



Effect of replacing saw dust litter with dry Neem leaves for improving litter quality, immunity and foot pad condition of broiler birds

R K YADAV¹, N SINGH^{2✉}, S SHARMA³, G GUJAR⁴, V CHANDRA⁵ and A FOZDAR⁶

*Post Graduate Institute of Veterinary Education and Research,
Jaipur (Rajasthan) NH-11, Agra Road, Jamdoli, Jaipur-302 031*

Department of Livestock Production Management, Rajasthan University of Veterinary and Animal Sciences, Bikaner

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ABSTRACT

This experiment was an attempt to study the impact of replacing saw dust with dry neem leaves in litter for improving litter quality, immunity, and foot pad condition of broiler birds. The experiment was carried out in the institutional poultry farm during June 2024. Eighty White Leghorn broiler chicks (Cobb strain) were randomly divided into treatment and control group (40 in each) with each group having four replications having 10 birds each. The broiler chicks were reared in deep litter housing system made of saw dust (94%) along with dry Neem leaves (6%) with the control group reared on saw dust litter without dry neem leaves. The litter quality parameters viz., pH, moisture, water holding capacity (WHC), ammonia emission, nitrogen content and cake formation score; broiler immunity expressed as antibody titre against Newcastle disease and broiler welfare in terms of footpad condition were recorded weekly over the period of 6 weeks. The data was analysed using one way ANOVA followed by post hoc DMRT. Results revealed that the treatment group had a significantly higher antibody response against NDV (145.50 ± 3.50 vs. 14.00 ± 1.33), indicating enhanced immunological status. Welfare indicators such as foot pad condition (0.57 ± 0.28 vs. 1.29 ± 0.64) and reduced cake formation in litter (4.00 ± 0.00 vs. 5.00 ± 0.00) also reflected positive effects. Litter quality was substantially better in the treatment group, with lower moisture content (18.37 ± 0.17 vs. 26.77 ± 0.18), reduced pH (6.40 ± 0.04 vs. 6.95 ± 0.06), decreased ammonia emission (10.80 ± 0.46 vs. 23.05 ± 0.55), lower nitrogen content (15.89 ± 0.15 vs. 20.91 ± 0.11), and reduced parasitic load (11.67 ± 0.06 vs. 25.47 ± 0.06). Microbial load was also diminished, as reflected in the lower total viable count (6.64 ± 0.05 vs. 8.19 ± 0.06). Although water holding capacity did not differ significantly between groups, the overall findings demonstrate that the treatment group achieved superior immune response, improved welfare, and enhanced litter quality, underscoring its effectiveness in broiler management.

Keywords: Ammonia Emission, Dry Neem Leaves, Foot Pad Condition, Immune Response, Litter

Poultry industry is a rapidly expanding, energyefficient livestock sector that reduces production cost and increases protein availability through genetic improvement, optimized nutrition, and improved management. Poultry, particularly broiler production contributes substantially to human nutrition and food security by supplying highquality animal protein and by diversifying farm income between crop and livestock enterprises (Augustine and Shukla, 2015). Despite genetic gains and favourable feed conversion, a huge gap remains between recommended and actual percapita consumption in India (ICMR target: 10.8 kg meat and 180 eggs per year; actual availability: 7.10 kg meat and 101 eggs per year; Basic Animal Husbandry

Statistics2023). Realizing the full genetic potential of modern strains therefore, requires enhanced management and welfare practices. Globally, broilers are predominantly reared on deeplitter system, where litter composition and management strongly influence their health, welfare, and production (Dukic Stojcic et al. 2016). Poor litter quality promotes pododermatitis (footpad dermatitis, a painful condition that impairs gait), reduces feed intake and weight gain, and constitutes a major welfare concern (Mayne et al. 2007; Hester et al. 1997; Berg 2004). Inadequate litter management also elevates ammonia concentrations, which are corrosive and toxic, and are associated with conjunctivitis, immunosuppression, respiratory lesions, and renal disorders. Consequently, litter management is a critical determinant of broiler performance and meat quality. To mitigate litterrelated problems, producers apply chemical, microbiological, and organic amendments to improve moisture control, pH, and physical structure, thereby reducing incidence of footpad dermatitis and

Present address: 1, 2, 3, 4, 5 and 6, Department of Livestock Production Management, Post Graduate Institute of Veterinary Education and Research, Jaipur (Rajasthan) NH-11, Agra Road, Jamdoli, Jaipur-302031. ✉Corresponding author Email: dmnavavsinghdhaker@gmail.com

respiratory and ocular conditions (Shepherd et al. 2010). Earlier reports indicate that incorporating neem leaves into litter can improve litter physical properties (pH, moisture, cake formation), reduce parasitic load, and enhance feed intake, feed conversion ratio, and immune responses in broilers (Yadav et al., 2025).

Neem (*Azadirachta indica*) is a fast-growing perennial tree which is easily available, and it is native to India and commonly used in Ayurveda, Unani, and Homeopathy system of medicine. The necessity for ancient medicinal practice for meeting the primary health needs of the people of developing countries like India, Bangladesh, etc. has been recognized by WHO. Neem leaves have several medicinal properties viz. antibacterial, antifungal, anti-malarial, antiviral, anti-cancer (Agarwal 2002; Subapriya and Naigin 2005) and anti-coccidial (Panday et al. 2018). Neem is easily available and has a potential of being used as litter material in poultry due to its medicinal properties. Earlier reports have suggested that inclusion of neem leaves in saw dust litter enhances quality and ultimately improves the performance (Panday et al., 2018) and immunity (due to the medicinal properties of Neem) of the broiler (Bishnoi et al. 2021). Taking note of those above facts, the current study was designed to compare the litter quality, immunity and foot pad condition of broiler birds reared under conventional saw dust litter and saw dust litter when partially replaced with dry neem leaves.

MATERIALS AND METHODS

Location and period of study: The present experiment was conducted at Livestock Farm Complex, Post Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur.

Ethics Statement: The current study was conducted in accordance with the ethical guidelines established by the institute for the ethical handling and welfare consideration of birds.

Experimental birds and management: The current study involved 80-day old White Leghorn broiler birds of cobb strain. Before the commencement of the actual experiment, the experimental pens, watering, and feeding troughs were cleaned, disinfected, and sprayed against external parasites. The experimental birds were provided identical care and management and strict hygienic measures were undertaken during the whole experimental period as per standards. The birds were maintained under standard management practices regarding feeding, watering, and disease control throughout the experimental period. The commercial poultry mash was procured from the local market and fed *ad-lib*. Starter feed was offered up to the end third week and thereafter finisher feed was offered.

Experimental design: The broiler birds were randomly distributed into two groups with four replications, with each replication including ten birds. Birds in the experimental group- T were reared on litter made of 94 percent Saw dust and 6 percent dry neem leaves and birds in control group (C) was reared only on saw dust litter in a deep litter house.

Recording of parameters: Estimation of antibody response in broilers: The antibody response against the New Castle Disease virus (NDV) was determined by haemagglutination inhibition (HI) tests. Briefly, on 7th and 14th day after vaccination, 2 ml of blood samples were aseptically collected from 4 birds of each group using a sterile hypodermic needle and kept aside to coagulate for an hour on the bench in a slanting posture, the sera were extracted and carefully poured into a fresh container labelled with the sample number, and treatment group. The sera were used to assess antibody titre and antibody specific to NDV by HI test following standard procedure (Allan and Gough, 1974).

Footpad condition: The footpad condition was assessed and scored on a 4-point scale as adapted and modified from the works of McWard and Taylor, (2000). The scores ranged from 0 to 3. 0 =no burn, scab, or lesion, 1 = pad burn (dermis only), 2= pad scab (healing) on one or both feet and 3 = pad lesions (open score) on one or both feet.

Moisture percentage: At the end of each week, 50-60 grams of litter samples were taken from four corners and one from centre at certain areas within each group, properly combined to create a representative sample. Moisture content Brake et al. (1992), and litter moisture (AOAC 1990) were then examined in each litter material every week upto 42 days of age as:

$$\text{Moisture \%} = \frac{\text{Loss in weight (W1g - W2g)}}{\text{Weight of sample (W1 - W2)}} \times 100$$

Where,

Wg : Weight of empty tin

W1g : weight of empty tin + sample before drying

W1-Wg : weight of sample taken

W2 g : weight of empty tin + sample after drying

W2 g-W1 g : loss in weight

pH evaluation of litter material: The pH of the litter was tested following Brake et al. (1992). At each sample location, the top 10 cm of the litter was removed, and brought back to the lab to measure the pH using a pH meter.

Total viable count: The total viable count was estimated using the methodology described by the American Public Health Association (1992) with some customizations and expressed as colony forming unit per gram (cfu/g).

Ammonia emission: At the end of the investigation, the litter condition was evaluated. The ammonia released from litter samples was measured using Hernandez and Cazetta's (2001) method based on gaseous ammonia fixation by micro-diffusion.

Nitrogen content: The nitrogen content of the litter was measured using the Kjeldahl Method (AOAC 1990).

Cake formation score in litter material: The cake formation score was calculated using the methodology outlined by Andrews (1972) and Carter et al. (1979). Litter material was picked up from four distinct group locations in each group and clenched into a fist. The compaction of the litter was measured using the scale from 0 (no litter cake development) to 5 (the whole pen covered with caked

litter).

Water-holding capacity of litter material: After adding 20 grams of a dried sample of litter material to the plastic pan, which was 4 cm deep, water was added, and the pan was allowed to sit at room temperature for an hour. The sample was weighed, and the percentage of water detected was calculated on a dry matter basis after any surplus water was drained.

Parasitic load in litter: The count of coccidial oocysts was done using the technique of Long *et al.* (1975) and recorded as oocyst per gram (OPG) of litter.

Statistical Analysis: Data collected were tabulated and analysed by Snedecor and Cochran (1994) followed by Duncan's Multiple Range Test (DMRT; Duncan 1955) analysis using SPSS software version 24.

RESULTS AND DISCUSSION

The results revealed that the mean antibody titre against NDV was significantly ($p < 0.01$) higher in the treatment group (145.50 ± 3.50) compared to control group (14.00 ± 1.33). Similar findings were observed by Bishnoi *et al.*

(2021) and Jawad *et al.* (2013) with inclusion of organic litter material with conventional litter. Findings of Landy *et al.* (2011) also supported this study and it could be said that Neem leaves increased humoral immune response against NDV.

The foot pad score was 1.29 ± 0.64 in the control group compared to 0.57 ± 0.28 in the treatment group. This reduction was highly significant ($p < 0.01$), suggesting improved welfare and reduced foot pad lesions in the treatment group. This could be the result of antibacterial nature of neem (Agarwal 2002; Subapriya and Naigin 2005) that played a role in immunomodulatory properties to reduce total viable count, moisture percent (Bishnoi *et al.* 2021), pH (Bishnoi *et al.* 2021, Panday *et al.* 2018) and consequently low cake formation score (Bishnoi *et al.* 2021) leading to reduction of foot pad lesion.

Moisture content was recorded as $26.77 \pm 0.18\%$ in the control group and $18.37 \pm 0.17\%$ in the treatment group. The difference was highly significant ($p < 0.01$; Fig. 2), showing that the treatment litter maintained comparatively drier conditions. The Post hoc DMRT revealed significant

Table 1. Mean values of Various Parameters in different experimental groups

S.No.	Parameters	Unit	Control group	Treatment group	Significance Level
1.	Antibody response in broiler	Antibody titer against NDV	14.00 ^e ±1.33	145.50 ^a ±3.50	**
2.	Foot pad condition	Score 0 to 4	1.29 ^d ±0.64	0.57 ^{ab} ±0.28	**
3.	Moisture per cent	per cent	26.77 ^c ± 0.18	18.37 ^a ± 0.17	**
4.	pH evaluation of litter material	pH	6.95 ^c ± 0.06	6.40 ^b ± 0.04	**
5.	Total Viable Count	CFU/gm	8.19 ^b ± 0.06	6.64 ^a ± 0.05	*
6.	Ammonia Emission	ppm/100gm litter	23.05 ^c ± 0.55	10.80 ^b ± 0.46	**
7.	Nitrogen content	g/kg	20.91 ^c ± 0.11	15.89 ^b ± 0.15	**
8.	Cake formation score in litter material	Score 1 to 5	5.00 ^d ± 0.00	4.00 ^{ab} ± 0.00	**
9.	Water holding capacity of litter material	per cent	114.41 ± 0.19	142.31 ± 0.18	NS
10.	Parasitic load in litter	Oocysts/G	25.47 ^f ± 0.06	11.67 ^a ±0.06	**

Means bearing different superscripts (a, b, c, d, e, f) differ significantly at (*)5% level ($P < 0.05$) and (**)1% level ($P < 0.01$)

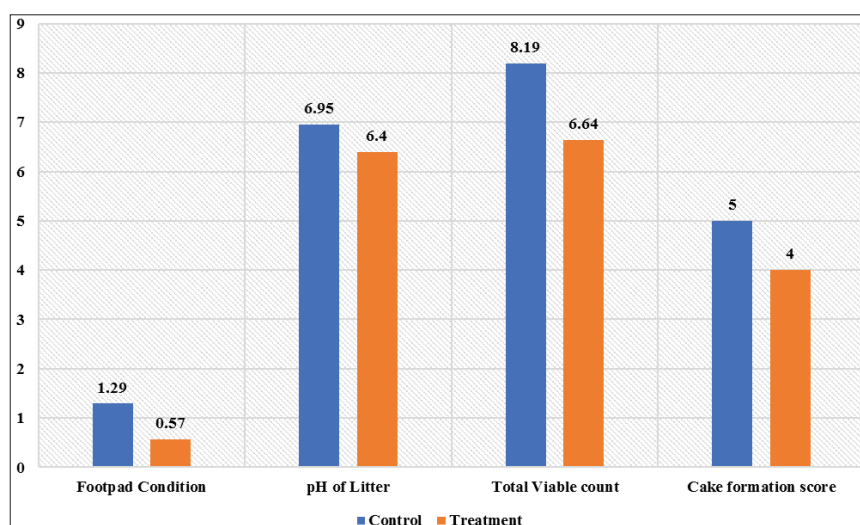


Fig. 1 Graph showing mean values of Footpad Condition, Litter pH, Total Viable Count and Cake Formation Score in control and treatment groups

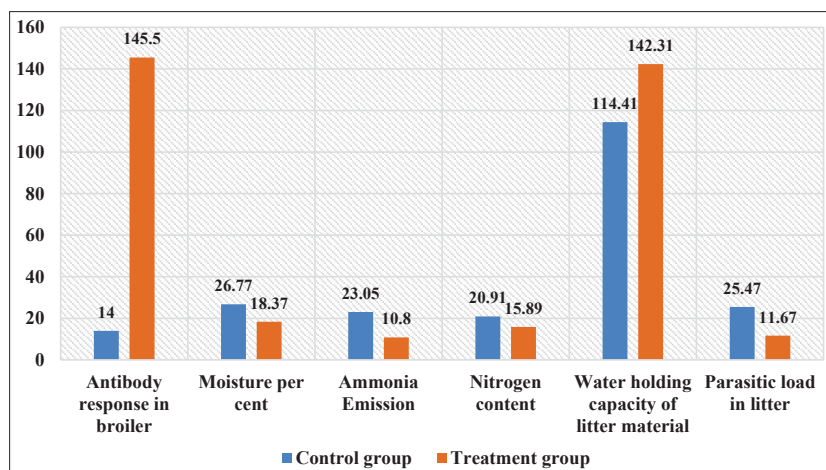


Fig. 2 Average Values of Various Parameters recorded in Control and Treatment Groups

variation of weekly moisture percent between treatment and control group and found lower moisture percent in treatment group as compared to control group. Similar findings have been earlier reported by Panday *et al.* (2018) and Bishnoi *et al.* (2021). Neem leaves inclusion in litter increases the water releasing capacity of litter leading to low moisture content.

The pH of litter material was 6.95 ± 0.06 in the control group and 6.40 ± 0.04 in the treatment group. Highly significant ($p < 0.01$; Fig. 1) effect of dry neem leaves supplementation was seen on pH of litter. These results are similar with the findings of Panday *et al.* (2018) and Bishnoi *et al.* (2021). The pH of neem supplemented litter material was to be found low which indicated low ammonia production and leads to improved broiler performance.

The microbial load (CFU/gm) was 8.19 ± 0.06 in the control group and 6.64 ± 0.05 in the treatment group. The total viable count significantly ($p < 0.05$; Fig. 1) varied between treatment and control groups with lower total viable count in treatment group as compared to control group. Neem also has antibacterial, antifungal (Agarwal 2002, Subapriya and Naigin 2005), and anti-coccidial (Bishnoi *et al.* 2021 and Chakma *et al.* 2012) properties which reduced the total viable count and parasitic load. Inclusion of neem leaves reduced pH (Bishnoi *et al.* 2021; Panday *et al.* 2018) and moisture percent (Panday *et al.* 2018, Bishnoi *et al.* 2021) in litter matter that led to a decrease in total viable count and parasitic load.

Cake formation score was 5.00 ± 0.00 in the control group and 4.00 ± 0.00 in the treatment group (Fig. 1). The difference was highly significant ($p < 0.01$), indicating reduced caking in the treatment litter. Neem leaves inclusion in poultry litter results in good water releasing capacity and in turn, low moisture content which decreases the cake formation score in litter (Panday *et al.* 2018 and Bishnoi *et al.* 2021).

Ammonia emission was 23.05 ± 0.55 ppm/100 g litter in the control group and 10.80 ± 0.46 ppm/100 g litter in the treatment group (Fig. 2). The difference was highly significant ($p < 0.01$), demonstrating reduced ammonia

release under treatment conditions. In dry neem leaves inclusion group, there was significant reduction of ammonia emission of litter material as compared to control group. Shishir *et al.* (2013) had also reported similar findings regarding ammonia emission of litter material. This could be due to antibacterial properties (Agarwal 2002, Subapriya and Naigin 2005) of neem against ureolytic bacterium which leads in reduction of ammonia production.

This study revealed nitrogen content to be 20.91 ± 0.11 g/kg in the control group and 15.89 ± 0.15 g/kg in the treatment group (Fig. 2). The reduction was highly significant ($p < 0.01$), suggesting lower nitrogen accumulation in the treatment litter. The antimicrobial properties of neem leaves (Agarwal 2002, Subapriya and Naigin 2005) and dry neem leaves contribute to a reduction in nitrogen content of the litter. Nitrogen is a key component of ammonia and by inhibiting its production, lower overall nitrogen levels in the litter is found. Similar finding has been reported by Panday *et al.* (2018). Significant ($p < 0.01$; Fig. 3) variation of water holding capacity between treatment group and control group was also recorded. The water holding capacity of litter material supplemented with dry neem leaves significantly increased as compared to control group till 5th week but in the last week no significant difference was observed. Dry neem leaves have good water releasing capacity and reduce moisture levels that might have to increase water holding capacity in litter materials. Garces *et al.* 2013 and Bishnoi *et al.* 2021 also reported similar finding concurring with our results.

Parasitic oocyst count was 25.47 ± 0.06 /g in the control group and 11.67 ± 0.06 /g in the treatment group. The data revealed significant ($p < 0.01$) variation of parasitic load in treatment group and control group and found lower parasitic load in treatment groups as compared to control group (Fig. 2). In the present study, parasitic load (oocyte/ gm) increased up to 6th week in treatment group but comparatively the concentration of oocytes was lower in neem leaves group as compared to control group probably owing to medicinal properties of neem leaves (Agarwal 2002, Subapriya and Naigin, 2005, Panday *et al.* 2018; Bishnoi *et al.* 2021).

This led to an improvement in the performance and better health in broiler. Similar findings were reported earlier also (Chakma *et al.* 2012, Bishnoi *et al.* 2021).

Inclusion of dry neem leaves in conventional litter material resulted in low parasitic load and better immune response of broilers compared to broilers reared on conventional poultry litter like sawdust. Inclusion of neem leaves at 6% level in litter material could improve litter characters (pH, Moisture, total viable count, ammonia emission, cake formation score and foot pad lesion) and quality of litter remains good throughout rearing period. It was concluded from the above experiment that using neem leaves as a partial replacement of saw dust litter material improved the litter quality, immunity and foot pad condition of broiler birds through improved litter hygiene.

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