



# Neem (*Azadirachta indica*) Leaf Powder as Phytogetic Feed Additives Improves the Production Performance, and Immune Organ Indices of Broiler Chickens

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**Abstract** | In recent years, incorporating phytogetic feed additives as an alternative to synthetic antibiotics is promising in poultry production. It is commonly added to poultry diets to boost immunity and improve production performance. This study was conducted to ascertain the potential of neem leaf powder (NLP) on broiler chickens' production and cell-mediated immunity. Sixty Cobb broiler chickens were distributed to four experimental treatments containing graded levels of NLP, 0% (T<sub>1</sub>), 2% (T<sub>2</sub>), 4% (T<sub>3</sub>), and 6% (T<sub>4</sub>) and arranged in a Completely Randomized Design experimental set-up. Each treatment was replicated three times, with five birds in each replication. The bi-weekly body weight gain (BWG), average daily gain (ADG), voluntary feed intake (VFI), feed conversion ratio (FCR), cell-mediated immunity, and return above feed and chick costs were observed within 42 days experimental period. The results showed significant differences ( $p < 0.05$ ) from all parameters, except for the cell-mediated immunity. The feed intake of broiler chickens was significantly reduced, whereas birds without NLP got the highest feed intake. Although feed intake of broiler chickens fed with NLP was significantly reduced, the body weight gain was not affected. Also, the feed conversion ratio of broiler chickens fed with 4% NLP showed better than birds fed with 0%, 2%, and 6% NLP. In return above feed and chick cost, birds fed with 4% NLP is more profitable, and the income generated increases as high as 25.73% compared to the birds fed without NLP in the diet. In conclusion, 4% NLP could be incorporated into the diets of broiler chickens without fear of compromising growth and immunity responses.

**Keywords** | Neem leaf powder, feed additives, growth performance, weight gain, cell-mediated immunity

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## INTRODUCTION

Poultry production is one of the most dynamic and ever-expanding sectors in the world. It helps fill the gap between the requirement and availability of high-quality protein for human consumption (Alkhalaf et al., 2010). However, producing good quality meat and eggs without harmful residues within a short time interval is challenging in the poultry industry (Uko and Kamalu, 2008). In 2019,

the Philippines chicken industry increased up to 14.72% of the total value of the agriculture industry, equivalent to 25,941 billion compared to the third quarter of 2017 and 2018 accounted for 13.27% and 13.96%, respectively (PSA, 2019; Galang, 2019). The production volume grew from 8.48% to 465,150 metric tons during the third quarter of 2019 (PSA, 2019; Galang, 2019). This was brought by increased demand as consumers shifted from pork to chicken meat due to the African Swine Fever outbreak.

Thus, there is an increasing demand for chicken meat and its by-products in the market.

One of the significant constraints in poultry production is the feed cost, accounting for 70–80% of the production cost, especially in developing countries (Siahan et al., 2021; Zacaria and Ampode, 2021). Also, incorporating synthetic antibiotics into poultry diets to boost production performance and meet animal protein demand is a public health concern (Lagua and Ampode, 2021). Synthetic antibiotics as growth promoters are expensive and have an adverse effect on the consumer's health due to the survival of the residues of antibiotics in the tissues of the birds (Ezzat et al., 2018). Hence, the ban on adding antibiotics into poultry diets has sought producers to utilize alternative natural feedstuffs to meet the demands (Hossain et al., 2012). It was found out that phyto-genic feed additives such as *Curcuma longa*, *Moringa oleifera*, *Echinacea purpurea*, *Azadirachta indica*, and other herbs boost production performance by the increasing growth rate, better feed conversion ratio, greater livability, enhanced immune stimulation, lower mortality in poultry and reduced total production costs (Kafi et al. 2017, Maass et al., 2005; Roth-Maier et al., 2005, Windisch et al., 2007).

Neem tree (*Azadirachta indica*) is considered a perennial tree under the mahogany Meliaceae family. It is a herbal plant that exhibits various beneficial pharmacological properties, including immunomodulatory effects in broilers (Jawad et al., 2013; Upadhyay et al., 1992). Several feeding trials using neem leaves in animal production were conducted, especially as an anti-helminthic agent. However, most studies used neem seeds as a protein source in animal feed (Aruwayo et al., 2011). The medicinal properties of this plant as antifungal, antiviral, antibacterial, and growth promoter manifested its significance without adverse effects on chickens (Ubua et al., 2019). However, neem leaf meal has anti-nutritional content such as sodium nimbolide, gallic acid, azadirachtin, and nimbodin, affecting nutrient utilization (Kharde and Soujanya, 2014). Hence, its usage is drastically reduced in feeds to capture beneficial effects with less adverse effects. The addition of neem leaves into ruminant feeds also enhanced plants' utilization and decreased the severe feed inadequacy during the dry season (Tiwarly & Pandey, 2008).

Neem leaves have been found to have a higher crude protein concentration than any other non-leguminous tree leaves (Adjorlolo et al., 2016). Moreover, it has low fiber and is considered a suitable protein supplement for ruminants in poor-quality diets. Neem leaves were also discovered to be a useful dry season fodder species where ruminant feeding during the prolonged dry season is a significant concern (Adjorlolo et al., 2016). However, few studies about neem

leaf powder as feed additives have reported no adverse effect of feeding to poultry and livestock. Thus, this study investigates the potential of Neem leaf powder for broiler chickens' production and cell-mediated immunity.

## MATERIALS AND METHODS

### BIRDS, DIETS, AND MANAGEMENT

The study was carried out following the standard rearing of farm animals as stipulated in the Good Animal Husbandry Practices of the Philippines concerning animal farming, health, and welfare (PNS/BAFPS, 2008). A week before the arrival of the experimental birds, the brooding house was constructed at an elevated type made of bamboo slats, disinfected, and cleaned thoroughly. A total of 60 at one-day-old Cobb broiler chicks were purchased from a reliable Agrivet supply in Tacurong City, Philippines, and housed at Ala, Esperanza, Sultan Kudarat. During the brooding period, artificial light was provided for twenty-four (24) hours for fourteen (14) days to regulate the birds' body temperature. The bulb was placed at the center of the brooding cage to allow the equal distribution of heat.

After the brooding period, the experimental birds were randomly distributed into four treatments and replicated three times, with five birds in every replication. All broilers were raised in a wire-floored pen, measuring 1x1 square meter per bird, fed *ad libitum*, and individual waterer and feeding trough was provided for each pen. The feeding trial lasted for 42 days with two feeding periods, the starter and the finisher phase. The experimental birds were given a starter ration from 15 to 28 days and gradually shifted to a finisher ration from 29 to 42 days. The formulated diets met the nutrient requirements based on the Philippine Recommends Livestock Feed Formulation (PCAARRD, 2000). The experimental diet was incorporated with graded levels of Neem Leaf Powder at 0% ( $T_1$ ), 2% ( $T_2$ ), 4% ( $T_3$ ), and 6% ( $T_4$ ) at the starter and finisher phases (Table 1).

### COLLECTION AND PREPARATION OF NEEM LEAF POWDER

The fresh neem leaves were collected in the locality of Ala, Esperanza, Sultan Kudarat. The fresh leaves were washed thoroughly using clean water to remove dirt and other unwanted matters and air-dried for seven (7) days. The dried leaves were ground using an attrition mill, sieved through a 1 mm sieve to produce neem leaf powder, and stored in large plastic containers with tight-fitting lids until needed (Dumaup and Ampode, 2021). The neem leaf powder was subjected to the proximate analysis following the AOAC (2016) procedure, and the chemical analysis was used in formulating the experimental rations.

**Table 1:** Composition and Chemical Analysis of Starter and Finisher Ration

Ingredients (% as fed basis)	Finisher							
	T <sub>1</sub> 0%	T <sub>2</sub> 2%	T <sub>3</sub> 4%	T <sub>4</sub> 6%	T <sub>1</sub> 0%	T <sub>2</sub> 2%	T <sub>3</sub> 4%	T <sub>4</sub> 6%
Ground Yellow Corn	50.00	49.00	50.00	50.00	54.30	51.40	50.05	50.20
Rice Bran D <sub>1</sub> *	11.00	12.80	12.00	12.00	12.00	12.00	12.00	11.00
Soybean (US)	20.00	18.00	14.00	20.00	14.00	15.00	14.00	14.00
Fish Meal, 60%	7.00	7.20	7.00	6.00	8.00	8.00	8.00	8.00
Copra Meal	10.00	10.00	7.00	5.00	10.00	10.00	10.00	10.00
Neem Leaf Meal	0.00	2.00	4.00	6.00	0.00	2.00	4.00	6.00
Dicalcium phosphate	0.40	0.20	0.16	0.16	0.40	0.40	0.50	0.20
Limestone	1.00	0.20	0.20	0.10	0.90	0.80	1.00	0.20
Lysine HCL	0.10	0.10	0.20	0.20	0.05	0.05	0.10	0.05
D-L Methionine	0.10	0.10	0.10	0.20	0.05	0.50	0.05	0.05
L Threonine	0.10	0.10	0.10	0.10	0.05	0.05	0.05	0.05
L Tryptophan	0.10	0.16	0.16	0.10	0.05	0.05	0.05	0.05
Vit. Premix <sup>1</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Calculated Analysis (%DM)								
Moisture	10.44	10.43	10.22	10.79	12.79	12.61	12.90	12.67
Crude Protein	20.04	20.54	20.57	20.66	19.91	19.59	19.08	19.85
ME (kcal/kg) <sup>2</sup>	2884	2864	2905	2926	2791	2887	2869	2870
Ash	5.29	4.63	4.38	4.58	11.24	11.44	10.27	8.05
Calcium	0.84	0.86	0.88	0.84	0.84	0.82	0.83	0.84
Available Phosphorus	0.48	0.49	0.45	0.47	0.43	0.45	0.46	0.42
Lysine	1.13	1.11	1.13	1.13	1.06	1.10	1.14	1.09
Methionine	0.45	0.46	0.44	0.47	0.44	0.48	0.50	0.51
Meth + Cys	0.51	0.55	0.53	0.58	0.44	0.48	0.50	0.53
L-Threonine	0.92	1.09	1.27	1.45	0.80	1.01	1.18	0.74
Tryptophan	0.31	0.29	0.27	0.29	0.31	0.28	0.31	0.29

\*D<sub>1</sub> is a category of rice bran which has fine quality/texture.

<sup>1</sup>Vitamin Mineral Premix: Vit. A 12000000iu, Vit D3 2000000iu, Vit. E 15000mg, Vit K3 2000mg, Vit. C 10000 mg, Vit. B1 2000mg, Vit. B2 4000mg, Vit. B6 3000mg, Vit. B12 25000mg, Folic Acid 700mg, Pantothenic Acid 15000mg, Biotin 10000mg, Niacin 25000mg.

<sup>2</sup>Metabolizable Energy.

**GROWTH PERFORMANCE**

The initial weight (g/bird) was taken on the 15<sup>th</sup> day (right after brooding) and recorded at the start of the study. The final weight was determined at the end of the study (after 42 days) using a digital weighing scale maximum of 40kg with a difference of 5g. The birds' final weight was subtracted from their initial weight to observe the weight gain, and the body weight gain (BWG) was measured every two (2) weeks to monitor their weight gain. The voluntary feed intake (VFI) was determined by offering a weighted amount of feed and subtracted by the feed refused every morning. The feed conversion ratio (FCR) was computed by com-

puting the amount of feed consumed to the broilers' body weight gain.

**CELL-MEDIATED IMMUNITY**

The broiler chickens were slaughtered following the guidelines of the Philippine National Standard (*PNS/BAFS 103:2016*) Code of Halal Slaughtering Practices for Poultry. Before slaughtering, the broiler chickens undergo fasting for twelve (12) hours, and birds nearest to the mean weight in every replication were slaughtered to determine the immune response by weighing the lymphoid organs, the bursa of Fabricius, and spleen. The bursa of Fabricius

**Table 2:** Proximate composition of Neem Leaf Powder (NLP)

Parameters	Analysis (%)
Dry Matter	88.94
Moisture Content	11.06
Crude Protein	20.58
Crude Fiber	14.13
Ash	11.53

The analysis was performed in triplicate samples following the methods described by the AOAC (2016)

**Table 3:** Effects of Neem leaf powder on the growth performance of broiler chickens

Parameters (days)	Treatments				P - value
	T <sub>1</sub> 0%	T <sub>2</sub> 2%	T <sub>3</sub> 4%	T <sub>4</sub> 6%	
Body Weight (g)					
15-28	924.73±3.05 <sup>d</sup>	960.73±2.76 <sup>a</sup>	952.20±1.64 <sup>b</sup>	937.00±3.86 <sup>c</sup>	0.000**
29-42	1492.67±5.96 <sup>b</sup>	1492.67±6.62 <sup>b</sup>	1706.00±5.72 <sup>a</sup>	1468.67±4.04 <sup>c</sup>	0.000**
Body Weight Gain (g)					
15-28	640.67±4.21 <sup>d</sup>	674.33±2.08 <sup>a</sup>	665.27±3.00 <sup>b</sup>	653.00±3.54 <sup>c</sup>	0.000**
29-42	567.93±8.39 <sup>b</sup>	531.93±4.84 <sup>c</sup>	753.80±4.92 <sup>a</sup>	531.67±3.82 <sup>c</sup>	0.000**
15-42	1208.60±37.39 <sup>b</sup>	1206.27±42.64 <sup>b</sup>	1419.07±70.59 <sup>a</sup>	1184.67±50.31 <sup>b</sup>	0.002**
Daily Gain (g)					
15-28	45.76±0.30 <sup>d</sup>	48.17±0.14 <sup>a</sup>	47.52±0.21 <sup>b</sup>	46.64±0.25 <sup>c</sup>	0.000**
29-42	40.57±0.59 <sup>b</sup>	38.00±0.35 <sup>c</sup>	53.84±0.35 <sup>a</sup>	37.98±0.27 <sup>c</sup>	0.000**
15-42	43.16±1.33 <sup>b</sup>	43.08±1.52 <sup>b</sup>	50.68±2.52 <sup>a</sup>	42.31±1.79 <sup>b</sup>	0.002**
Feed Intake (g)					
15-28	740.93±5.62 <sup>a</sup>	736.53±3.61 <sup>a</sup>	719.33±5.91 <sup>b</sup>	742.60±4.93 <sup>a</sup>	0.002**
29-42	1625.33±11.01 <sup>a</sup>	1406.87±2.48 <sup>c</sup>	1431.33±10.60 <sup>b</sup>	1417.40±1.00 <sup>bc</sup>	0.000**
15-42	2366.27±16.58 <sup>a</sup>	2143.40±5.96 <sup>b</sup>	2150.67±4.93 <sup>b</sup>	2160.00±4.61 <sup>b</sup>	0.000**
Feed Conversion Ratio					
15-28	1.15±0.01 <sup>c</sup>	1.09±0.01 <sup>a</sup>	1.08±0.01 <sup>a</sup>	1.11±0.01 <sup>b</sup>	0.000**
29-42	2.86±0.05 <sup>c</sup>	2.64±0.02 <sup>b</sup>	1.90±0.02 <sup>a</sup>	2.67±0.01 <sup>b</sup>	0.000**
15-42	1.96±0.04 <sup>b</sup>	1.78±0.07 <sup>b</sup>	1.52±0.08 <sup>a</sup>	1.83±0.08 <sup>b</sup>	0.000**

<sup>abcd</sup> Means±SD with different superscripts in the same row differ significantly.

\*\* (P<0.01)

**Table 4:** Effects of Neem leaf powder on the immune organs of broiler chickens

Parameters	Treatments				P - value
	T <sub>1</sub> 0%	T <sub>2</sub> 2%	T <sub>3</sub> 4%	T <sub>4</sub> 6%	
Bursa weight, g	3.63	4.40	3.93	3.93	0.979 <sup>ns</sup>
Spleen weight, g	0.83±.11 <sup>a</sup>	0.67±.057 <sup>ab</sup>	0.83±.06 <sup>a</sup>	0.63±.06 <sup>b</sup>	0.020**
Spleen Index	0.06±.00	0.04±.00	0.05±.00	0.04±.00	0.053 <sup>ns</sup>
Bursa Index	0.24±.20	0.29±.13	0.23±.08	0.27±.15	0.951 <sup>ns</sup>

<sup>ab</sup>Means±SD with different superscripts in the same row differ significantly. ns: not significant; \*\*: P<0.01

**Table 5:** Return above feed and chick cost of broiler chickens fed with graded levels of Neem leaf powder

Parameters	Treatments			
	T <sub>1</sub> 0%	T <sub>2</sub> 2%	T <sub>3</sub> 4%	T <sub>4</sub> 6%
Final live weight, g	1492.67	1492.67	1706.00	1468.67
Price/kg live weight (PhP)	130.00	130.00	130.00	130.00
Gross return/head (PhP)	194.05	194.05	221.78	190.93
Cost of DOC/head (PhP)	30.00	30.00	30.00	30.00
Feed Consumption (kg/head)				
Chick Booster Mash (kg)	0.33	0.33	0.33	0.33
Starter ration (kg)	0.74	0.74	0.72	0.74
Finisher ration (kg)	1.63	1.41	1.43	1.42
Price/kg of feed (kg)				
CBM (kg)	32.00	32.00	32.00	32.00
Starter ration (kg)	22.98	22.71	21.39	22.49
Finisher ration (kg)	22.47	22.95	21.93	21.70
Total Feed Cost (PhP)				
Chick Booster Mash (kg)	10.56	10.56	10.56	10.56
Starter ration (kg)	17.01	16.81	15.40	16.64
Finisher ration (kg)	36.63	32.36	31.36	30.81
Total Cost (PhP)	94.19	89.72	87.32	88.02
RAFCC (PhP)*	99.86	104.33	134.46	102.91

# All costs were shown in PhP (Philippine peso); 1 USD = 50.30 PhP

\* RAFCC: Return above feed and chick cost

and spleen were immediately removed and individually weighed (g) using the digital weighing scale following the actual visual appraisal. The cell-mediated immunity was determined following the methods of Dumaup and Ampode (2020) and Latif et al. (2014), using the formulae:

$$\text{Spleen Index} = \frac{\text{Spleen Weight (g)} \times 100}{\text{Body Weight (g)}}$$

$$\text{Bursa Index} = \frac{\text{Bursa of Fabricius (g)} \times 100}{\text{Body Weight (g)}}$$

### COST AND RETURN ANALYSIS

The return above feed and chick cost (RAFCC) was determined by subtracting the cost of chicks and feeds (feed supplemented expenses) from the sales of the birds at the termination of the study. The cost and return analysis measured the gain or loss generated by the study.

### STATISTICAL ANALYSIS

The data collected were subjected to a one-way Analysis of Variance (ANOVA) using the Statistical Package of Social Science (SPSS) computer software version 21, and significant differences were compared using Tukey's Honest Sig-

nificant Difference (HSD) at  $p < 0.05$  level.

## RESULTS

### PROXIMATE COMPOSITION OF NLP

The chemical analysis of Neem leaf powder (NLP) exhibited 88.94% dry matter, 20.50% crude protein, 14.13% crude fiber, 11.06% moisture content, and 11.53% ash (Table 2).

### GROWTH PERFORMANCE

The broiler chickens fed with different levels of NLP showed a significant effect ( $p < 0.05$ ) on the final weight, bi-weekly and average daily weight gain, voluntary feed intake, and feed conversion ratio at 15-42 days (Table 3). Numerically, birds fed with 4% NLP (T<sub>3</sub>) obtained the highest values among the parameters, including the final weight  $1706.00 \pm 5.72$ , BWG ( $1411.0 \pm 71.44$  g/bird), ADG ( $50.39 \pm 2.55$  g/bird), and FCR with  $1.52 \pm 0.08$  compared to the birds without NLP in the diet. The values recorded for the cumulative mean of the final weight ranges from  $1492.67 \pm 5.96$  to  $1706.00 \pm 5.72$  g/bird, body weight gain  $1184.67 \pm 50.31$  to  $1419.07 \pm 70.59$  g/bird, average daily weight gain  $42.31 \pm 1.79$  to  $50.68 \pm 2.52$  g/bird, and mean daily feed intake was  $2143.40 \pm 5.96$  to  $2366.27 \pm 16.58$  g/bird. On the other hand, broiler chickens fed with 2% NLP (T<sub>2</sub>) showed the least values from the BWG, ADG,



and VFI. The feed conversion ratio ranged from  $1.52 \pm 0.08$  (better) and  $1.96 \pm 0.047$ , and it was significantly ( $p < 0.05$ ) different between dietary treatments where birds fed with 4% NLP ( $T_3$ ) had the lowest value. In the present study, a FCR of  $1.52 \pm 0.08$  ( $T_3$ ) means that the chickens gained 1 kilogram of weight for every  $1.52 \pm 0.08$  kilograms of feed intake (Table 3).

### CELL-MEDIATED IMMUNITY

The broiler chickens fed with graded levels of NLP had no significant differences ( $p > 0.05$ ) on the bursa weight, spleen and bursa indices of broiler chickens (Table 4). Although not significant, the numerical values of bursa weight and bursa index in birds fed with NLP are higher than those without NLP in the diet. On the other hand, the spleen weight (lymphoid organ) showed a significant difference ( $p > 0.05$ ) between each treatment where birds fed with 4% NLP ( $T_3$ ) got the highest value but still comparable to the birds fed with 0% NLP ( $T_1$ ) and 2% NLP ( $T_2$ ) in the diet. Moreover, the broiler chickens fed with 6% ( $T_4$ ) NLP got the lowest spleen weight but were statistically comparable to the birds with 2% ( $T_2$ ) NLP in the diet.

### RETURN AND ABOVE FEED AND CHICK COST

With the same amount of price per kilo (Php130/kilo), broiler chickens fed with 4% NLP ( $T_3$ ) got the highest final weight with 1706.00g/bird was a good asset for a higher market with 221.78Php gross income per chicken compared to the birds fed with 6% NLP ( $T_4$ ) with 190.93Php, and birds fed with 0% ( $T_1$ ) and 2% ( $T_2$ ) NLP with 194.05Php/bird (Table 5). As a whole, feed diets incorporated with NLP showed lower production costs than diets without NLP. With this result, broiler chickens fed with 4% ( $T_3$ ) NLP got the highest return above feed and chick cost amounting to 104.33 Php/bird than the birds without NLP ( $T_1$ ) 148.02 Php/bird (Table 5).

## DISCUSSION

### GROWTH PERFORMANCE

In the present study, the inclusion of graded levels of NLP into the diet significantly improved ( $p < 0.05$ ) the growth performance of broiler chickens. However, the significant effect of NLP on the growth performance of broiler chickens is contrary to the findings of Deore et al. (2005), who reported that supplementation of neem oil in broilers resulted in poor performance in feed consumption and body weight, showing a dose-dependent adverse effect on the production performance. In addition, Shihab et al. (2017) reported that the inclusion of neem leaf powder in broiler diets had no significant ( $p > 0.05$ ) effect on the body weight and weight gains of broiler chickens. On the other hand, the result of the current study confirmed the investigation of Kharde and Soujanya (2014), who reported that male

Venn Cobb broiler chickens fed with neem leaf powder had heavier weight gains than the birds without neem leaf powder in the diet. The improvement in weight gains might be due to anti-protozoal and immunostimulatory properties of neem leaves that help reduce the microbial load and improve broiler chickens' performance (Wankar et al., 2009; Kharde and S. Soujanya, 2014).

In terms of voluntary feed intake, the data revealed that the inclusion of NLP significantly reduced ( $p < 0.05$ ) the feed intake of broiler chickens where birds fed without NLP ( $T_1$ ) has higher feed intake compared to broiler chickens fed with 2%, 4%, and 6% NLP. This result is contrary to Landy et al. (2011), who reported that neem leaf powder at a rate of 7grams and 12 grams/kg to broiler diets at the age of 42 days had no significant effect on the feed intake of broiler chickens. However, the present study revealed that feed intake was significantly affected, where birds fed with NLP have lower feed intake than broiler chickens without NLP in the diet. Although birds fed with NLP have lower feed intakes, it was observed that the body weight gain of birds fed with 4% NLP ( $T_3$ ) is statistically heavier ( $p < 0.05$ ) compared to the birds with 0% NLP ( $T_1$ ) in the diet. This result may be due to the antimicrobial and anti-protozoal properties of neem leaf powder, which help reduce the microbial organisms of the birds as neem leaf might have suppressed the growth of harmful microorganisms. As a result, it creates a conducive environment for the active substances to aid digestion and improve production performance (Ketkar, 1976; Ezzat et al., 2018; Adeyemo and Akanmu, 2012; Kharde and Soujanya, 2014).

The cumulative feed conversion ratio (FCR) of broiler chickens fed with 4% NLP ( $T_3$ ) got the lowest (better) FCR compared to other treatments. It should be noted that FCR measures the efficiency of poultry and livestock in converting animal feed into the desired output. The FCR indicates that the lower the value, the more efficient the birds convert feed to live weight (Ampode et al., 2020). In this study, broiler chickens fed with 4% NLP have the lowest FCR of  $1.52 \pm 0.08$  than those fed with 0%, 2%, and 6% NLP. This result is similar to Kharde and Soujanya (2014), who reported that supplementation of NLP in broiler chicken diets significantly improved the feed conversion ratio. The higher body weight gains might be due to growth-promoting and antimicrobial properties of neem leaves that helped reduce birds' microbial load and improved feed efficiency (Jong et al. 2009; Wankar et al. 2009).

### CELL-MEDIATED IMMUNITY

In avian species, adaptive immunity encompasses both humoral and cell-mediated immune responses (Erf, 2004). The humoral or antibody-mediated immune responses ef-

fectively combat the extracellular antigens. On the other hand, cell-mediated immunity is focused on eliminating intracellular antigens that have infiltrated cells, such as viral proteins and proteins originating from neoplastic cell transformation (Erf, 2004; Eladia and Ampode, 2021).

In the present study, the indicators of cell-mediated immunity, i.e., bursa and spleen indices, were not significantly affected ( $p>0.05$ ) when NLP was incorporated in the diet. However, a significant difference ( $p<0.05$ ) was observed in the spleen weight. The spleen weight of broiler chickens fed with 4% NLP ( $T_3$ ) is higher but statistically comparable to the birds fed with 0% ( $T_1$ ) and 2% ( $T_2$ ) NLP and birds fed with 6% NLP ( $T_4$ ) got the lowest spleen weight but statistically comparable to the birds fed with 2% ( $T_2$ ) NLP. The spleen and bursa indices are indicators of cell-mediated immunity, which means that the higher the immunity index, the stronger the broiler chickens' immune response (Fu Chang et al., 2004; Dumaup and Ampode, 2020).

Many studies reported that neem leaf powder plays a vital role in strengthening the immune system (Zahid et al., 2013, Al-Samarrai, 2012, Talpur and Ikhwanuddin, 2013). However, the cell-mediated immunity of the current study fed with NLP was not significantly affected. Although not significant, it was observed that the body weight gains of broiler chickens fed with NLP are significantly higher than birds without NLP in the diet. This might be attributed to neem leaf powder in the diet, which increased humoral and cell-mediated immune responses (Sadekar et al. 1998). At the same time, it killed or slowed down the growth of many organisms such as bacteria, viruses, and fungus, which boost the production performance of broiler chickens (Sadekar et al., 1998).

Moreover, the findings of Jawad et al. (2013) reported that neem leaf powder had good immunomodulatory effects against Newcastle disease (ND) and infectious bursal diseases (IBD) as indicated by the serum antibody titers. Also, it showed higher mean antibody titer values against ND compared to the negative control group (Jawad et al., 2013). Hence, the variations of the findings might be due to the level of NLP incorporated in the diet, breeds or strain of the experimental animals, and the quality of the neem leaf powder used in the study.

#### RETURN ABOVE FEED AND CHICK COST

The total expenses were reduced by up to 7.29% of total inputs when broiler chickens were fed with 4% ( $T_3$ ) neem leaf powder. Also, the market explained that the income generated for this study would increase as high as 25.73% of the return above feed and chick cost. Thus, the utilization of NLP in poultry diets seems potential as phytogenic feed additives for safe and efficient broiler production.

## CONCLUSION

The feed intake of broiler chickens fed with graded levels of NLP was significantly reduced, where birds without NLP in the diet got the highest feed intake. The body weight gain was significantly improved in broiler chickens fed with 4% NLP as compared to other treatments. Also, the feed conversion ratio of broiler chickens fed with 4% NLP showed better than birds fed with 0%, 2%, and 6% NLP. Moreover, no significant difference was observed in the cell-mediated immunity, and no mortality of birds was recorded. The return above feed and chick cost of broiler chickens fed with 4% NLP is more profitable, and the income generated increases as high as 25.73% compared to the birds fed without NLP in the diet. However, a future digestibility study using a large population of experimental animals is recommended to assess the nutrient flow and retention from the digestive sites. In conclusion, 4% NLP could be incorporated into the diets of broiler chickens without fear of compromising growth and immunity responses.

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## NOVELTY STATEMENT

Several studies using phytogenic feed additives or supplements to broiler chickens were conducted. However, the information utilizing neem leaf powder as phytogenic feed additives to broiler chickens and investigating its potential on the production performance, economic traits, and cell-mediated immunity are scarce.

## AUTHORS CONTRIBUTION

Both authors contributed equally to this work.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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