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A study on immune response to Newcastle disease virus in broiler chickens fed with different levels of shrimp waste meal replacing fishmeal

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Abstract

An experiment was conducted with 300 day old Cobb broiler chicks which were randomly allotted into five groups: T₁- basal diet with 10% FM+0%SWM, T₂-7.5%FM+2.5%SWM, T₃-5%FM+5%SWM, T₄-2.5%FM+7.5%SWM+ synthetic lysine and methionine and T₅ - 0%FM+10%SWM+synthetic lysine and methionine, with three replicates of 20 birds each for 35 days. Humoral immune response was assessed on 0, 7th, 21st, 28th and 35th day of pre and post vaccination. The mean HI titre (log₂) values were not significant ($p > 0.05$) between different treatments for the antibody titres against NDV. The antibody titre (log₂) were higher (above 5) in pre-vaccination serum of all groups on 7th day. The T₅ group had higher titre of 4.33 ± 0.76 in post-vaccination serum on 35th day. It can be concluded that the replacement of fish meal with steam processed shrimp waste meal+synthetic lysine and methionine in broiler diets up to 100% showed no significant adverse suppression on hemagglutination inhibition titres.

Keywords: Broiler, Shrimp waste meal, Fish meal, Immune response

1. Introduction

Feed expenditure accounting for more than two-third of the total cost of production of broiler farming. Indian poultry feeds are commonly formulated based on corn-soya ingredients. Among the animal protein sources, Fish meal (FM) is mostly preferred in poultry feed. However, Nowadays, the fish meal inclusion in poultry diets is limited due to its unavailability of feed grade fish meal, higher cost, lack of uniformity, adulteration, pathogenic salmonella contamination and presence of fish smell and flavour in meat and eggs. This has created a need for new, cheap and non-conventional alternate animal protein sources, not competed by man and easily available and in large quantity for poultry farmers.

Shrimp waste meal (SWM) is one of the cheaper sources of animal protein which can be utilized to formulate economic diet for poultry. The SWM has potentiality as protein source feedstuff with a high-quality protein, chitin, minerals, carotenoids, such as astaxanthin, and lipids that are high in n-3 fatty acids (Sanchez *et al.*, 2011) ^[10] which can be used partially or completely replacing fish meal in broiler diet. The proximate composition of SWM (g/100g DM) was revealed as CP 46.3%, CF 4.3%, EE 9.04%, ash 17.04%, chitin 9.82%, calcium 7.0%, phosphorous 3.03% and metabolizable energy (ME) 2500 kcal/kg dry matter (Okoye *et al.*, 2005) ^[9]. Shrimp waste contains chitin, which can be processed into chitosan. Chitosan has been approved by the Food and Drug Administration in 2001 in United States of America (Wang *et al.*, 2020) ^[13]. Chitosan originates from alkaline deacetylation of chitin in the exoskeleton of shrimp, crabs, squid, insects, and fungal biomass (Tommeraa *et al.*, 2001) ^[12]. Chitosan is biocompatible, safe, non-toxic and has the biological qualities like hemostatic, anti-tumor, anti-cholesteremic, fungistatic and immunoadjuvant. (Younes and Rinaudo, 2015) ^[15]. Swiatkiewicz *et al.* (2015) ^[11] chitosan used as a feed additive for poultry and pigs has some beneficial, biological effects, including immunomodulatory, anti-oxidative, anti-microbial and hypocholesteremic properties which were reflected in improved growth performance in broiler chickens and weaned pigs. Chitosan act as an adjuvant for vaccines and

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drugs delivery (Zhao *et al.*, 2017) ^[16] due to its ability to carry and deliver compounds through the different administration routes.

Newcastle disease (ND) is one of the most devastating infectious diseases affecting poultry which causes very high mortality in chickens and huge productivity losses. Vaccination against ND is the most effective means of controlling the disease in commercial and backyard poultry. Assessing chicken's immune status helps farmers time vaccines correctly. It shows if birds have enough protection or need a booster shot. Testing antibody levels prevents weak responses and keeps the flock healthy. Since shrimp waste is a natural source of chitin and chitosan which has immunomodulatory property, its role on immune status of broiler chickens needs to be explored. However, limited research only exists on effect of shrimp waste meal included diets on immune response to Newcastle disease virus in broilers under Indian climatic conditions. Hence, a study on immune response to Newcastle disease virus in broiler chickens fed with different levels of shrimp waste meal replacing fishmeal was designed and conducted.

2. Materials and Methods

2.1 Geographic coordinates

The present study was carried out at ICAR experiential learning broiler house, Madras Veterinary College, Chennai-7, for a period of 35 days. The college is located between 13.0864° N, and 80.2683° E. The minimum temperature ranged from 20 to 32 °C and relative humidity ranged from 60 to 70% during the experimental period. All the experimental procedures were performed as per the guidelines of institute's ethics committee in this study.

2.2 Procurement, preparation and proximate analysis of shrimp waste meal

The fresh shrimp waste samples consisting of shrimp head and shell was collected from the six shrimp processing plants at Tuticorin in Tamil Nadu, India and were oven dried at 90° C for 24 hrs. then ground into powder in grinder. The collected shrimp waste samples were analyzed for their proximate principles such as moisture, crude protein, crude fibre, ether extract, total ash and acid insoluble ash at the Institute of Animal Nutrition laboratory, Kattupakkam. The analysis was carried out according to AOAC (2012) ^[3] and results were expressed as% on dry matter basis.

2.3 Biological experiment

A total of 300 day old commercial Cobb broiler chicks were procured for this study. The chicks were randomly allotted into five treatment groups with three replicates having twenty chicks per replicate. Three phases of experimental diets (broiler pre-starter, broiler starter and broiler finisher) were formulated as per BIS (2007) ^[4] specifications with locally available feed ingredients. The rations for the five treatment groups were iso-caloric and iso-nitrogenous corn soya-based diets with the addition of 10% animal protein source. The groups were fed with the diets containing steam processed SWM gradually replacing FM and the treatment diets of T₁, T₂, T₃, T₄ and T₅ groups consisted of basal diet with 10% FM +0% SWM, 7.5% FM + 2.5% SWM, 5% FM + 5% SWM, 2.5% FM + 7.5% SWM and 0% FM + 10% SWM, respectively. All the birds were reared under the standard managemental conditions and fed ad libitum till 35 days of age. Ingredient and nutrient composition (%) of the experimental broiler pre-starter, starter and finisher feed are furnished in Table: 1- 3.

Table 1: Ingredient and nutrient composition (%) of the experimental broiler pre-starter feed

Name of the Ingredients	T ₁	T ₂	T ₃	T ₄	T ₅
Maize	54.46	55.01	55.67	55.00	55.23
Soyabean meal	31.00	30.75	30.50	30.70	30.40
Fish meal	10.0	7.5	5.0	2.5	0.0
Shrimp waste	0.0	2.5	5.0	7.5	10.0
Oil (palmolein)	2.00	1.75	1.30	1.40	1.20
Poultry mineral mixture	1.00	0.83	0.40	0.28	0.20
Di-calcium phosphate	0.08	0.20	0.60	1.00	1.27
Salt	0.50	0.50	0.50	0.50	0.50
Feed additives #	0.86	0.86	0.86	0.86	0.86
Lysine	0.00	0.00	0.05	0.11	0.18
Methionine	0.10	0.10	0.12	0.15	0.16
Total	100	100	100	100	100
Nutrient composition of the experimental broiler pre-starter feed					
Crude protein (%)	23.05	23.05	23.00	23.00	23.00
Metabolizable energy (Kcal/kg)	3005	3008	3000	3001	3007
Calcium (%)	1.16	1.16	1.15	1.11	1.21
Av. phosphorus (%)	0.53	0.49	0.47	0.45	0.46
Lysine (%)	1.37	1.31	1.30	1.30	1.33
Methionine (%)	0.51	0.50	0.49	0.51	0.50

- Feed additives includes Ultravite – M, Toxin binder, Synercox, Oxinex, Choline chloride, Spectra DFM and Ultra – B12.

T₁-FM10/SWM0, T₂-FM7.5/SWM2.5, T₃-FM5/SWM5, T₄-FM2.5/SWM7.5, T₅-FM0/SWM10

Table 2: Ingredient and nutrient composition (%) of the experimental Broiler starter feed

Name of the Ingredients	T ₁	T ₂	T ₃	T ₄	T ₅
Maize	55.48	56.00	56.30	56.50	56.30
Soyabean meal	28.50	28.25	28.10	28.00	27.87
Fish meal	10.0	7.5	5.0	2.5	0.0
Shrimp waste	0.0	2.5	5.0	7.5	10.0
Oil (palmolein)	3.56	3.25	3.00	2.80	2.76
Poultry mineral mixture	1.00	0.80	0.47	0.10	0.07

Di-calcium phosphate	0.00	0.21	0.60	1.01	1.33
Salt	0.5	0.5	0.5	0.5	0.5
Feed additives #	0.86	0.86	0.86	0.86	0.86
Lysine	0.00	0.00	0.02	0.08	0.14
Methionine	0.10	0.13	0.15	0.15	0.17
Total	100	100	100	100	100
Nutrient composition of the experimental broiler starter feed					
Crude protein (%)	22.04	22.02	22.02	22.04	22.01
Metabolizable energy (Kcal/kg)	3102	3103	3103	3101	3100
Calcium (%)	1.15	1.15	1.15	1.17	1.14
Av. phosphorus (%)	0.52	0.48	0.46	0.45	0.45
Lysine (%)	1.31	1.24	1.21	1.21	1.21
Methionine (%)	0.5	0.51	0.51	0.51	0.5

- Feed additives includes Ultravite – M, Toxin binder, Synercox, Oxinex, Choline chloride, Spectra DFM and Ultra – B12.

T₁-FM10/SWM0, T₂-FM7.5/SWM2.5, T₃-FM5/SWM5, T₄-FM2.5/SWM7.5, T₅-FM0/SWM10

Table 3: Ingredient and nutrient composition (%) of the experimental broiler finisher feed

Name of the Ingredients	T ₁	T ₂	T ₃	T ₄	T ₅
Maize	59.51	59.80	60.00	59.82	59.50
Soyabean meal	23.30	23.16	23.00	23.00	23.00
Fish meal	10.0	7.5	5.0	2.5	0.0
Shrimp waste	0.0	2.5	5.0	7.5	10.0
Oil (palmolein)	4.75	4.50	4.27	4.28	4.22
Poultry mineral mixture	1.22	1.12	1.00	0.70	0.70
Di-calcium phosphate	0.0	0.2	0.5	0.9	1.2
Salt	0.5	0.5	0.5	0.5	0.5
Feed additives #	0.62	0.62	0.62	0.62	0.62
Lysine	0.00	0.00	0.00	0.05	0.10
Methionine	0.10	0.10	0.11	0.13	0.16
Total	100	100	100	100	100
Nutrient composition of the experimental broiler finisher feed					
Crude protein (%)	20.11	20.16	20.13	20.15	20.08
Metabolizable energy (Kcal/kg)	3206	3208	3214	3216	3219
Calcium (%)	1.16	1.16	1.18	1.2	1.23
Av. phosphorus (%)	0.53	0.49	0.49	0.48	0.49
Lysine (%) *	1.17	1.11	1.05	1.05	1.03
Methionine (%) *	0.47	0.46	0.44	0.45	0.46

- Feed additives includes Ultravite – M, Toxin binder, Synercox, Oxinex, Choline chloride, Spectra DFM and Ultra – B12.

T₁-FM10/SWM0, T₂-FM7.5/SWM2.5, T₃-FM5/SWM5, T₄-FM2.5/SWM7.5, T₅-FM0/SWM10

- **Poultry Mineral Mixture (MMP)** contains Calcium-23%, Phosphorus -12%, Magnesium-6.5%, Iron - 0.5%, Iodine - 0.026%, Copper-0.077%, Manganese-0.12%, Cobalt-0.012%, Zinc -0.38%, Sulphur - 0.5%, Fluorine - 0.07% and Selenium - 0.3 ppm.
- **Ultravite M** Each 2.5 kg contains Vitamin A- 1,25,00,000 IU, Vitamin D3- 25,00,000 IU, Vitamin B2 - 5 gm, Vitamin B6- 0.4 gm, Vitamin B12- 15 mg, Vitamin E - 800 IU, Vitamin K - 2 gm, Niacinamide - 10 gm, Calcium d-Pantothenate - 4 gm, Choline chloride - 300 gm, Calcium- 760gm, Copper-2 gm, Iodine-2 gm, Iron - 20 gm, Manganese - 55 gm, Zinc - 52 gm and Cobalt - 100 mg.

2.4 Humoral immune response (Haemagglutination inhibition titre against Newcastle disease virus)

Immune response of the experimental birds as influenced by different levels of shrimp waste meal replacing fishmeal was evaluated in terms of humoral immune response.

2.4.1 Collection of serum samples

Blood was collected from wing vein on 0, 7th, 14th, 21st, 28th and 35th day of pre and post vaccination against NDV to determine the antibody titre level. Whole blood was collected from 30 birds i.e., 2 birds selected randomly from each replicate contributing 6 birds per treatment. Serum was harvested from the blood and was subjected to

Haemagglutination (HA) and Haemagglutination Inhibition (HI) test to assess the antibody titre as a measure of humoral immune response to Newcastle disease.

2.4.2 Materials required

2.4.3 Viral antigen

Newcastle disease vaccine (LaSota strain) is used as known antigen obtained from Indovax Private Limited, Hissar, Haryana.

2.4.4 Alsever's solution

Alsever's solution is an isotonic, balanced salt solution which is routinely used as an anticoagulant or blood preservative, used for storing erythrocytes during experiment. It contains the following ingredients:

Dextrose	2.5 gm
Sodium citrate	0.8 gm
Sodium chloride	0.4 gm
Distilled water to make	100 ml

The final pH of the solution was maintained at 5.5

2.4.5 Chicken erythrocytes

Blood was collected from specific antibody negative chicken in equal quantity of Alsever's solution and washed three times in normal saline until clear supernatant was obtained. From the pellet formed of RBC, the 1% (v/v) suspension was

prepared to carry out Haemagglutination (HA) and Haemagglutination Inhibition (HI) tests.

2.4.6 Phosphate buffer saline

Phosphate buffer saline was prepared and used for dilution of serum while performing the tests.

Sodium chloride	8.0 gm
Potassium chloride	0.2 gm
Potassium dihydrogen phosphate	0.2 gm
Disodium hydrogen phosphate	1.133 gm
Distilled water to make	1000 ml

2.4.7 Humoral immune response (Haemagglutination inhibition titre against Newcastle disease virus) determination methods

1. Haemagglutination Test
2. Haemagglutination Inhibition Test

1. Haemagglutination Test

Haemagglutination Test (HA) was carried out as per the standard procedure outlined by Allan *et al.* (1978) [2] to quantify the amount of Newcastle disease virus in a suspension. In this method, phosphate buffered saline (PBS) (0.025 ml) was dispensed into each well of a plastic 96 well V-bottomed microtitre plate. Virus suspension (0.025 ml) was placed in the first well. Two fold dilutions of 0.025 ml volume of the virus suspension were made across the plate and 0.025 ml of PBS was dispensed to each well. Chicken RBCs (0.025 ml of 1% (v/v) was dispensed to each well. The solution was mixed by tapping the plate gently. The RBCs were allowed to settle for about 40 min at room temperature and HA was determined by tilting the plate and observing for the presence or absence of tear shaped streaming of the RBCs. The titration was read to the highest dilution giving complete HA (no streaming) which represented 1 HA unit (HAU).

2. Haemagglutination Inhibition Test

Haemagglutination Inhibition (HI) test was done according to the procedure outlined by Allan and Gough (1974) [1]. In this procedure, PBS (0.025 ml) was dispensed into each well of a 96 well plastic V-bottomed microtitre plate. Serum (0.025 ml) was placed into the first well of the plate. At two fold dilution of 0.025 ml volume of the serum were made across the plate. 4 HAU antigen in 0.025 ml was added to each well and the plate was left for a minimum of 30 min at room temperature. Then chicken RBCs (0.025 ml of 1% v/v) were added to each well and after gentle mixing, the RBCs were allowed to settle for about 40 min at room temperature. The HI titre was the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing 0.025 ml RBCs and 0.05 ml PBS only) was considered to show inhibition.

Data recorded in this biological experiment were subjected to one way analysis of Variance (ANOVA) as per the procedures of statistical analysis system (SPSS, version 20.0). Post hoc test was carried out to compare means by Duncan multiple range comparison test (Duncan, 1955) [5] with level of significance ($p < 0.05$). The results of the analysis were interpreted accordingly.

3. Results and Discussion

The mean ($\pm$ S.E) Mean ($\pm$ SE) antibody titre ($\log_2$) against NDV in broilers as influenced by different levels of SWM

replacing FM is presented in Table 4.

3.1 Influence of SWM feeding on HI titre against NDV

The mean HI titre ($\log_2$) values for antibody titre against NDV on 7th, 21st, 28th and 35th days were not significant ($p > 0.05$) between T₁, T₂, T₃, T₄ and T₅ treatments fed with diets containing different levels of SWM replacing FM. The HI titre value of 2⁴ and above (OIE, 2012) [8] is considered to be protective. The antibody titre ($\log_2$) were higher (above 5) in pre-vaccination serum of all groups on 7th day. In post-vaccination serum on 35th day, the T₅ group had higher titre of 4.33 $\pm$ 0.76 and T₃ group recorded lowest titre of 2.66 $\pm$ 0.80.

The mean HI titre ($\log_2$) values for antibody titre against NDV on 7th, 21st, 28th and 35th days were not significant ($p > 0.05$) between T₁, T₂, T₃, T₄ and T₅ treatments fed with diets containing different levels of SWM replacing FM. This is in concurrence with the results of Wati *et al.* (2014) [14], who utilized chitosan prepared from SWM and turmeric extract in broiler chicken as an immunomodulator and they observed no significant ($p > 0.05$) difference in antibody titres against NDV in the eight treatment groups fed with different combinations of basal diet, chitosan and turmeric extract. However, Mahata *et al.* (2026) [7], reported that the broilers fed the 5% shrimp meal diet showed better antibody titers against IBDV and NDV, indicating that shrimp meal boosted immunological competence. High-quality proteins, vital amino acids (particularly arginine), and bioactive substances including chitin, chitosan, and astaxanthin-all of which are known to enhance immunological modulation-are abundant in prawn meal.

All the groups had HI titre ($\log_2$) values above 5 in the pre vaccination serum on 7th day which might be due to the presence of maternal antibodies in chicks. Since live ND vaccine was used for immunization, the low antibody titre values were obtained on 21st and 28th d. At the end of the experiment i.e., on 35thd, the T₅ group fed with 10% SWM had numerically higher titre values (4.33 $\pm$ 0.76) than the other groups. This observation was partly in line with Jan *et al.* (2012) [6] who recorded significantly ($p < 0.05$) higher ND antibody levels in birds fed with chitosan and its derivative in the water and postulated that the chitosan could enhance immunity in broilers. The present study indicate that broilers fed with different levels of SWM and FM combinations, had comparable antibody titers against NDV, indicating a better immune response and overall health. Feeding broilers with shrimp protein derivatives maintains normal immunocompetence and baseline antibody response levels against common challenges like Newcastle Disease Virus, ensuring that alternative protein substitution does not compromise immune protection.

Table 5: Mean ($\pm$ SE) antibody titre ($\log_2$) against NDV in broilers as influenced by different levels of SWM replacing FMat different levels

Treatments	7 th day	21 st day	28 th day	35 th day
T ₁ (FM10/SWM0)	5.50 $\pm$ 0.43	3.33 $\pm$ 0.21	2.67 $\pm$ 0.423	3.83 $\pm$ 0.60
T ₂ (FM7.5/SWM2.5)	5.17 $\pm$ 0.48	4.00 $\pm$ 0.45	1.50 $\pm$ 0.22	4.00 $\pm$ 0.73
T ₃ (FM5/SWM5)	5.50 $\pm$ 0.22	3.50 $\pm$ 0.43	3.50 $\pm$ 0.67	2.66 $\pm$ 0.80
T ₄ (FM2.5/SWM7.5)	5.50 $\pm$ 0.43	3.17 $\pm$ 0.40	2.50 $\pm$ 0.22	3.00 $\pm$ 0.82
T ₅ (FM0/SWM10)	5.50 $\pm$ 0.43	3.17 $\pm$ 0.31	2.50 $\pm$ 0.56	4.33 $\pm$ 0.76
F value	0.13 ^{NS}	0.87 ^{NS}	2.42 ^{NS}	0.88 ^{NS}

n= 6, NS - Not significant ($p > 0.05$), * - Significant ($P < 0.05$), ** - Highly significant ($p < 0.01$).

Mean values within each column bearing common superscript do not differ significantly ($p > 0.05$).

4. Conclusion

The present study indicates that the replacement of fish meal with steam processed shrimp waste meal (SWM) in broiler diets up to 100% showed no statistically significant adverse suppression on hemagglutination inhibition (HI) titres, suggesting that steam processed shrimp waste meal (SWM) is generally safe for overall health especially when supplemented with synthetic lysine and methionine amino acids.

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Conflicts of Interest

The authors declare that they have no conflicts of interest to report regarding the present study.

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