Lysine HCl	0.11	0.13	0.05
Pellet binding agent	0.00	0.50	0.50
Total	100.00	100.00	100.00

Table 3b. *Diet composition and computed content of standard diet with 2 % brown seaweed fed to broiler chickens of the trial.*

	Starter diet (0-14 d)	Grower diet (15-25 d)	Finisher diet (16-35 d)
Calculated analysis			
Metabolizable energy(kcal/g)	2.90	2.95	3.05
Crude protein(%)	22.00	20.00	19.00
Calcium(%)	0.96	0.80	0.74
Available phosphorus(%)	0.48	0.40	0.37
Sodium(%)	0.18	0.18	0.18
Lysine(%)	1.32	1.19	1.05

Hatchery Feeding Trial

Post-hatch feeding trial utilized 792 viable embryos at the hatchery. At day18th of incubation, hatcher eggs were randomly assigned to the three post-hatch diets, each with three replications (88 viable eggs per replicate); PH1 (no feed and water/control), PH2 (standard starter and water), and PH3 (starter with 2% brown seaweed and water). Hatcher trays equipped with were filled with the experimental diets, eggs were standardized based on their size to ensure uniformity for newly-hatched chicks. Control groups were deprived of feed and water until grower phase. Hatcher chicks under feeding treatments were allowed to eat and drink for 24 hours before transportation to the grower-finisher pens for the grow-out trial.

Grower-Finisher Feeding Trial

The newly hatched chicks of Ross 308 broiler chicken from hatcher trial were used. A total of 576 mixed-sex chicks transported from post-hatch feeding was immediately selected from the replications. Upon arrival at the Atlantic Poultry Research Centre (APRC), chicks from three post-hatch treatments (4 replicates per grower-finisher trial) were randomly distributed to each of the 24 pens (24 birds/pen; 12 replicates per diet) with a pen size of 1.4m × 2.14 m. A depth of 4 cm wood shavings was used as beddings in the experimental rooms. For the first 48 hours, chicks received continuous light, followed by 18-hour light and 6-hour dark photoperiod from days 5 until 35(marketable age). A rheostat was use in light control that automatically reduced light intensity (lux) from 20 lux on day 0 to 5 lux on day 35. During the first week, 32°C room temperature was maintained and decreased weekly by 3°C until 21°C on day 28, that remained throughout the rearing period. Feed and water were provided ad libitum. Health status was monitored on a daily basis during 08:00 in the morning.

Blood Sample Collection

At day 35, one bird per pen (24 birds) were selected and humanely euthanized by cervical dislocation for blood sample collection. Whole blood was withdrawn from the jugular vein using a knife and collected using BD Vacutainer Sodium Heparin Blood Collection Tube (anticoagulant coated/heparinized tube).

Blood Smear Preparation

Immediately after euthanasia and blood collection on day 35, blood smears were prepared to preserve cellular morphology. The Fisherbrand™ (Thermo Fisher Scientific) microscope slide was properly labeled based on treatment group and a small drop of fresh whole blood was placed near one end of the slide using a microhematocrit capillary tube. To distribute the blood evenly, second slide held as spreader

at approximately 45° to produce a thin monolayer smear with a feathered edge. At room temperature, smears were air-dried and stained with Wright-Giemsa stain through standard protocols for avian hematology to have clear differentiation of blood cells.

Leukocyte Counting

Leukocyte populations was identified based on nuclear morphology and cytoplasmic characteristics. A Leica DM-series brightfield microscope by Leica Microsystems (Germany) equipped with a 100× oil immersion objective (1000× total magnification) was used to examine the stained smear. Using the standard avian hematological criteria, leukocyte counting was done from the monolayer region of the slide. A minimum of 100 leukocytes per cell type that includes heterophils, lymphocytes, monocytes, eosinophils, and basophils were classified. To determine the overall immune and physiological response, the heterophil-to-lymphocyte (H/L) ratio was calculated from each sample.

Statistical Treatment of Data

Research data was recorded manually and encoded in MS Excel for statistical analysis. The SAS 9.4 by PROC GLIMMIX for factorial analysis using mixed model procedure was used. Mean differences were compared with the Least Significant Difference (LSD) test at a 5% probability level.

RESULTS AND DISCUSSION

The immune response and leukocyte profile of Ross 308 broiler chicken under post-hatch and grower-finisher feeding of 2% brown seaweed is shown in Table 4 and 5. Blood samples collected on day 35 was measured for its leukocyte cell count and heterocyte-to-lymphocyte (H/L) ratio to assess the immune and physiological response of the Ross 308 broiler chicken.

Post-hatch leukocyte profile of birds

Post-hatch feeding of 2% brown seaweed does not significantly affect ($p>0.05$) the leukocyte population and H/L ratio of the Ross 308 broiler chicken (Table 4). Basophils population of PH2 (1%) was relatively lower as compared to PH1 and PH3 treatment groups, a sign of healthier animal. However, birds fed with 2% brown seaweed (1.44%) have the highest basophil profile. According Maxwell et al. (1990), basophils could only be the leukocyte to be affected by food deprivation such as during post-hatch and can be associated with higher basophils. Eosinophil cell count (0.60%) of birds fed with 2% brown seaweed posed the lowest percentage. This means that birds were healthier with lower mean of eosinophil. Wamboi et al. (2020) showed that higher eosinophils were significantly ($p=0.0363$) related in chickens with gastrointestinal parasitic infection. In terms of heterophil counts, PH3 only accounts 27.89 % of the total leukocyte which indicated the lowest among post-hatch treatment. Conversely, Sayed et al. (2023) evaluated prebiotic as prophylaxis in ross 308 broiler chicken that increases ($p\leq 0.0001$) % heterophil of the birds. Lymphocyte's profile (67%) from 2% brown seaweed addition incurred the highest population. Monocyte population (3.00%) was higher in birds fed with PH3 in correlation with control group. Based on the H/L ratio of birds, PH3 (0.4330) was lower in comparison with control groups. Similarly, sabah Abdulameer (2018) found that probiotic (*Lactobacillus plantarum* K KKP 529/p, 1.0×10^6 (cfu/g) and *Thymus vulgaris* increased ($p<0.05$) growth performances, lymphocyte cells and decreased ($p<0.05$) heterophiles and of H/L Ratio ($P<0.05$) of broiler chickens equating from diet incorporated with antibiotic (Virginiamycin).

Table 4. *Post-hatch feeding trial leukocyte profile of Ross 308 broiler chicken.*

% Leukocyte	Post-Hatch Diet			P-value
	PH1	PH2	PH3	
Basophils	1.43	1.00	1.44	0.3578
Eosinophils	1.43	1.62	0.67	0.0536
Heterophils	32.43	28.75	27.89	0.4094
Lymphocytes	62.14	65.13	67.00	0.2993
Monocytes	2.57	3.50	3.00	0.4716
H/L Ratio	0.4583	0.4488	0.4330	0.9128

Note: PH1, control/no feed and water; PH2, standard starter diet and water; PH3, standard starter diet with 2 % brown seaweed; H/L, heterocyte-to-lymphocyte ratio.

Grower-finisher leukocyte profile of birds

Grower-finisher feeding of 2% brown seaweed does not significantly affect ($p>0.05$) the leukocyte population and H/L ratio of the Ross 308 broiler chicken (Table 5). Different leukocyte populations were not significantly affected ($p>0.05$) by the brown seaweed infused ration, but basophil cell count from birds fed with GF2 (standard broiler diet with 2% brown seaweed) projected a higher number (1.50%) than the other group. Correspondingly, Kim et al. (2011) documented that 0.5% prebiotics fructo-oligosaccharide (FOS) elevated heterophil/lymphocyte ratio and basophil level. Zanu et al. (2020) associated that increased concentration of white blood cells (WBC) such as basophils ($p<0.001$) was related to *Clostridium perfringens* infection. Necrotic enteritis (NE) induced by *C. perfringens* in broiler chicken, a major global challenge to poultry production is linked to absence of antimicrobial growth promoters in feeds (Abd El-Hack et al., 2022; Naeem, 2026). Whereas, eosinophil population was comparable from both GF1 and GF2 ration with a 1.17% each. In terms of Heterophil counts, PH3 only accounts 27.89 % of the total leukocyte which indicated the lowest among post-hatch treatment. Alongside, the concentration of the birds' lymphocytes (27.08%) from GF2 diet indicated a lower number than the GF1 (31.92%) diets. Likewise, Al-Khalaifah et al. (2022) also proved that brown algae (*Sargassum sp.*) in the feed ration of broiler chickens induced a higher cellular response represented by T-cell ($p=0.037$), and enhanced IgA antibody titers, however heterophils was decreased that is disadvantageous. Monocyte (3.67%) was significantly higher ($p=0.0286$) in GF2 fed birds compared to control groups. Hernández-Granados et al. (2021) supported this claim, indicating that prebiotic (standard diet with 1% Agave fructans) increased the monocyte (7.4%) count of broiler chickens compared to AGPs (standard diet with colistin sulfate and zinc bacitracin (0.25 g/kg of feed) diet. A lower immune response based on H/L ratio was observed in birds fed with GF2 diet (0.4184). Tang et al. (2017) reported that prebiotic in layer improved immunity by lower heterophil-to-lymphocyte (H/L) ratio. A lower H/L ratio indicates healthier birds free from physiological stress.

Table 5. *Grower-finisher feeding trial leukocyte profile of Ross 308 broiler chicken.*

% Leukocyte	Grower-Finisher Diet		P-value
	GF1	GF2	
Basophils	1.08	1.50	0.1427
Eosinophils	1.17	1.17	0.8231
Heterophils	31.92	27.08	0.0835
Lymphocytes	63.42	66.50	0.2241
Monocytes	2.42	3.67	0.0286
H/L Ratio	0.4750	0.4184	0.2645

Note: GF1, standard broiler diet; GF2, standard broiler diet with 2 % brown seaweed; H/L, heterocyte-to-lymphocyte ratio.

CONCLUSIONS AND RECOMMENDATIONS

The feeding strategy using 2% brown seaweed in Ross 308 broiler chicken during early feeding at post-hatch and grower-finisher stage improved immunological response of birds. Across experimental diet, standard broiler diet with 2% brown seaweed as prebiotic indicated a lower heterophil-to-lymphocyte (H/L) ratio, that implies better health, status and disease resistance in birds. However, grower-finisher feeding of 2% brown seaweed increased the total cell counts of basophils and monocyte as compared to other treatment groups. Higher immune cells predict a possible pathogenic infection. Gross pathological assessment of immune organs and gut microbiome analysis is recommended to confirm the case.

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