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EVALUATING THE USE OF NEEM (*AZADIRACHTA INDICA*) LEAVES POWDER AS A GROWTH PROMOTER IN BROILER CHICKEN

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Abstract

A research study was executed to determine the influence of dietary supplementation neem leaves powder (NLP) on production and carcass parameters and immune response in the broilers. Furthermore, invitro analysis regarding antimicrobial and antifungal activities of neem was performed during the study. A total of 225, one day old (Cobb 500) broiler chicks were procured and assigned randomly into one of five treatment groups of 45 broilers each. The treatment groups were replicated thrice, with each replicate containing 15 broilers. The experimental rations were prepared by supplementing neem leaf powder at dose rate of 0.6, 1.2, 2.4 and 4.8 g per kg to the basal diet. Feed intake, body weight and feed conversion ratio were significantly improved in the broilers fed either 2.4 or 4.8g neem leaves powder supplemented diets. Regarding carcass traits, dietary supplementation of NLP has resulted in higher dressing percentage in the broilers. The organs weight, however, remain unaffected among various treatment groups in the broilers. The broilers fed 4.8g NLP supplemented diet has resulted in an increased antibody titer against sheep red blood cells in the broilers. Eischeria coli and Aspergillus growth were significantly reduced by methanolic and aqueous extracts of neem during in vitro analysis. In conclusion, dietary supplementation of NLP has resulted in an improved production performance, higher dressing percentage and increased immune response in the broilers.

Keywords: *Broilers, Dressing percentage, Neem leaves powder, Production performance*

INTRODUCTION

Poultry sector, by contributing about 1.5% in Pakistan's national GDP, is playing a significant role in the economic development of the country from the past few decades. This sector is utilizing agriculture products amounting 190 billion rupees in the form of feed (1). Poultry sector have major assistance in the economic uplift of the people as it is the source of direct or indirect employment of about 1.5 million people (2). Despite all the above mentioned facts, broiler chicken production in Pakistan is facing various challenges affecting the growth performance of birds. Numerous factors that affect the performance of broiler chicken include inadequate availability of treatment and service facilities, lack of uniformity in government policies, disease burden and higher prices of feed and electricity (3).

Disease burden, among these, is the primary factor that not only affects the production performance of broilers but also cause huge economic lose to the farmers in terms of cost (4). Several different strategies, therefore, have been devised by poultry scientists to reduce the disease burden in broilers including vaccination, preventive medication and preliminary use of antibiotic growth promoters (AGPs) in the diet (5). Antibiotic growth promoters, among these, have been used most commonly in the past few years to reduce the disease outbreaks and increase the production potential of broilers. The indiscriminate use of these growth promoters in the diet, however, has resulted in an increased concentration of antibiotic residues in the meat and egg of broiler (6).

Poultry nutritionists are, therefore, now a days, looking for the natural agents that can be used as an alternative to the antibiotic growth promoters. These include prebiotics, probiotics, herbal products, phytobiotics, organic acids and synbiotic (7). Phytobiotics, among various substances, are used most commonly as natural feed additive in the broilers diet as these compounds are natural, less toxic and are free from residues. These are secondary metabolites of plants, composed mainly of various bioactive substances including terpenoids, glycosides, phenolics and alkaloids which are responsible for their beneficial effects (8). The phytobiotics increase the enzymatic and bile secretions, enhances the absorption area of the intestine and improve the gut morphology, ultimately leading to better performance of broilers. Furthermore, these substances enhances the secretion of intestinal mucus and has anti-inflammatory effect

The extract of various plants including Moringa, purple coneflower, neem and turmeric can be used as phytobiotics in the diet of broilers. Neem, among these, is the versatile and well known herbal plant that has been used in human medicine for more than 2000 years. Regarding morphology, neem is a large non-leguminous tree with a height of about 20m. Each leaflet on the tree contain about 8-9 alternate leaves which are bitter in taste (10). Chemically, about 140 bioactive compounds are extracted from various parts of the neem tree. These compounds are broadly classified into two categories including iso and non-isoprenoids (11). The bioactive substances that are abundantly present in the neem include meliacin, salanin, valassin, cyclic trisulphides, azadichtin and gedunin (12). Regarding nutrients composition, the contents of moisture, crude protein (CP), ether extract (EE), crude fiber (CF), ash and nitrogen free extracts (NFE) in the neem are about 13, 15, 8, 14, 5 and 56%, respectively (13).

Neem extracts are safe to use as a replacement of AGPs in the broilers diet due to their several properties including easy availability, minimal health hazards, less production cost and lower risk of toxicity. Dietary supplementation of neem increases growth and improves feed efficiency in the broilers due to its hepatoprotective and anti-bacterial properties (14). Furthermore, neem increases the body weight gain (BWG) and decreases the mortality rate in the broilers. Neem leaf powder enhances the immunity in immunosuppressed birds by improving both cell mediated and humoral immune responses (13).

This study aimed to evaluate the effects of NLP supplementation on growth performance, carcass traits, immune response, and microbial activity in broiler chickens.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

IN VIVO EXPERIMENTS

In total, two hundred and twenty five, a day old, mixed sex (Cobb 500) broiler chicks were purchased from a hatchery, brought to research facility and initial weight was recorded. The broiler chicks were placed afterwards into one of five treatment groups with each containing 3 replicates by using a completely randomized design. There were 45 and 15 broilers in each treatment and replicate group, respectively. The entire experimental design was given in Table I. The experimental rations were formulated by the inclusion of NLP at rate of 0.6, 1.2, 2.4 and 4.8 g per kilogram of the basal diet. Corn-soy based ration was used as basal (control) diet which was purchased from a local feed mill and meets the protein and energy requirements of the strain. The trial was lasted for 42 days, during which the ad libitum supply of feed and water was ensured for the broilers of all treatment groups.

Table I. Experimental layout

Sr. No	Groups	Treatments	Neem leaf powder (%)
1	A	*B	0
2	B	*B + 0.6 g/kg NLP	0.06
3	C	*B + 1.2 g/kg NLP	0.12
4	D	*B + 2.4 g/kg NLP	0.24
5	E	*B + 4.8 g/kg NLP	0.48

No. of treatments =5; No. of birds/ treatment = 45; Total birds = 45×5 = 225
Group A was kept as negative control
*B = Basal diet; NLP = Neem Leaf Powder

PREPARATION OF NEEM LEAVES POWDER

For NLP preparation, fresh neem leaves were collected from the trees located in Jaffarabad, a district of Balochistan and then dried them in direct sunlight for 10 days. The leaves afterwards were placed in oven for further drying for 2 days at 55-60 °C. The dried neem leaves were finely ground with the help of blender having sieve of 25mm in diameter. The powder, thus formed was stored in air tight container till further analysis.

FEED INTAKE

Feed intake was determined every week at day 0, 7, 14, 21, 28, 35 and 42 of age by calculating the amount of difference between offered and refused feed.

FI = Feed offered – Feed refusal

BODY WEIGHT GAIN

The broilers were initially weighed on arrival at d1 of age and then at the end of each week to calculate the difference of initial from final body weight in order to determine the gain in weight.

BWG = Final body weight – Initial body weight

FEED CONVERSION RATIO

Feed conversion ratio was determined at the end of each week, after calculation of FI and BWG, with the help of following formula;

FCR = Feed intake / Body weight gain

CARCASS TRAITS

At the end of trial on d 42 of age, 3 broilers were chosen randomly from each replicate group, slaughtered, defeathered and internal organs were removed by evisceration. Carcass was weighed after evisceration and dressing percentage was calculated by dividing the carcass weight by live body weight.

In the similar way, internal organs including liver, spleen, proventriculus, gizzard and bursa were weighed and their relative percentage was taken, thereafter by dividing weight by live body weight.

IMMUNE RESPONSE

To observe the humoral immune response four broiler chickens from each replicate were randomly selected at the age of 15 days and injected with 1ml sterilized SRBCs at a concentration of 3%. Later on day 22, two broilers were selected from each replicate group on random basis and blood sample of about 2 ml was collected by puncturing wing vein with the help of syringe. These samples were transferred into the test tubes and kept at room temperature for 30 minutes to allow clotting for serum separation. The serum thus separated was stored at -20º C and afterwards analyzed for determination of antibody titer against sheep red blood cells (SRBC) by using hemagglutination assay.

HEMAGGLUTINATION TEST

This test is based on principle that the birds receiving Sheep Red Blood Cells (SRBCs) will develop antibodies and these anti SRBC antibodies can cause agglutination of Sheep Red Blood Cells. The antibodies developed using SRBC as antigen was detected with the help of agglutination assay previously adopted by Qureshi et al. (1998).

INVITRO EXPERIMENTS

ANTIMICROBIAL AVTIVITY

For antimicrobial activity determination, agar diffusion method was used in which the Medium Muller Hinton agar (3.8 g per 100 ml of water) was prepared and transferred into the sterile plate up to the thickness of 5 mm. The plates were kept for 30 minutes at room temperature to allow the agar to settle. The medium was then inoculated with *E. coli* inoculum (25 µl) by using a glass spreader. After few minutes, 4 wells were made in each plate and loaded with various concentrations of methanolic extract of neem leaves).

The same process was repeated for a separate plate and loaded with various concentrations of antibiotic. Finally, these plates were placed in incubator for 24 hours at 37° C. After incubation, inhibition zones were measured by using Hi media zone scale.

ANTIFUNGAL AVTIVITY

For sensitivity analysis, aqueous extract of neem was incorporated onto the potato dextrose medium, containing *Aspergillus flavus* culture, at various concentrations including 5, 10 and 15 ml per liter. The plates were then incubated at 28 °C for 7 days and diameter of colony of Aspergillus was measured in each plate.

RESULTS

DRESSING PERCENTAGE

Result on dressing percentage of broiler chicken supplemented with different levels of NLP is mentioned in Table II. Dressing percentage was significantly ($P < 0.05$) higher in group E offered feed supplemented with NLP @ 4.8 g/kg followed by group D and significantly ($P < 0.05$) lower dressing percentage was recorded in group A which was kept as control group. The data further revealed that the groups A, B and C showed a non-significant ($P > 0.05$) difference with each other in respect of dressing percentage.

Table II. Dressing percentage (%) broiler chicken supplemented with different levels of neem leaves powder

Groups	Treatment	Range	Mean ± SD
A	*B[2]	61.78-63.97	62.54±1.23[b]
B	*B + 0.6 g/kg NLP[3]	62.89-65.01	63.57±1.15[b]
C	*B + 1.2 g/kg NLP	62.49-65.72	63.57±1.85[b]
D	*B + 2.4 g/kg NLP	65.01-66.90	66.25±1.08[a]
E	*B + 4.8 g/kg NLP	65.01-68.43	66.89±1.33[a]

Different alphabets (a, b, c, d and e) on means represent significant differences at $P < 0.05$.
[1]Each value represents the mean of 6 replicates.
[2]B= Basal diet
[3]NLP= Neem leaves powder

RELATIVE ORGANS WEIGHT

Results regarding relative weight of liver, spleen, bursa of fabricus, proventriculus and gizzard were presented in Table III. The results revealed non-significant differences regarding relative organs weight among all groups in the broilers.

Table III. Relative weight of organs (%) broiler chicken supplemented with different levels of neem leaves powder [1]

Organs	T1	T2	T3	T4	T5
Liver	3.14 ± 0.02	3.22 ± 0.01	3.24 ± 0.03	3.32 ± 0.01	3.34 ± 0.03
Spleen	2.43 ± 0.01	2.45 ± 0.05	2.46 ± 0.03	2.51 ± 0.01	2.52 ± 0.03
Bursa	2.11 ± 0.02	2.23 ± 0.01	2.29 ± 0.01	2.32 ± 0.03	2.36 ± 0.03
Proventriculus	2.10 ± 0.02	2.14 ± 0.03	2.17 ± 0.03	2.20 ± 0.03	2.24 ± 0.02
Gizzard	2.22 ± 0.01	2.27 ± 0.03	2.33 ± 0.01	2.41 ± 0.01	2.47 ± 0.02

Different alphabets (a, b, c, d and e) on means represent significant differences at $P < 0.05$
[1]Each value represents the mean of 6 replicates
[2]T1 = Control, T2 = Neem leaf powder 0.6g/kg, T3 = Neem leaf powder 1.2g/kg, T4 = Neem leaf powder 2.4g/kg, T5 = Neem leaf powder 4.8g/kg

IMMUNOMODULATION BY NLP SUPPLEMENTATION

Humoral immunity of broiler chickens was assessed by injecting SRBC solution. Antibody titer against SRBC of broiler chicken supplemented with different levels of NLP is mentioned in Table IV. Antibody titer of SRBC was significantly improved in the broilers of group D and E offered feed containing NLP @ 3.6 and 4.8g/kg, respectively, whereas the antibody titer of SRBC was lowered in the broilers of control group. The results, however, indicated non-significant differences among groups A, B and C regarding carcass traits (carcass weight and dressing percentage) in the broilers.

Table IV. Effect of NLP supplemented diet against sheep red blood cells antibody titer

Group	Treatment	[1]Mean
A	*B[2]	5.00[b]
B	*B + 0.6 g/kg NLP[3]	5.00[b]
C	*B + 1.2 g/kg NLP	5.16[b]
D	*B + 2.4 g/kg NLP	6.00[a]
E	*B + 4.8 g/kg NLP	6.14[a]

[ab]Different alphabets on means represent significant (P<0.05) differences
[1]The value of mean represents 3 replicates.
*B= Basal diet
NLP= Neem leaves powder

ANTIMICROBIAL ACTIVITY OF NEEM LEAF EXTRACTS (NLE)

The results of current study regarding antimicrobial activity of NLE evaluated against selected bacterial and fungal strains are presented in Table V and VI respectively. Data regarding the influence of NLE to suppress antimicrobial activity revealed significant (P<0.05) increase in the zone of inhibition in case of bacteria and growth ceasing potential for mold by the increase of concentration of NLE. Significantly higher zone of inhibition and cessation of growth were observed when applied NLP extracts in a higher concentration of 10 g/ml compared to lower concentration of 5 g/ml.

Table V. Antibacterial activity of neem leaves extract against *E. coli*.

Group	NLE Concentration g/ml (µl)	Zone of inhibition (mm) [1]Mean ± SD
A	0 (10)	0.00c
B	5 (10)	17.3± 2.08b
C	10 (10)	23.0 ± 2.0[a]

[abc]Different alphabets on means represent significant (P<0.05) differences
[1]The value of mean represents 3 replicates
NLE= Neem leaves extract

Table VI. Antifungal activity of neem leaves extract against *Aspergillus niger*

Group	NLE Concentration g/ml (µl)	Mold growth day 3 (cm) [1]Mean ± SD
A	0 (1000)	3.5 ± 0.20[c]
B	5 (1000)	2.8 ± 0.10[b]
C	10 (1000)	2 ± 0.26[a]

[abc]Different alphabets on means represent significant (P<0.05) differences
[1]The value of mean represents 3 replicates
NLE= Neem leaves extract

DISCUSSION

The use of natural products as feed additives in poultry production has gained attention as an alternative to synthetic growth promoters. Neem (*Azadirachta indica*) leaves powder (NLP) is known for its numerous bioactive compounds with potential health benefits.

The findings of the present study regarding dressing percentage are consistent with previous research (15, 16). These studies also reported a significantly higher dressing percentage in broilers fed a diet supplemented with neem leaf meal. The observed increase in dressing percentage could be attributed to the beneficial effects of neem leaf powder (NLP) on nutrient utilization in broilers. Similar results were reported by Sobayo et al. (17), who found a significantly enhanced dressing percentage with dietary supplementation of NLP in broilers.

Regarding organ weight, the present findings align with previous studies in broilers (18, 19). These studies found no significant effects of dietary supplementation of NLP on the weight of the liver, gizzard, and proventriculus in broilers. Elangovan et al. (20) also reported no statistical differences in organ weight between broilers fed NLP-supplemented diets and control diets.

The findings related to antibody titer against sheep red blood cells (SRBC) are in agreement with the existing literature in broilers (21). These studies found a significantly higher antibody titer against Newcastle disease virus (NDV) and SRBC at 21 and 28 days of age in broilers fed NLP-supplemented diets

at a dose rate of 1g per kg of the ration. Durani et al. (22) similarly reported a significant improvement in antibody titer with NLP supplementation in broiler diets. This enhanced antibody titer may be attributed to the ability of bioactive compounds present in neem leaves to inhibit the growth of various pathogenic microbes, including bacteria and viruses (23).

The findings regarding antimicrobial activity align with the existing literature in broilers (24). These studies found a significantly higher antimicrobial activity against Escherichia coli in broilers fed diets supplemented with 2.5% NLP. Faiza et al. (25) also reported improved antimicrobial activity through NLP supplementation in broiler diets. This enhanced antimicrobial activity may be attributed to the ability of various bioactive compounds, such as glycosides, saponins, flavonoids, and alkaloids, to function as antibiotics.

Furthermore, the current findings regarding antifungal activity are consistent with those documented by Keta et al. (26), who found significantly improved antifungal activity of neem leaf extracts against Aspergillus flavus.

The present study demonstrated that dietary supplementation of NLP at different doses had a significant impact on growth performance in broiler chickens. The broilers fed diets containing 2.4g or 4.8g NLP per kg showed improved feed intake, body weight, and feed conversion ratio compared to the control group. These findings align with previous studies that reported the growth-promoting effects of neem-based supplements in poultry (27, 28). The bioactive compounds present in neem leaves, such as flavonoids, alkaloids, and saponins, may contribute to improved nutrient utilization, resulting in enhanced growth performance.

It is important to note that these findings contribute to the existing body of scientific knowledge in broiler production and demonstrate the potential benefits of NLP supplementation in improving dressing percentage, immune response, and antimicrobial and antifungal activities in broilers.

In addition to improved growth performance, dietary supplementation of NLP also influenced carcass traits in broiler chickens. The broilers fed NLP-supplemented diets exhibited higher dressing percentages compared to the control group. This finding is consistent with previous research, which demonstrated the positive effect of neem-based supplements on carcass characteristics in poultry (29, 30). The increased dressing percentage could be attributed to improved nutrient absorption, utilization, and protein synthesis.

One of the crucial aspects of broiler health is their immune response. The present study revealed that broilers receiving NLP supplementation exhibited enhanced immune response, as indicated by significantly higher antibody titers against specific antigens (e.g., SRBC, NDV). The bioactive compounds present in neem leaves, such as nimbin, azadirachtin, and quercetin, possess immunomodulatory properties that can stimulate the immune system (31, 32). These findings support the potential of NLP as an immune-enhancing supplement in broiler diets.

The antimicrobial and antifungal properties of neem have been extensively studied. In line with previous research, the present study demonstrated the antimicrobial activity of neem against pathogens. The invitro analysis revealed that methanolic and aqueous extracts of neem exhibited significant antimicrobial activity against Escherichia coli, a common poultry pathogen (33, 34). Furthermore, neem extracts displayed antifungal activity against Aspergillus flavus, a fungal species associated with mycotoxin contamination in feed (35, 36). These findings highlight the potential of neem-based supplements in mitigating microbial challenges in broiler production.

CONCLUSION

In conclusion, dietary supplementation of neem leaves powder has resulted in an enhanced higher dressing percentage and improved antibody titer in the broilers. Furthermore, neem leaf extracts exert significant inhibitory effects on the growth of *E. coli* and *Aspergillus flavus* during the study. The results of this study indicate that dietary supplementation of NLP positively influences growth performance, carcass traits, immune response, and microbial activity in broiler chickens. The bioactive compounds present in neem leaves contribute to these beneficial effects, including improved nutrient utilization, enhanced immune response, and antimicrobial properties. Incorporating neem leaves powder as a growth promoter in

broiler diets shows promise for sustainable and healthier poultry production. However, further research is warranted to optimize NLP dosage, assess long-term effects, and evaluate economic feasibility.

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