Indian Journal of Animal Sciences **95** (5): 430–437, May 2025/Article
https://doi.org/10.56093/ijans.v95i5.168006

Optimizing broiler production: Effect of inclusion of inorganic litter supplements on litter characteristics, growth, immunity, and welfare of broiler chickens

RAJESH KUMAR YADAV[1], NAVAV SINGH[1✉], SANJITA SHARMA[1] and GAYATRI GUJAR[1]

Post Graduate Institute of Veterinary Education and Research, Jaipur, Rajasthan

Received: 19 June 2025; Accepted: 11 August 2025

ABSTRACT

Broiler production is a key component of poultry systems, and efforts to enhance bird performance and welfare are ongoing. Among the key interventions used to enhance broiler production and welfare, various litter amendments are being used to optimize broiler production. The current study was thus an attempt to holistically assess the impact of adding two common inorganic litter supplements, calcium carbonate and alum sulfate, alone or in combination to saw dust bedding on the growth performance, litter quality, immune response and welfare of birds. A total of 160 White Leghorn chicks were randomly assigned to four groups: T1 (sawdust only), T2 (sawdust + calcium carbonate at 50 g/kg), T3 (sawdust + alum sulfate at 25 g/kg), and T4 (sawdust with both supplements). Birds in T2, T3, and T4 showed significantly higher weekly body weight and weight gain than the control group (T1), while the T1 group had higher feed intake and feed conversion ratio. Immune responses were also significantly improved in the treatment groups. Additionally, litter from T2, T3, and T4 had lower moisture content, ammonia levels, nitrogen, viable count, pH, parasitic load, and reduced cake formation compared to T1. Welfare indicators, such as footpad health, were better in the treatment groups, emphasizing the benefits of adding inorganic supplements to litter for improved bird health, performance, and environmental conditions.

Keywords: Alum, Broiler production, Growth performance, Inorganic litter material, Welfare

The demand for animal source food has grown multi fold in the last decade owing to a booming world population. The demand is further going to increase due to change in food habits and increase in purchasing power of developing world where most population increase is expected to happen (FAO 2011). As a result, the demand for poultry products, mostly eggs and meat has been booming in the last decade. The broiler production has shown exponential growth in the Indian poultry world, with a compound annual growth rate of 4.33 per cent (BAHS 2023). The broiler industry contributes to more than half of India's meat production with 5 million tonnes of meat. Of this, 80-85% of the broiler production occurs through commercial organized farms while 15-20 per cent is handled by the unorganized sector (BAHS 2023), showing a popularization of intensive farming. The broiler industry worldwide has seen intensive intensification in the past two decades, on account of maximizing output per unit input (Mottet and Tempio 2017). Intensification in broiler production is associated with plethora of challenges for the birds as well as environment.

All broilers of poultry are commonly reared in deep litter systems. Poultry farming in deep litter systems affects health, welfare, and production. Litter material is any dry material used on the floor of chicken houses and defined as a combination of bedding material, excreta, feathers, spilled feed, and water (Casey *et al.* 2005). Deep litter management has an important say in the health and welfare of birds. The litter material plays an important role in ensuring the health, welfare, productivity as well as environmental sustainability of poultry. Traditionally, organic materials like wood shavings, straw, or sawdust are used as litter as they are readily available and cost less. However, all type of conventional litter material suffers from some drawbacks like extent of moisture retention, ammonia emission, supporting viable microbial population, which directly or indirectly impacts the birds' health and welfare. Various solutions have been tested over the years to address issues associated with the quality and limitations of commonly used litter materials in poultry housing. These include adding supplementary litter materials (Lopes *et al.* 2015), differential litter management strategies (Pepper and Dunlop 2021), using unconventional litter materials (Benabdeljelil and Ayachi 1996) or using housing amendments (Oliveira *et al.* 2019). The addition of litter supplements are principally aimed at reducing ammonia emission (Poudel *et al.* 2024) or reduction of viable microbial count (Soliman *et al.* 2018), decreasing moisture retention (Mohammadi-Aragh *et al.*

Present address: [1]Post Graduate Institute of Veterinary Education and Research, Jaipur (Rajasthan) NH-11, Agra Road, Jamdoli, Jaipur- 302031 ✉Corresponding author email: drnavavsinghdhaker@gmail.com

2025) or decreased cake formation (Watts *et al.* 2012) and enhancing overall welfare in birds.

Among poultry litter amendments, calcium carbonate ($CaCO_3$) and aluminium sulfate [$Al_2(SO_4)_3$] have been sparingly used to supplement conventional litter materials. Calcium carbonate, a naturally occurring mineral, has been investigated for its potential as a poultry litter material and has been found to significantly reduce ammonia emissions and increase litter pH and nitrogen content (Lee *et al.* 2013). As a hygroscopic substance, calcium carbonate has a propensity to absorb water from its surroundings. By decreasing pH, microbial load, and pathogenic microbiota, it lowers the moisture content of moistened litter and renders it unsuitable for microbial development and activity. On the other hand, when aluminium sulfate is added to poultry litter, it acidifies the litter and changes the volatile ammonia into the non-volatile ammonium ions. Reduced ammonia levels in the poultry house, fewer viable microbes in the litter, and better bird performance have been reported with aluminium sulfate supplementation to poultry litter, on account of notable decreases in pH (Kim and Choi 2009). Despite their acidifying potential and other physio-chemical attributes that could gainfully enhance litter quality and improve birds' health and welfare, limited studies have been undertaken to evaluate the role of calcium carbonate and aluminium sulfate on poultry performance and litter characteristics. With the above stated facts in consideration, this study was designed to holistically assess the impact of adding two common inorganic litter supplements, calcium carbonate and aluminium sulfate, alone or in combination to saw dust bedding on the growth performance, litter quality, immune response and welfare condition of birds.

MATERIAL AND METHODS

Experimental design: The experiment was carried out at the institutional Poultry Farm of Livestock Farm Complex (LFC), Post Graduate Institute of Veterinary Education and Research (PGIVER) Jaipur. For the trial, one hundred and sixty (160) one day old broiler chicks (Cobb strain) were procured from a hatchery (Sangam hatchery Jobner, Jaipur), randomly assigned to four groups, with each group having four replications having ten birds each. Chickens in the first experimental group T1 were reared on sawdust litter material and acted as control, the T2 and T3 treatment groups were reared on saw dust with calcium carbonate as litter supplement (50 gm per kg litter) and saw dust with alum sulfate (25 gm per kg litter) as litter supplement, respectively. While the T4 group was reared on saw dust supplemented with a combination of calcium carbonate (50 gm per kg litter) along with alum sulfate (25 gm per kg litter). Proper health and housing management was ensured throughout the trial to minimize stress and discomfort to the birds.

Growth parameters: The growth performance of birds was recorded on a weekly basis in terms of Weekly body weight (g), weekly body weight gain (g), weekly feed consumption (g) and weekly feed conversion ratio (FCR) using standard methods.

Litter characteristics: Litter samples were collected weekly from each group and each replicate from five locations within the pen (four peripheral, equidistant locations from each pen corner and one from centre of the pen) and properly mixed to obtain material representative of that entire pen. The litter samples were analysed to quantify the litter moisture percentage, pH, Water-holding capacity using methodology of Brake *et al.* (1992). Ammonia emission from litter was determined by microdifusion method (Hernandez and Cazetta 2001) and nitrogen content of litter was determined as per the Kjeldahl Method (AOAC 1990) with slight modifications. The cake formation score of litter was determined using the methodology outlined by Andrews (1972) and Carter *et al.* (1979). The total viable count and parasitic load of litter was estimated following the methods of American Public Health Association (1992) and Long *et al.* (1975), respectively.

Immune and welfare status of birds: The antibody response against the new castle disease virus was determined by haemagglutination (HA) and 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 labelled. The sera were used to assess antibody titre and antibody specific to New Castle Virus (NDV) was also evaluated in the sera by haemagglutination inhibition (HI)

Table 1. Effect of inorganic supplementary litter material with saw dust on average weekly body weights (g) of broilers

Age (Weeks)	Treatment group				
	T_1	T_2	T_3	T_4	Significance level
0 Day	46.42 ± 0.43	46.75 ± 0.59	46.12 ± 0.55	46.37 ± 0.71	NS
1st	165.82^a ± 0.44	167.75^b ± 0.43	171.47^c ± 0.49	174.00^d ± 0.49	**
2nd	397.32^a ± 2.25	405.70^b ± 0.38	409.12^c ± 0.53	414.35^d **± 1.05**	**
3rd	882.25^a ± 1.56	887.77^b ± 0.69	892.27^c ± 1.68	898.42^d ± 1.41	**
4th	1419.30^a ± 3.11	1427.17^b ± 0.63	1432.47^c ±0.82	1437.75^d ± 1.07	**
5th	1923.70^a ± 1.38	1929.35^b ± 0.98	1936.42^c ± 1.19	1943.22^d ± 0.96	**
6th	2324.65^a ± 0.78	2329.80^b ± 0.57	2336.17^c ± 0.66	2337.6^c ± 2.47	**

Mean bearing different superscripts (a,b,c,d) within a row differ significantly (p<0.01), **:Highly Significant, ^{NS}: Non-significant

Table 2. Effect of inorganic supplementary litter material with sawdust on average weekly body weight gain (g) of broilers

Age (Weeks)	Treatment group				Significance level
	T_1	T_2	T_3	T_4	
1st	$119.40^a \pm 0.81$	$121.00^a \pm 0.41$	$125.35^b \pm 0.86$	$127.62^c \pm 0.74$	**
2nd	$231.50^a \pm 2.25$	$237.95^b \pm 0.67$	$237.65^b \pm 0.88$	$240.35^b \pm 1.15$	**
3rd	484.92 ± 1.96	482.07 ± 1.01	483.15 ± 2.07	484.07 ± 1.75	NS
4th	537.05 ± 3.43	539.40 ± 1.28	540.20 ± 1.31	539.32 ± 0.69	NS
5th	$504.40^a \pm 3.53$	$502.17^a \pm 1.42$	$503.95^a \pm 1.12$	$505.47^a \pm 0.39$	**
6th	$400.95^c \pm 1.38$	$400.45^c \pm 1.08$	$399.75^c \pm 0.62$	$394.37^b \pm 1.78$	**
Overall	$379.70^d \pm 68.69$	$380.50^c \pm 67.92$	$381.67^c \pm 67.61$	$381.87^c \pm 67.16$	*

Mean bearing different superscripts (a,b,c,d) within a row differ significantly ($p<0.01$), **: Highly Significant, NS: Non-significant

test following standard procedure (Allan and Gough 1974). The welfare status of birds was adjudged based on their footpad condition, 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.

Statistical analysis: The experimental data was subjected to statistical analysis in SPSS software Ver. 24.0 using one-way analysis of variance (ANOVA) as described by Snedecor and Cochran (1994) to test for significant variation between treatment groups and post-hoc Duncan's Multiple Range Test (DMRT; Duncan 1955). The results are interpreted and expressed as means ± SEM.

RESULTS AND DISCUSSION

The data on growth performance of broilers are presented in Tables 1, 2, 3, and 4. The ANOVA revealed highly significant ($p<0.01$) effect of inorganic supplementary litter material on weekly body weight, with significantly higher body weight recorded in T3 and T4 groups (Table 1). Similarly, ANOVA also revealed highly significant ($p<0.01$) effect of inorganic supplementary litter material on average body weight gain at all weeks except at 3rd and 4th weeks (Table 2). All treatment groups had higher body weight gain compared to control group. Significantly ($p<0.01$) higher body weight gain was observed in combination T4 group among treatment groups, while feed consumption significantly ($p<0.01$) lower was in the three treatment groups compared to control (Table 3), across six weeks. Significantly higher ($p<0.01$) weekly FCR was recorded in control groups compared to the three treatment groups (Table 4). These findings on bird growth performance concurs with the reports of Asaniyan *et al.* (2007), El-Deek *et al.* (2011), Koli *et al.* 2017 and Sigroha *et al.* (2017), who found significant effect of supplementation of various litter material on body weight in broiler. Similar results of positive effect of calcium carbonate and alum sulfate as supplementary litter material on growth performance of poultry birds were also reported by Rashid *et al*. (2017) and, Kim and Choi (2009) who found increase in the body weight, and weekly body weight gain of chicks raised on litter supplemented with calcium carbonate as compared to control group. Similarly, Moore *et al*. (2000), Mcward and Taylor (2000), and Sahoo *et al*. (2017) reported the broilers grown on alum treated litter material had significantly ($p<0.05$) better body weight and weekly body weight gain in comparison with birds raised on untreated litter. Like our findings, Guinotte *et al.* (1990), Ataee *et al*. (2011), and De Toledo *et al.* (2020) also reported higher feed intake in control group compared to birds with supplementation of litter with calcium carbonate. However, Rashid *et al.* (2017), and Kim and Choi (2009) observed no significant ($p<0.05$) difference in the feed consumption of birds reared on litter material treated with calcium carbonate.

Table 3. Effect of inorganic supplementary litter material with sawdust on average weekly feed consumption (g) of broilers

Age (Weeks)	Treatment group				Significance Level
	T_1	T_2	T_3	T_4	
1st	$208.72^f \pm 0.55$	$205.95^e \pm 0.67$	$203.07^d \pm 0.61$	$196.65^c \pm 0.64$	**
2nd	429.57 ± 0.11	375.02 ± 50.37	422.87 ± 0.46	419.97 ± 0.19	NS
3rd	$632.50^f \pm 0.20$	$627.52^e \pm 0.58$	$624.27^d \pm 0.66$	$619.62^c \pm 0.71$	**
4th	$827.00^f \pm 0.91$	$821.92^e \pm 0.52$	$814.95^d \pm 1.98$	$806.47^c \pm 0.74$	**
5th	$908.15^f \pm 0.61$	$905.25^e \pm 0.43$	$901.87^d \pm 0.26$	$887.02^c \pm 0.17$	**
6th	$1077.30^f \pm 0.31$	$1071.60^e \pm 0.50$	$1066.27^d \pm 0.38$	$1061.20^c \pm 0.35$	**
Overall	$4083.25^f \pm 131.47$	$4057.27^e \pm 134.63$	$4033.32^d \pm 130.76$	$3990.95^c \pm 130.09$	*

Mean bearing different superscripts (c,d,e,f) within a row differ significantly ($p<0.01$); **: Highly Significant, NS: Non-significant)

Table 4. Effect of inorganic supplementary litter material with sawdust on average average weekly feed conversion ratio of broilers

Age (Weeks)	Treatment group				Significance Level
	T_1	T_2	T_3	T_4	
1st	$1.74^{f} \pm 0.01$	$1.70^{e} \pm 0.05$	$1.62^{d} \pm 0.01$	$1.54^{c} \pm 0.01$	**
2nd	1.85 ± 0.02	1.57 ± 0.21	1.77 ± 0.01	1.74 ± 0.01	NS
3rd	$1.30^{d} \pm 0.01$	$1.30^{d} \pm 0.01$	$1.29^{cd} \pm 0.01$	$1.28^{bc} \pm 0.01$	**
4th	$1.54^{e} \pm 0.01$	$1.52^{d} \pm 0.01$	$1.50^{cd} \pm 0.01$	$1.49^{c} \pm 0.01$	**
5th	$1.80^{c} \pm 0.01$	$1.80^{c} \pm 0.01$	$1.78^{c} \pm 0.01$	$1.75^{b} \pm 0.01$	**
6th	$2.68^{a} \pm 0.01$	$2.67^{a} \pm 0.01$	$2.66^{a} \pm 0.01$	$2.69^{ab} \pm 0.01$	*
Overall	$1.82^{d} \pm 0.19$	$1.76^{c} \pm 0.19$	$1.77^{c} \pm 0.19$	$1.75^{c} \pm 0.20$	*

Mean bearing different superscripts (c,d,e,f) within a row differ significantly (p<0.01); **: Highly Significant, ^{NS}: non-significant

Thus, the addition of acidifying agents positively enhances the productive performance of the birds in terms of better weight gain and final body weight. This might result from lowered pH of litter resulting from an acidic litter formed due to addition of calcium carbonate and alum sulphate due to their acidifying effect. The acidic environment reduces ammonia volatilization and reduces microbial proliferation, enhancing the performance of birds (Younis *et al.* 2016). No mortality was recorded in the birds of all experimental groups during the trial.

Data on the physiochemical properties of litter has been presented in Fig. 1 and Table 5. Among the litter characteristics, significant (p<0.01) effect of inorganic supplementary litter material on moisture percentage, pH, ammonia emission, litter nitrogen content, water holding capacity, and caking score was observed at all weeks. The moisture percentage was significantly (p<0.01) lower in all treatment groups compared to control group. Among treatments, significantly (p<0.01) lower moisture percent was recorded in T4 group. Similarly, the litter pH was significantly (p<0.01) lower in all three treatment groups compared to control, with lowest moisture percentage recorded in the T4 group. Similar reports of lower litter moisture and pH level of litter was reported by other workers on addition of acidifiers like alum sulphate (Anderson *et al.* 2020) and calcium carbonate (Do *et al.* 2005). The lowered moisture level in calcium carbonate and alum supplemented litter might result from lowered ammonia volatilization in the treatment groups. In consonance with our findings, concurrently lower ammonia emission and litter moisture has been reported in earlier works of Do *et al*. (2005) with litter amendment having alum and calcium carbonate. However, contradictory results of both positive (Nahm 2002) and negative (Ferguson *et al.* 1998) correlation between ammonia emission and moisture percentage of litter has been reported.

The ammonia emission from litter was significantly (p<0.01) lower in the three treatment groups compared to control group. While the litter nitrogen content was significantly (p<0.01) higher in the three treatment

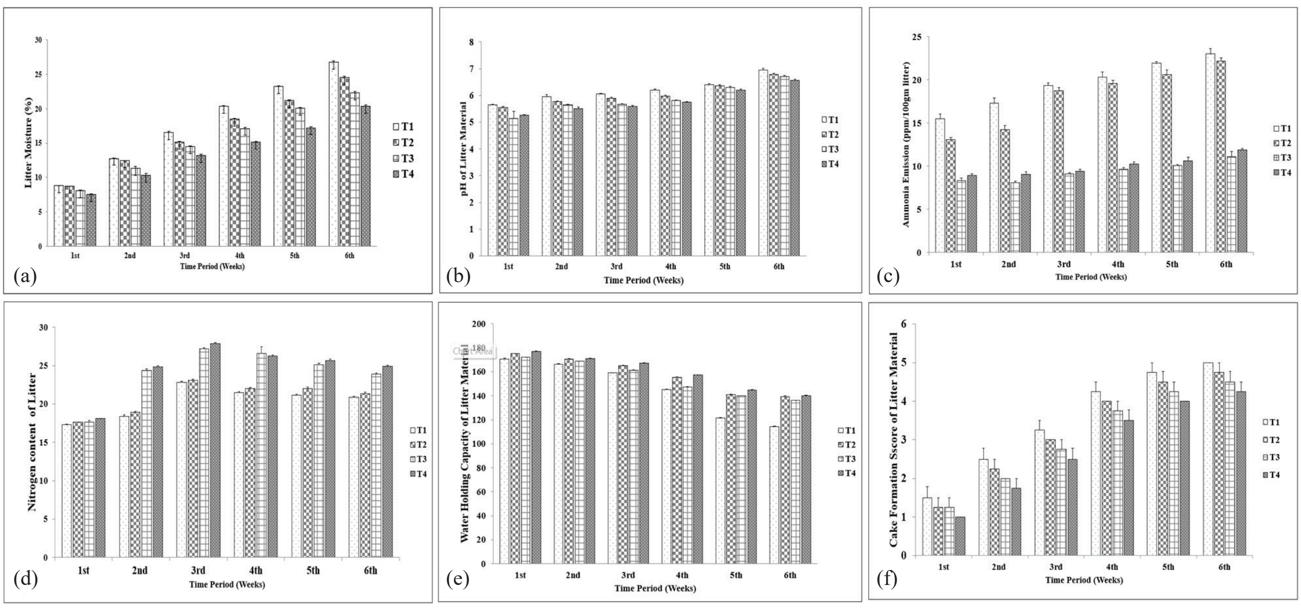

Fig. 1. Physiochemical properties of litter in different treatment groups, (a) litter moisture (%), (b) pH of litter, (c) ammonia emission from litter, (d) nitrogen content of litter, (e) water holding capacity of litter, (f) cake formation score of litter

Table 5. Effect of inorganic supplementary litter material with sawdust on litter quality parameters, parasitic and microbial load of litter

		Weeks					
Parameter		1	2	3	4	5	6
Litter Moisture (%)	T1	$8.85^{a} \pm 0.02$	$12.77^{a} \pm 0.04$	$16.51^{a} \pm 0.23$	$20.35^{a} \pm 0.09$	$23.24^{a} \pm 0.12$	$26.77^{a} \pm 0.18$
	T2	$8.71^{a} \pm 0.05$	$12.42^{a} \pm 0.08$	$15.18^{b} \pm 0.07$	$18.50^{b} \pm 0.13$	$21.19^{b} \pm 0.10$	$24.51^{b} \pm 0.22$
	T3	$8.11^{b} \pm 0.07$	$11.33^{b} \pm 0.28$	$14.46^{c} \pm 0.13$	$17.14^{c} \pm 0.12$	$20.10^{c} \pm 0.09$	$22.33^{c} \pm 0.18$
	T4	$7.51^{c} \pm 0.07$	$10.32^{c} \pm 0.25$	$13.21^{d} \pm 0.14$	$15.16^{d} \pm 0.10$	$17.23^{d} \pm 0.14$	$20.35^{d} \pm 0.19$
pH	T1	$5.65^{d} \pm 0.02$	$5.95^{d} \pm 0.08$	$6.05^{d} \pm 0.02$	$6.20^{e} \pm 0.04$	$6.40^{d} \pm 0.04$	$6.95^{e} \pm 0.06$
	T2	$5.55^{cd} \pm 0.05$	$5.77^{c} \pm 0.02$	$5.90^{c} \pm 0.04$	$5.97^{d} \pm 0.04$	$6.37^{d} \pm 0.04$	$6.80^{d} \pm 0.04$
	T3	$5.15^{ab} \pm 0.25$	$5.66^{c} \pm 0.02$	$5.67^{b} \pm 0.02$	$5.82^{c} \pm 0.02$	$6.30^{cd} \pm 0.04$	$6.70^{cd} \pm 0.04$
	T4	$5.27^{bc} \pm 0.02$	$5.50^{b} \pm 0.07$	$5.60^{b} \pm 0.04$	$5.75^{bc} \pm 0.02$	$6.20^{c} \pm 0.04$	$6.57^{c} \pm 0.04$
Litter Ammonia (ppm/100gm litter)	T1	$15.47^{e} \pm 0.57$	$17.28^{e} \pm 0.65$	$19.32^{e} \pm 0.33$	$20.29^{e} \pm 0.65$	$21.96^{d} \pm 0.17$	$23.05^{e} \pm 0.55$
	T2	$13.10^{d} \pm 0.25$	$14.25^{d} \pm 0.48$	$18.72^{d} \pm 0.41$	$19.57^{d} \pm 0.36$	$20.61^{c} \pm 0.55$	$22.19^{d} \pm 0.36$
	T3	$8.32^{b} \pm 0.29$	$8.08^{b} \pm 0.21$	$9.12^{c} \pm 0.14$	$9.66^{bc} \pm 0.17$	$10.06^{b} \pm 0.16$	$11.09^{b} \pm 0.62$
	T4	$8.96^{c} \pm 0.15$	$9.06^{c} \pm 0.28$	$9.41^{c} \pm 0.26$	$10.24^{c} \pm 0.29$	$10.63^{b} \pm 0.41$	$11.91^{c} \pm 0.15$
Litter Nitrogen	T1	$17.31^{c} \pm 0.08$	$18.41^{c} \pm 0.20$	$22.85^{c} \pm 0.13$	$21.46^{b} \pm 0.19$	$21.15^{c} \pm 0.14$	$20.91^{c} \pm 0.11$
	T2	$17.63^{c} \pm 0.03$	$18.93^{c} \pm 0.13$	$23.11^{c} \pm 0.12$	$22.04^{b} \pm 0.09$	$22.04^{d} \pm 0.17$	$21.38^{c} \pm 0.18$
	T3	$17.68^{cd} \pm 0.19$	$24.41^{d} \pm 0.19$	$27.19^{d} \pm 0.15$	$26.59^{c} \pm 0.88$	$25.10^{e} \pm 0.21$	$23.9^{d} \pm 0.16$
	T4	$18.11^{d} \pm 0.04$	$24.85^{d} \pm 0.14$	$27.90^{e} \pm 0.09$	$26.27^{c} \pm 0.14$	$25.68^{f} \pm 0.19$	$24.90^{e} \pm 0.15$
Litter Cake Formation Score	T1	1.50 ± 0.28	$2.50^{c} \pm 0.28$	$3.25^{d} \pm 0.25$	$4.25^{d} \pm 0.25$	$4.75^{c} \pm 0.25$	$5.00^{d} \pm 0.00$
	T2	1.25 ± 0.25	$2.25^{bc} \pm 0.25$	$3.00^{cd} \pm 0.00$	$4.00^{cd} \pm 0.00$	$4.50^{bc} \pm 0.28$	$4.75^{cd} \pm 0.25$
	T3	1.25 ± 0.25	$2.00^{abc} \pm 0.00$	$2.75^{bcd} \pm 0.25$	$3.75^{bcd} \pm 0.25$	$4.25^{abc} \pm 0.25$	$4.50^{bcd} \pm 0.28$
	T4	1.00 ± 0.00	$1.75^{abc} \pm 0.25$	$2.50^{abc} \pm 0.28$	$3.50^{abc} \pm 0.28$	$4.00^{abc} \pm 0.00$	$4.25^{abc} \pm 0.25$
Litter Water Holding Capacity	T1	$170.45^{a} \pm 0.80$	$166.35^{a} \pm 0.21$	$158.97^{a} \pm 0.23$	$145.37^{a} \pm 0.21$	$121.29^{a} \pm 0.43$	114.41 ± 0.19
	T2	$175.05^{c} \pm 0.17$	$170.72^{c} \pm 0.29$	$165.25^{c} \pm 0.30$	$155.41^{b} \pm 0.21$	$141.15^{c} \pm 0.33$	139.17 ± 310.94
	T3	$172.3^{b} \pm 0.16$	$168.8^{b} \pm 0.11$	$161.3^{b} \pm 0.18$	$147.31^{a} \pm 0.22$	$139.91^{b} \pm 0.16$	136.22 ± 0.19
	T4	$176.72^{d} \pm 0.48$	$171.17^{c} \pm 0.29$	$167.17^{d} \pm 0.22$	$157.4^{b} \pm 0.22$	$144.76^{d} \pm 0.24$	140.28 ± 0.18
Parasitic load (oocyts/g of litter)	T1	0.00 ± 0.00	$15.42^{e} \pm 0.18$	$18.47^{e} \pm 0.08$	$20.30^{f} \pm 0.21$	$23.05^{f} \pm 0.10$	$25.47^{f} \pm 0.06$
	T2	0.00 ± 0.00	$13.49^{d} \pm 0.19$	$16.65^{d} \pm 0.06$	$18.20^{e} \pm 0.12$	$21.20^{e} \pm 0.10$	$23.60^{e} \pm 0.12$
	T3	0.00 ± 0.00	$12.45^{c} \pm 0.06$	$14.37^{c} \pm 0.17$	$16.22^{d} \pm 0.12$	$18.45^{d} \pm 0.06$	$20.57^{d} \pm 0.08$
	T4	0.00 ± 0.00	$9.22^{b} \pm 0.12$	$12.30^{b} \pm 0.17$	$14.15^{c} \pm 0.13$	$16.15^{c} \pm 0.10$	$18.70^{c} \pm 0.08$
Total Viable Count (CFU/g of litter)	T1	$5.18^{d} \pm 0.05$	$6.06^{f} \pm 0.04$	$6.56^{f} \pm 0.03$	$7.03^{f} \pm 0.04$	$7.66^{e} \pm 0.08$	$8.19^{b} \pm 0.06$
	T2	$5.00^{c} \pm 0.04$	$5.87^{e} \pm 0.03$	$6.42^{e} \pm 0.03$	$6.90^{e} \pm 0.02$	$7.43^{d} \pm 0.04$	$7.78^{b} \pm 0.04$
	T3	$4.88^{b} \pm 0.02$	$5.74^{d} \pm 0.03$	$6.29^{d} \pm 0.02$	$6.73^{d} \pm 0.06$	$7.31^{c} \pm 0.03$	$7.51^{b} \pm 0.05$
	T4	$4.79^{b} \pm 0.04$	$5.62^{c} \pm 0.03$	$6.17^{c} \pm 0.03$	$6.54^{c} \pm 0.04$	$6.75^{b} \pm 0.04$	$6.89^{ab} \pm 1.22$

Note: Means bearing different superscript (a,b,c,d,e,f) within a column for particular parameter differ significantly ($p<0.05$)

groups compared to control group. This highlights that addition of alum and Calcium carbonate to litter reduced the ammonia volatilization from litter by lowering the pH of litter (Madrid *et al.* 2012, Anderson *et al.* 2020). The principal source of ammonia emission from poultry litter results from microbial breakdown of uric acid (Çelen *et al.* 2008). The temperature, moisture content, and pH of the litter that are conducive to bacterial breakdown of uric acid determine the rate of ammonia production. More ammonia is produced at alkaline pH and acidifying agents that reduce litter pH results in lowered ammonia emission (Chung 2019). Other reports have also found lesser cumulative atmospheric ammonia concentration when litter was treated with a combination of alum and Calcium carbonate (Do *et al.* 2005, Lee *et al.* 2013). Calcium carbonate shows particularly good antibacterial effect (Ataee *et al.* 2011, De Toledo *et al.* 2020), hence controls uratolytic bacterium (*Bacillus pasteurii*) that lead to decrease ammonia production. Decrease moisture (Do *et al*. 2005) also reduce growth of uratolytic bacteria that lead to decrease activity of ureolytic bacterium and reduction in ammonia production in litter (Terzich 1997 and Do *et al*. 2005). The data on higher litter nitrogen in treatment groups is consistent with the reports of Rashid *et al*. (2020), Sahoo *et al*. (2017) and Madrid *et al*. (2012). According to Moore *et al.* (1995), the acidity of the litter reduces gaseous losses of nitrogen by converting ammonia to ammonium, which in turn lowers ammonia volatilization, which raises the overall nitrogen content of the litter material. As a consequence, more nitrogen was retained in litter of treatment groups because the acidic composition of the litter prevented the free ammonium ion from converting to ammonia.

The data on water holding capacity and caking score of litter revealed a significant (p<0.01) effect of supplementary litter materials on both attributes. The water holding capacity of litter material supplemented with calcium carbonate, alum, and a combination of both, significantly (p<0.01) increased as compared to control group till 5th week but in the last week no significant difference was observed. The higher water holding capacity in the three treatment groups can be explained on account of the hygroscopic nature of both calcium carbonate and alum. However, the physical structure, particle size, and rate of compaction over time are the main factors influencing the litters' ability to bind and release water (Garces *et al.* 2013). In the present study, significant (p<0.01) reduction in the cake formation score of litter material supplemented with inorganic calcium carbonate and alum inclusion groups as compared to control group. Calcium carbonate has a high absorption capacity, which allows it to absorb excess moisture in the litter material. Inclusion of alum in poultry litter also reduces moisture level. This helps to keep the litter drier and reduces the formation of wet spots or cakes. Similar findings of lower cake formation in chemically treated litter has been reported, which aligns with our results (Nagaraj *et al*. 2007, Kim and Choi 2009, Madrid *et al.* 2012, Rashid *et al.* 2020).

The data on microbial and parasitic load of litter in different experimental groups is presented in table 5 along with figure 2a and 2b, respectively. The microbial load was significantly (p<0.01) lower in treatment groups as compared to control group. In earlier reports, both Calcium carbonate and Alum as litter supplements have shown a good antibacterial characteristic (Cook *et al.* 2008, Ataee *et al*. 2011, Sahoo *et al.* 2015, Rashid *et al.* 2020). When the pH levels turn towards acidic, ammonifying and putrefying bacteria are inhibited (Terzich *et al.* 2000). Similarly, the

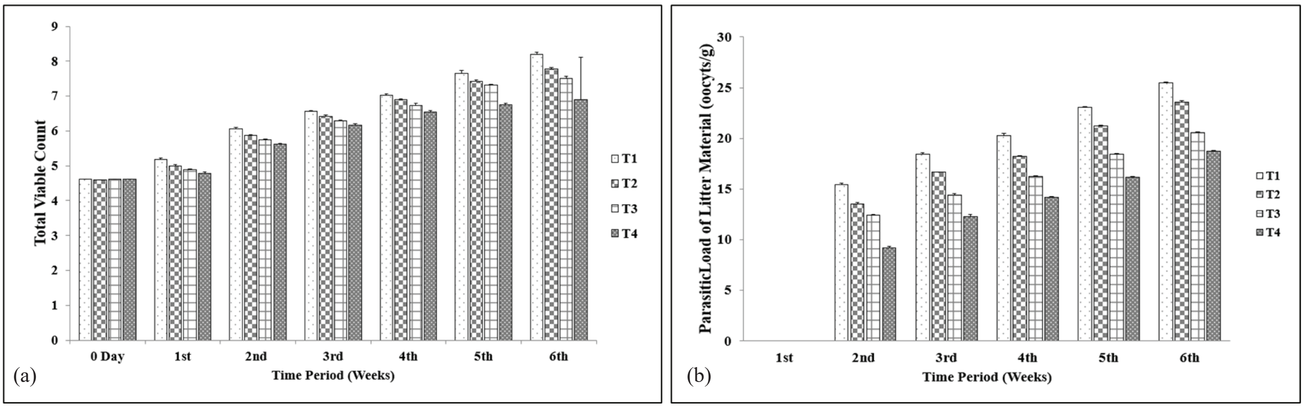

Fig. 2. Microbial and parasitic load of litter in different treatment groups, (a) total viable count (CFU/g), (b) parasitic load (oocytes/g)

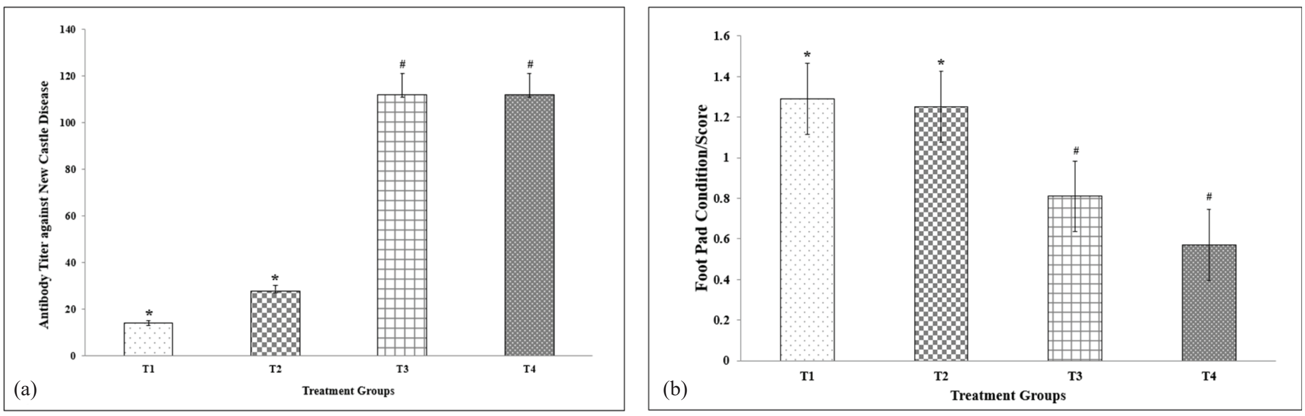

Fig. 3. (a) Antibody response of birds against new castle disease (b) welfare score in terms of foot pad condition

parasitic load was also significantly (p<0.01) lower in the three treatment groups compared to control. Sahoo *et al.* (2017) also reported a significantly lower parasitic load in litter treated with acidifying agents.

The data pertaining to immune status of broiler chicken reared in different groups in terms of immune response elicited against new castle disease virus is presented in Fig. 3a. The Mean antibody titre against new castle disease virus was significantly (p<0.05) higher in T3 and T4 treatment groups as compared to control group. The ANOVA revealed highly significant (p<0.01) effect of alum as supplementary litter material on immune response against new castle disease virus. However, no significant effect of calcium carbonate inclusion alone was seen on the antibody response to new castle disease. Earlier reports have also suggested a role of choice of litter material on the antibody response of broiler chickens (Toghyani *et al.* 2010). In agreement with our findings, Sahoo *et al.* (2015) and Younis *et al.* (2016) also reported high antibody titre in the birds reared on litter supplemented with acidifiers compared to non-supplemented litter group. The better antibody response in alum treated group might result from lowered ammonia levels and lowered pH of litter that inhibits the viable microbes and results in better health status of birds due to reduced stress (Kim and Choi 2009, Eid *et al.* 2021). Data on the footpad condition of broiler also revealed significant (p<0.01) positive effect of the three treatments compared to control, with lower score for T2, T3 and T4 groups (Fig. 3b). Among treatment, the T4 group had significantly lower foot pad score compared to both T2 and T3 groups. Concurring with our results, Rashid *et al.* (2020) also reported better footpad condition of birds reared on litter supplemented with either alum or calcium carbonate. Similar reports of positive foot pad condition with addition of acidifying litter amendments have been reported with addition of Calcium carbonate and Alum to litter (Rashid *et al.* 2020, Toppel *et al.* 2019). This reflects a more conducive growth environment in treatment groups on account of superior litter characteristics and lower microbial and parasitic load that improves the health of footpad of birds.

The findings of this study suggest that addition of calcium carbonate and aluminium sulfate alone or in combination as supplements to conventional litter material like saw dust has the ability to improve broiler performance and welfare by positively influencing the litter physiochemistry and reducing pathogenic load. These acidifying supplements can be used as a cost-effective method for welfare based economic poultry farming.

REFERENCES

Allan W H and Gough R E. 1974. A standard haemagglutination inhibition test for Newcastle disease: A comparison of macro and micro methods. *American Journal of Veterinary Research* **95**(6):120-23.

American Public Health Association (APHA). 1992. *Compendium of methods for the microbiological examination of foods*. Washington, D.C.

Anderson K, Moore Jr P A, Martin J and Ashworth A J. 2020. Effect of a new manure amendment on ammonia emissions from poultry litter. *Atmosphere* **11**(3): 257.

Andrews L D. 1972. Cage rearing of broilers. *Poultry Science* **51**: 1194-97.

AOAC. 1990. *Association of Analytical Chemist, Official Methods of Analysis*, 15th ed., Collegiate Press, Washington, D.C.

Asaniyan E K, Agbede J O and Laseinde E A O. 2007. Impact assessment of different litter depths on the performance of broiler chickens raised on sand and wood shaving litters. *World Journal of Zoology* **2**(2): 67-72.

Ataee R A, Derakhshanpour J, Tavana A and Eydi A. 2011. Antibacterial effect of calcium carbonate nanoparticles on *Agrobacterium tumefaciens*. *Journal of Military Medicine* **13**(2): 65-70.

Benabdeljelil K and Ayachi A. 1996. Evaluation of alternative litter materials for poultry. *Journal of Applied Poultry Research* **5**(3): 203-09.

Brake J D, Boyle C R, Chamblee T N, Schultz C D and Peebles E D. 1992. Evaluation of the chemical and physical properties of hardwood bark used as a broiler litter material. *Poultry Science* **71**: 467-72.

Carter T A, Allison R C, Mills W C and West J R. 1979. Wood chips for poultry litter. *Poultry Science* **58**: 994-97.

Casey W R, Brian D F and Lacy M P. 2005. Litter quality and broiler performance. *Cooperative Extension Service Bulletin* 1267. Citrus pulp pellet for broiler. *World Poultry* **17**: 30-31.

Çelen M F, Kozat S, Ekin S, Yörük B H and Alki E. 2008. Effects of adding aluminum sulfate to different litters on selected trace elements and vitamin concentrations in broilers. *African Journal of Biotechnology* **7**: 3363-66.

Chung T H. 2019. Evaluation of liquid and dry aluminum chloride treatment of litter-on-litter quality and performance of duck. *Indian Journal of Animal Research* **53**(12): 1684–87.

Cook K L, Rothrock M J, Warren J G, Sistani K R and Moore P A. 2008. Effect of alum treatment on the concentration of total and ureolytic microorganisms in poultry litter. *Journal of Environmental Quality* **37**: 2360-67.

De Toledo T D S, Roll A A P, Rutz F, Dallmann H M, Dai Pra M A, Leite F P L and Roll V F B. 2020. An assessment of the impacts of litter treatments on the litter quality and broiler performance: A systematic review and meta-analysis. *PLoS (Public Library of Science) One* **15**(5): 1-26.

Do J C, Choi I H and Nahm K H. 2005. Effects of chemically amended litter on broiler performances, atmospheric ammonia concentration, and phosphorus solubility in litter. *Poultry Science* **84**(5): 679-86.

Duncan D B. 1955. Multiple range and multiple F tests. *Biometrics* **11**: 1-42.

Eid Y Z, Amber K A, Hassan M S, Hassan R A and Abo-ouf A M. 2021. Efficacy of aluminum sulphate addition to poultry litter on productive performance of laying hens, ammonia emissions, and litter quality. *Brazilian Journal of Poultry Science* **24**: 1–8.

El-Deek A A, Al-Harthi M A, Khalifah M M, Elbanoby M M and Alharby T. 2011. Impact of newspaper as bedding material in arid land on broiler performance. *Egyptian Poultry Science* **31**(4): 715-25.

Ferguson N S, Gates R S, Taraba J L, Cantor A H, Pescatore A J, Straw M L, Ford M J and Burnham D J. 1998. The effect of dietary protein and phosphorus on ammonia concentration and litter composition in broilers. *Poultry Science* **77**: 1085-93.

Garcês A P J T, Afonso S M S, Chilundo A and Jairoce C T S. 2013. Evaluation of different litter materials for broiler production in a hot and humid environment: 1. Litter characteristics and quality. *Journal of Applied Poultry Research* **22**(2): 168-76.

Guinotte F, Nys Y and De Monredon F. 1990. The effects of particle size and origin of calcium carbonate on performance and ossification characteristics in broiler chicks. *Poultry Science* **70**(9): 1908–20.

Hernandez R and Cazetta J O. 2001. Simple and affordable method to determine ammonia released by the avian bed. *Revista Brasileira de Zootecnia* **30**(3): 824-29.

Kim Y J and Choi I H. 2009. Effect of alum and liquid alum on pH, electrical conductivity, moisture, ammonium, and soluble phosphorus contents in poultry litter during short term: A laboratory experiment. *Poultry Science* **46**: 63–67.

Koli S S, Dhumal M V, Mishra G, Ingle V D and Koli R S. 2017. Effect of use of different litter material combinations on growth performance and economics of broiler production. *Indian Journal of Animal Health* **56**(2): 239-48.

Lee G D, Kim S C and Choi I H. 2013. Using anhydrous aluminium chloride with calcium carbonate to reduce ammonia volatilization and increase nitrogen content from poultry litter. *The Journal of Poultry Science* **50**(2): 172-76.

Long P L, Tompkins R V and Millard B J. 1975. Coccidiosis in broilers: Evaluation of infection by the examination of broiler house litter for oocysts. *Avian Pathology* **4**(4): 287-94.

Lopes M, Leite F L, Valente B S, Heres T, Dai Prá M A, Xavier E G and Roll V F B. 2015. An assessment of the effectiveness of four in-house treatments to reduce the bacterial levels in poultry litter. *Poultry Science* **94**(9): 2094-98.

Madrid J, López M J, Orengo J, Martínez S, Valverde M, Megías M D and Hernández F. 2012. Effect of aluminum sulfate on litter composition and ammonia emission in a single flock of broilers up to 42 days of age. *Animal* **6**(8): 1322-29.

McWard G W and Taylor D R. 2000. Acidified clay litter amendment. *Journal of Applied Poultry Research* **9**: 518-29.

Mohammadi-Aragh M K, Norris K L, Chesser Jr G D, Lowe J W, Evans J D, Purswell J L and Linhoss J E. 2025. Comparison of biochar and poultry litter treatment (PLT) amendments on broiler litter quality and bird performance. *Journal of Applied Poultry Research* **34**(1): 100499.

Moore P A, Daniel T C, Edwards D R and Miller D M. 1995. Effect of chemical amendments on ammonia volatilization from poultry litter. *Journal of Environmental Quality* **24**: 293-300.

Moore P A, Daniel T C and Edwards D R. 2000. Reducing phosphorus runoff and inhibiting ammonia loss from poultry manure with aluminum sulfate. *Journal of Environmental Quality* **29**: 37-49.

Mottet A and Tempio G. 2017. Global poultry production: Current state and future outlook and challenges. *World's Poultry Science Journal* **73**(2): 245-56.

Nagaraj M, Hess J B and Bilgili S F. 2007. Evaluation of a feed-grade enzyme in broiler diets to reduce pododermatitis. *Journal of Applied Poultry Research* **16**: 52–61.

Nahm K H. 2002. Efficient feed nutrient utilization to reduce pollutants in poultry and swine manure. *Critical Reviews in Environmental Science and Technology* **32**: 1–16.

Oliveira J L, Xin H, Chai L and Millman S T. 2019. Effects of litter floor access and inclusion of experienced hens in aviary housing on floor eggs, litter condition, air quality, and hen welfare. *Poultry Science* **98**(4): 1664-77.

Pepper C M and Dunlop M W. 2021. Review of litter turning during a grow-out as a litter management practice to achieve dry and friable litter in poultry production. *Poultry Science* **100**(6): 101071.

Poudel S, Chakraborty D and Prasad R. 2024. Evaluation of the efficacy of amendment types and rates in reducing ammonia emissions from broiler litter. *Poultry Science* **103**(12): 104467.

Rashid A, Banday M T, Adil S, Khan A A and Ali H. 2020. Effect of chemical treatment of litter on its quality and footpad lesions of broiler chicken. *Indian Journal of Hill Farming* **33**(2): 331-36.

Rashid A, Banday M T, Adil S, Khan A A, Qureshi S, Untoo M and Pal M A. 2017. Effect of chemically treated litter on ammonia emission, performance and carcass characteristics of broiler chicken. *Journal of World's Poultry Research* **7**(2): 88-93.

Sahoo S P, Kaur D, Sethi A P S, Chandrahas and Saini A L. 2015. Efficacy of acidified litter treatment on broiler production during rainy season. *Indian Journal of Poultry Science* **50**(3): 323-26.

Sahoo S P, Kaur D, Sethi A P S, Sharma A, Chandra M and Chandrahas. 2017. Effect of chemically inclusion in litter-on-litter quality and broiler performance in winter. *Journal of Applied Animal Research* **45**(1): 53337.

Sigroha R, Bidhan D S, Yadav D C, Sihag S S and Malik A K. 2017. Effect of different litter materials on the performance of broiler chicken. *Journal of Animal Research* *7*(4): 665-71.

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8th Edition, IOWA State University Press, Ames, Iowa, USA.

Soliman E S, Sallam N H and Abouelhassan E M. 2018. Effectiveness of poultry litter amendments on bacterial survival and *Eimeria* oocyst sporulation. *Veterinary World* **11**(8): 1064.

Terzich M A C. 1997. Ammonia and pH level of the bed. *Conference of Science and Technology*, Sao Paulo, SP, Brazil: 141–46.

Terzich M A C, Pope M J, Cherry T E and Hollinger J. 2000. Survey of pathogens in poultry litter in the United States. *Applied Poultry Research* **9**: 287–91.

Toghyani M, Gheisari A, Modaresi M, Tabeidian S A and Toghyani M. 2010. Effect of different litter material on performance and behavior of broiler chickens. *Applied Animal Behaviour Science* **122**(1): 48–52.

Toppel K, Kaufmann F, Schön H, Gauly M and Andersson R. 2019. Effect of pH-lowering litter amendment on animal-based welfare indicators and litter quality in a European commercial broiler husbandry. *Poultry Science* **98**(3): 1181–89.

Watts D B, Smith K E and Torbert H A. 2012. Impact of poultry litter cake, cleanout, and bedding following chemical amendments on soil C and N mineralization. *International Journal of Agronomy* **2012**(1): 204629.

Younis M E, Bazh E K, Ahmed H A and Elbestawy A R. 2016. Broiler performance, carcass characters and litter composition after management of fresh litter with two types of acidifier amendments. *International Journal of Agriculture Innovation and Research* **4**: 1050-56.