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Influence of Neem Leaves Powder in Litter Contamination, and Welfare Indicators of Broiler (Ross 308) Exposed to Heat Stress

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ABSTRACT: The increase in the emergence of the resistance of pathogenic bacteria against antibiotics in poultry farms increased the need to find therapeutic alternatives to raise the level of biosecurity, especially the high rate of disease infection during heat stress, which reduces broiler welfare. 320 chicks one-day old were used and were divided into four groups at random, each containing 80 chicks, Each group was made up of four replicates, and each group received a dose of 0, 200, 400, and 600 mg of neem leaf powder/ kg of diet for G1, G2, G3, and G4, respectively. All chicks were, exposed to (HS) (28-35-28 c◦). A significant heights ($P \leq 0.05$) for G2, G3, and G4 groups in benefits bacteria (Lactobacilli) of litter compared G1 group that increased significantly in E. coli bacteria and total bacteria. A significant heights ($P \leq 0.05$) in moisture and pH of litter and, feces weight for the G1 group compared to another group, in broiler welfare significant improvement ($P \leq 0.05$) in footpad dermatitis and feather hygiene percentage for G2, G3, and G4 groups compared G1 group. These results conclude that the neem leaf powder added to the broiler feed increased the number of beneficial bacteria and reduced the number of harmful bacteria, as well as improved the physical characteristics of the litter, which achieved positive results in the welfare of broilers raised under conditions of heat stress.

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KEYWORDS: Biosecurity, broiler welfare, HS, therapeutic alternatives.

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1. INTRODUCTION

It is important to promote the idea of biosecurity in the farms and projects of poultry farming for its significant contribution to preventive medicine and its economic implications for reducing infections in chicken farms (Al-Salhi et al., 2022; Hwang and Singer, 2020). Due to the challenge of regulating microbes, especially pathological species, this aspect has become an area of interest for many researchers, and the use of antibiotics to reduce pathological bacteria to develop preventive programs in poultry farming, but the rise of some bacterial species resistant to these treatments, like salmonella and coliform, which are common in poultry farms, has a negative impact (Alnajjar and Alemadi, 2017) for they have the characteristic of resistance against some types of antibiotics, which led to preventing the use of these antibiotics because of preventing the formation of bacterial resistance on the one hand and preventing its transmission to the consumer on the other hand (Agboola et al., 2015), also, paying attention to the health of the digestive system of chicks and maintaining the microbial balance in it improves the health status of the chicken. HS is caused by increased water intake and decreased feed intake, the nutrients are less likely to be absorbed, additionally, HS increases intestinal secretion (Al-Jebory et al., 2023). HS also negatively impacts the intestinal mucosa, causing oxidative stress and inflammation, it also reduces populations of helpful bacteria (*Lactobacillus* and *Bifidobacterium*), which are then replaced by harmful bacteria like (*Coliforms* and *Clostridium*) (Abdelqader and Al-Fataftah, 2016; Xing et al., 2019), from this, it is concluded that the increased drinking of water with the decrease in the consumption of feed due to HS increases the excretion of feces with the increase in the moisture content in it, which raises the moisture of the litter and the multiplication of harmful bacteria. The neem plant (*Azadirachta indica*), whose leaves have high levels of protein, carbs, and dietary fiber, is frequently utilized in traditional medicine (Saleem et al., 2018; Al-Jebory et al., 2023). In addition, the leaves have a fair amount of fat and minerals (Bonsu et al., 2012). According to Nnenna and Okey (2013), chicken feed might be supplemented with dried neem leaf extract without impairing growth. Neem leaves are plentiful in ferulic acid and other phenolic substances like gallic acid (Shewale and Rathod, 2018), which have significant antioxidant and antibacterial activity (Heyman et al., 2017). Neem leaves contain anti-microbial compounds (Valarmathy et al., 2010) and one of the most vital components of neem is sodium nimbidate, which is anti-inflammatory, anti-fungal, and anti-virus (Ezzat et al., 2018). Therefore, the current study aims to investigate the effect of neem leaves powder on the number of bacteria produced from broiler chickens, the feces weight, and their effect on the characteristics of the litter and broiler welfare under HS circumstances.

2. MATERIAL AND METHODS

320 chicks of one-day-old broilers (Ross 308) that had not been sexed were used, with HS conditions (28-35-28 c◦) for the period (1-5 weeks). The experiment took place in the Al- Anwar Poultry Company from the period November 29, 2022, through January 4, 2023, the chicks have divided randomly into four groups each group 80 chicks for four subgroups, and the groups were 0, 200, 400, and 600 mg Neem (*Azadirachta Indica*) leaf powder/ Kg diet feed for G1, G2, G3, and G4 respectively. Rice husk litter was, used during the study period.

2.1 Feed treatment

According to NRC (1994), the chicks were feed starter and finisher diets were used throughout the experiment (ad libitum), starter diet contained a crud protein of 23% and metabolic energy of 3027 kcal/kg, and the finisher diet contained a crud protein of 20% and metabolic energy of 3195.3 kcal/kg.

2.2 Studied traits

Litter samples were collected three times a week, then the average was taken and the bacterial count was conducted for total bacteria, *Lactobacilli*, and *E.coli* by pouring dishes according to the method (Da Silva et al., 2019). Litter samples were, taken from five locals from each replicate, after which the method was, followed to calculate litter moisture (Sluiter et al., 2008). The pH of the litter was, measured three times a week, and then the average was, taken according to (Hoskins et al., 2003). Feces weight was measured using paper litter and after feces collection, it was, treated according to the method (AOAC, 2019). Footpad dermatitis percentage calculate according to (Kaukonen et al., 2016), while feather hygiene percentage calculate according to (Karcher et al., 2013).

The statistical Analysis was conducted according to the following mathematical model (SAS, 2012: Duncan, 1955).

$Yij=\mu+Ti+eij$

3. RESULTS

3.1 Total litter bacterial contamination

Study the amount of litter contamination (Tables: 1, 2, 3, 4, and, 5), noted in the first week (table 2) significant increase (P≤0.05) for the G2 group in benefits bacteria (*Lactobacilli*) compared to other group, in the second week (table 3) significant height (P≤0.05) for G4 group in *Lactobacilli* and total bacteria count, as well significant increase (P≤0.05) for G1 group in *E. coli* bacteria.

Table 1: Effect of neem leaves powder in total litter bacterial contamination (Mean± stander error) CFU/gm of broiler (Ross 308) exposed to HS

Groups	First week		
	Lactobacilli	*E. coli*	Total bacteria
G1	2.45±0.01 b	1.14±0.05	4.52±0.04
G2	3.11±0.02 a	1.18±0.06	4.61±0.09
G3	2.51±0.01 b	1.11±0.03	4.55±0.02
G4	2.62±0.03 b	1.13±0.08	4.57±0.07
Significant	*	N.S	N.S
Means with deferent litter defer at level (P≤0.05): N.S not significant			

Table 2: Effect of neem leaves powder in total litter bacterial contamination (Mean± stander error) CFU/gm of broiler (Ross 308) exposed to HS

Groups	Second week		
	Lactobacilli	*E. coli*	Total bacteria
G1	4.78±0.01 c	2.63±0.01 a	7.88±0.01 b
G2	5.96±0.03 ab	1.39±0.02 c	7.61±0.03 b
G3	6.11±0.01 a	1.89±0.01 b	8.12±0.01 a

G4	5.41±0.02 b	1.92±0.01 b	7.33±0.02 c
Significant	*	*	*
This means different litter defers at level (P≤0.05).			

The third week (table 3) noted a significant superior (P≤0.05) for the G4 group in *Lactobacilli* as for significant improvement (P≤0.05) for G2, G3, and G4 groups in *E. coli* and, total bacteria compared to another group. In the fourth week (table 5) noted no significant difference between groups of *Lactobacilli* and *E. coli* bacteria, while significant increase (P≤0.05) for the G1 group in total bacteria compared to other groups. The fifth week noted significant improvement (P≤0.05) for neem plant group addition in *Lactobacilli*, *E. coli*, and total bacteria compared G1 group.

Table 3: Effect of neem leaves powder in total litter bacterial contamination (Mean± stander error) CFU/gm of broiler (Ross 308) exposed to HS

Groups	Third week		
	Lactobacilli	*E. coli*	Total bacteria
G1	5.71±0.01 b	3.11±0.02 a	9.10±0.01 a
G2	5.69±0.03 b	2.17±0.01 b	8.13±0.01 b
G3	5.18±0.02 b	1.58±0.02 c	7.86±0.02 c
G4	6.61±0.01 a	2.35±0.03 b	8.36±0.01 b
Significant	*	*	*
This means different litter defers at level (P≤0.05).			

Table 4: Effect of neem leaves powder in total litter bacterial contamination (Mean± stander error) CFU/gm of broiler (Ross 308) exposed to HS

Groups	Fourth week		
	Lactobacilli	*E. coli*	Total bacteria
G1	6.21±0.09	2.27±0.02	9.97±0.01 a
G2	6.33±0.07	2.26±0.06	8.69±0.04 b
G3	6.25±0.02	2.20±0.05	8.57±0.02 b
G4	6.27±0.05	2.25±0.05	8.63±0.03 b
Significant	N.S	N.S	*
Means with deferent litter defer at level (P≤0.05): N.S not significant			

Table 5: Effect of neem leaves powder in total litter bacterial contamination (Mean± stander error) CFU/gm of broiler (Ross 308) exposed to HS

Groups	Fifth week		
	Lactobacilli	*E. coli*	Total bacteria
G1	5.28±0.03 c	3.63±0.04 a	10.11±0.03 a
G2	7.34±0.02 a	1.35±0.01 b	9.25±0.03 b
G3	6.72±0.01 b	1.42±0.02 b	8.65±0.02 c
G4	6.97±0.02 b	1.65±0.01 b	8.79±0.01 c
Significant	*	*	*
This means different litter defers at level (P≤0.05).			

3.2 Litter percent moisture

Table 6: shows the effect of the study on litter percent moisture, with no significant difference in the first and fourth weeks, and a significant decrease (P≤0.05) in litter percent moisture for neem plant addition in the second, third, and, fifth weeks compared G1 group.

Table 6: Effect of neem leaves powder in litter moisture percentage % (Mean± stander error) of broiler (Ross 308) exposed to HS

Groups/ week	**Percent moisture %**				
	1 W	2 W	3 W	4 W	5 W
G1	20.16±0.75	26.15±0.25 a	28.75±0.18 a	30.14±1.00	32.56±1.01 a
G2	19.75±0.90	21.10±0.45 b	22.29±0.37 b	29.33±1.95	25.27±0.83 b
G3	19.65±0.63	22.25±0.15 b	24.13±0.42 b	28.79±1.55	24.75±1.25 b
G4	20.32±0.55	21.36±0.31 b	23.45±0.21 b	30.10±1.10	25.35±0.57 b
Significant	N.S	*	*	N.S	*
Means with deferent litter defer at level (P≤0.05): N.S not significant.					

3.3 Litter pH

Table 7: shows the effect of the study on litter pH, in the first week no significant difference between groups, while in the second week, a significant increase (P≤0.05) for G1, G2, and, G4 groups compared to G3 group, as well in a third-week significant increase (P≤0.05) for G1 compared other cells, in fourth and fifth weeks significant increase (P≤0.05) for G1 group compared neem plant groups.

Table 7: Effect of neem leaves powder in litter pH (Mean± stander error) of broiler (Ross 308) exposed to HS

Groups/ week	**Litter pH**				
	1 W	2 W	3 W	4 W	5 W
G1	7.12±1.63	7.44±0.05 a	7.97±0.02 a	8.13±0.02 a	8.32±0.04 a
G2	7.14±0.81	6.12±0.01 b	6.22±0.03 b	7.27±0.01 b	7.69±0.01 b
G3	7.32±1.24	5.25±0.01 c	6.31±0.02 b	7.36±0.03 b	7.72±0.02 b
G4	7.10±1.50	6.37±0.02 b	6.43±0.01 b	6.10±0.01 c	6.55±0.03 c
Significant	N.S	*	*	*	*
Means with deferent litter defer at level (P≤0.05): N.S not significant.					

3.4 Feces weight

Figure 1: shows feces weight (gm) of broiler through the experiment period, in the first week no significant difference between groups, in the second, third, and, fifth-week significant decrease (P≤0.05) for G2, G3, and, G4 groups compared G1 group, the fourth week showed a significant increase (P≤0.05) for G2 group compared other groups.

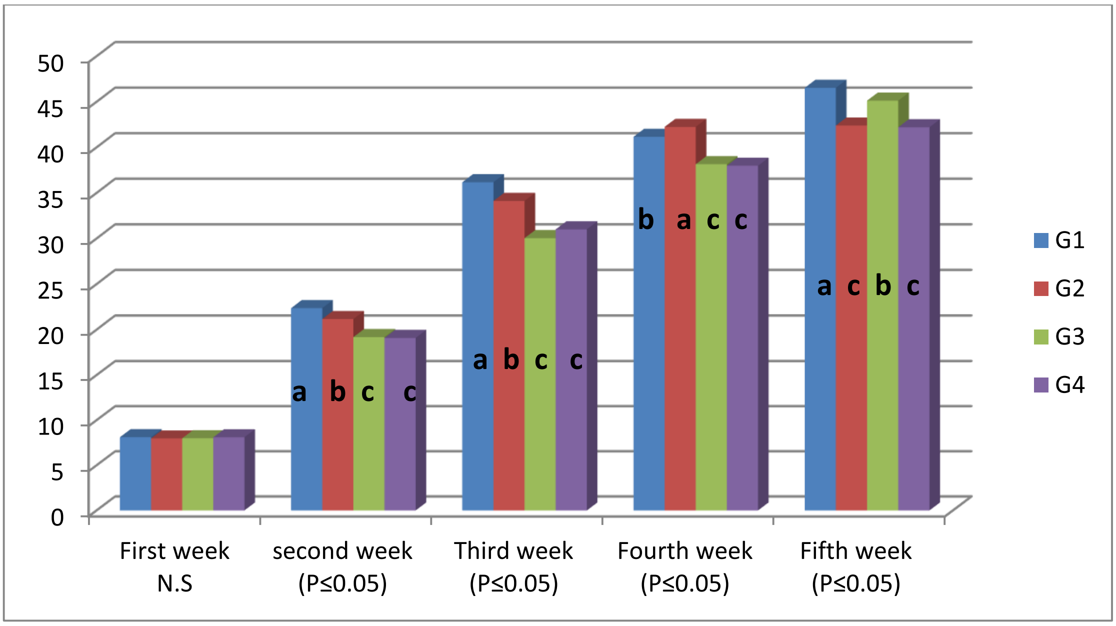

Figure 1: Effect of neem leaves powder in feces weight (g) of broiler (Ross 308) exposed to HS.

3.5 chickens welfare

3.5.1 Footpad dermatitis %

The effect of the study on footpad dermatitis (%) shown in (figure 2), noted a significant increase ($P \leq 0.05$) for the G1 group compared to other groups.

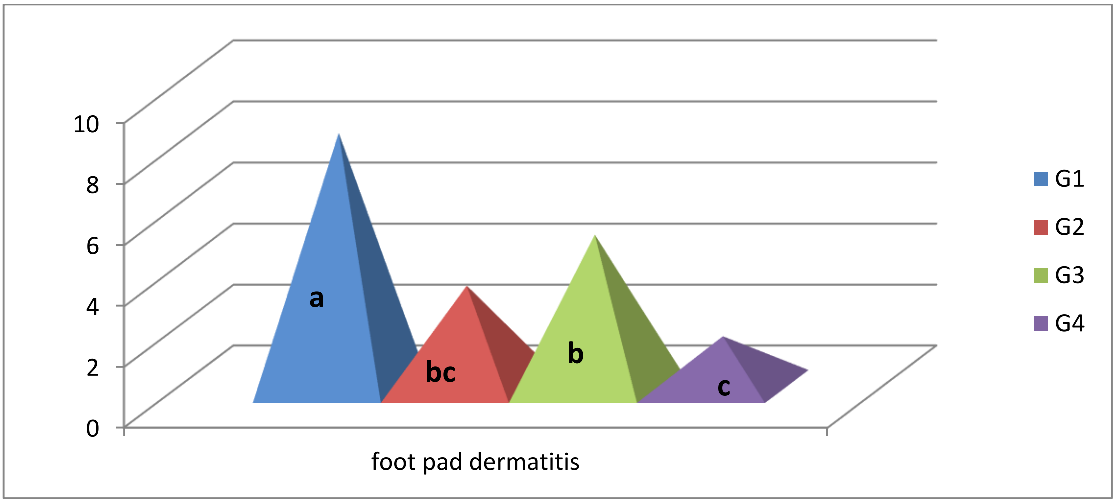

Figure 2: Effect of neem leaves powder in foot pad dermatitis % of broiler (Ross 308) exposed to HS

3.5.2 Feather hygiene percentage %

The groups of neem plant powder were the best in the proportion of feather cleanliness compared to the chickens of the control group, where the second group was significantly ($P \leq 0.05$) higher, followed by the third, fourth, and first groups, respectively.

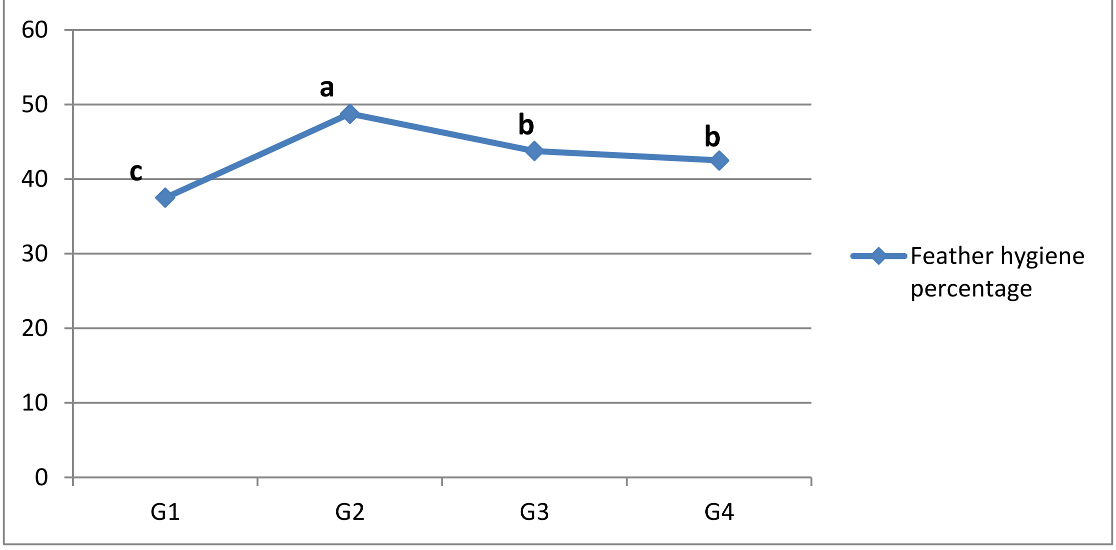

Figure 3: Effect of neem leaves powder in Feather hygiene percentage % of broiler (Ross 308) exposed to heat stress

4. DISCUSSION

The improvement in microbial contamination in the litter of neem plant groups is due to the role of neem leaf powder as an effective antibacterial, as it is characterized by its bitter taste and has properties similar to garlic as an antibacterial (Sonhafouo et al., 2019). Neem contains triterpenoid compounds such as azadirachtin, guidonin, nimbin, and nimbidine, which have antibacterial and antifungal properties (Makeri et al. 2007; Valarmathy et al. 2010), which may have a role in improving the amount of microbial contamination resulting from neem plant groups. In addition, the leaves of the neem plant contain gallic acid and ferulic acid (Shewale and Rathod, 2018), which have an effective role against harmful bacteria as they are phenolic acids that contain hydroxyl groups and an aromatic ring, which are toxic to harmful microbes (Stalikas, 2007: Wang et al., 2011) and are the same reasons that give these compounds antioxidant properties, where the higher the hydroxyl groups, the higher the antioxidant property (Samy et al., 2010: Borges et al., 2013) and this, in turn, improves the health of the intestines of broiler, which causes a balance of the microbiota and an increase in the beneficial bacteria and a decrease in the harmful bacteria as the gut microbes interfere with the compounds phenolic and polyphenols by using these compounds by microorganisms and converting them into other compounds and using them as an energy source. Thus, these compounds control the metabolic activity of the chicken gut microbiota (Correa et al., 2019). In addition, these acids have a high bioavailability and are absorbable by beneficial bacteria in particular. (Lillehoj et al., 2018) In addition, these phenolic compounds are absorbed by intestinal cells and villi and used as an energy source, which improves the activity and health of the intestinal tubule (Theilmann et al., 2017). Some types of beneficial bacteria such as *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, and *Eubacterium ramulus* are characterized by their ability the use glycosidic compounds as food substrates to increase their metabolic activity (Viveros et al., 2011: Espin et al., 2017).

The increase in the beneficial bacteria, especially *lactobacilli*, causes a decrease in the pH in the intestine, which reduces the growth of harmful bacteria such as *Salmonella* (Beal et al., 2006), *Campylobacter* and *coliforms* (Heres et al., 2004) due to the inability of these bacteria to live in acidic media lactic acid (produced by lactobacillus bacteria) can enter the bacterial cell, causing damage to the nucleic acid and protein (Moran et al., 2006) due to the action of lactic acid on lowering the pH inside the bacterial cell, which stops the action of enzymes and protein synthesis and its damage or may cause rupture the outer wall of the bacteria, causing its death (Alakomi et al., 2000), and this explains the decrease in pH in the litter of the neem plant groups, Garces et al., (2013) found that a decrease in the pH value of the litter is accompanied by a decrease in uric acid and ammonia, which increases the welfare of chickens. And the deterioration of these traits in the chickens of the first group may be due to exposure to HS, as HS causes heavy economic losses in the production of broiler chickens (Al-Jebory et al., 2023), as it causes a decrease in the rate of broiler production and a defect in its physiological performance, and the rate depends on the length of time and intensity of exposure to HS (Del vesco et al., 2015) and many physiological changes related to the immune system, oxidative stress, and intestinal integrity, and several other disorders that culminate in deterioration in chicken performance (Lara and Rostagno, 2013). It has also been shown that HS can affect intestinal integrity due to hypoxia of the intestinal epithelium and the impaired epithelial integrity that occurs during HS can cause the occurrence of intestinal inflammation, increased permeability of pathogens, and an imbalance in the balance of the microbial community of the intestine (Burkholder et al., 2008; Deng et al., 2012), which is reflected in avian health and microbial imbalance (Del Vesco et al., 2015, 2017; Gasparino et al., 2018). In addition, the improvement of the chicken gut microbiota will improve the readiness of the consumed feed and its digestibility by staying for a longer period in the digestive tract of the chicken, and thus less excretion from the feces to the outside (Al-Jebory and Naji, 2021 a,b) and the lack of excretion from the feces reduces the humidity of the litter and this the reason may be the low weight of the feces produced and the moisture of the litter from the chickens of the neem plant groups. The results of the study showed that there was a significant improvement in the percentage of footpad dermatitis and the quality of feather hygiene in the treatments of adding neem leaves powder, this is due to the direct and indirect role of neem in improving the microbial traits and litter traits and the ability to add neem to mitigate the effects of HS, as it was found that footpad dermatitis increased with wet litter and the type of litter and the incidence of contact dermatitis (Gonder and Barnes, 1987; Kamyab, 2001 : Mayne, 2005), suggesting that HS increases footpad dermatitis and poor feather exfoliation in broiler chickens.

5. CONCLUSION

The results presented confirmed the effective role of neem leaf powder in enhancing biosecurity and reducing the effects of HS through the improvement of the gut microbiota, this is reflected in the characteristics of the litter and the rate of microbial contamination in it, and then the welfare of the animal. As a result, neem leaf powder can be added to broiler diets to support biosecurity and under conditions of HS.

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