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ORIGINAL ARTICLE

Evaluation of a polyherbal formulation for the management of wet litter in broiler chickens: Implications on performance parameters, cecal moisture level, and footpad lesions

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ABSTRACT

Objective: The study was carried out to develop a wet litter model with magnesium chloride to assess the effectiveness of a polyherbal formulation (PHF) on growth performance, litter and cecal moisture (LCM) level, cecal consistency (CC) score, and footpad lesions (FPLs) score in Ross 308 broiler chickens.
Materials and Methods: 1,200 one-day-old chicks were assigned into five groups: normal control, negative control [NTC; treated with 1.7% magnesium chloride hexahydrate ($MgCl_2.6H_2O$)], and three treatment groups, T1, T2, and T3, where 750, 1,000, and 2,000 gm/ton of PHF, respectively, were supplemented. All the groups were fed a basal diet until day 7. However, the NTC and treatment groups were fed a diet with $MgCl_2$ from days 8 to 42.
Results: The addition of $MgCl_2$ for 35 days worsened the growth performance traits in broilers and induced wet litter problems (FPL, high LCM, and poor CC) in the NTC group. However, PHF (750, 1,000, and 2,000 gm/ton) ameliorated the negative effect of a diet with $MgCl_2$ on growth performance and wet litter problems, but a better response with respect to LCM and CC was observed in 2,000 gm/ton of PHF group, followed by that in 1,000 gm/ton of PHF group and 750 gm/ton of PHF group on day 42.
Conclusion: The wet litter broiler model was developed through excessive feeding of $MgCl_2$, which caused the performance parameters to worsen and the emergence of problems associated with the wet litter. Supplementation with PHF ameliorated these problems and, therefore, it can be used for the management of wet litter in poultry.

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Introduction

Deep litter system plays a key role in maintaining the bird's health, comfort, welfare, and production efficiency. Typically, litter materials (wood shavings, sawdust, peanut hulls, and rice husk) are continuously blended with spilled food, feathers, and poultry fecal waste, preventing the production of harmful pathogens in the poultry house [1,2]. Furthermore, it acts as a moisture absorber, but the capacity varies from 15% to 45% based on the type of litter material used in the shed [3,4]. However, the moisture-absorbing capacity of litter is completely saturated when the moisture content of chicken droppings (watery/loose) increases owing to the high water intake. As a result, the addition of water surpasses the removal of water (evaporation), leading litter moisture problems in the poultry sheds. Wet litter is the complex issue caused by multiple interrelated factors, such as management and housing of birds [5,6], disease and diet control [7], and gut health factors [8]. Additionally, it is one of the most significant factors that contribute to the induction of footpad lesions (FPLs), also called footpad dermatitis (FPD), which has become a critical concern in contemporary chicken meat production.

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This is supported by several authors [3,9,10] who observed a high incidence of FPD associated with high litter moisture concentrations in broiler sheds [9,11]. Furthermore, it was demonstrated that the high prevalence of FPD reduced the growth performance (weight gains by 7.75% and feed conversion efficiency by 4.16%) of 37-day-old meat-type chickens [12]. Therefore, attention must be given to the wet litter problem and it should be addressed to prevent production losses and uphold animal health and welfare.

Wet droppings can be effectively rectified by identifying the underlying causal factors, for instance, the management of drinkers and proper shed ventilation would prevent problems due to environmental or housing factors. Similarly, any nutritional disturbance alters the excreta quality of meat chickens, which can be improved by influencing the microbial phytase and dietary electrolyte balance in the feed [13,14]. Similarly, the addition of antibiotics at subtherapeutic concentrations reduces the prevalence of coccidiosis and necrotic enteritis infection, which is the most frequently cited condition among the relevant diseases. However, with the ban of in-feed antibiotics and zinc oxide, well-known anti-diarrheal agents in piglets [15], in European countries animal health deteriorated, which include increased loose droppings/diarrhea due to gut disturbances, weight loss, and high mortality of early post-weaning pigs and broilers [16,17]. Hence, there is an unmet need for natural alternatives to prevent the wet droppings, and as far as we know, no literature related to the effect of natural feed additives on the moisture of broilers excreta is published. The polyherbal formulation (PHF) under investigation, Stodi®, is a blend of several Indian medicinal plants (*Punica granatum*, *Andrographis paniculata*, *Acacia nilotica*, *Terminalia bellirica*, and *Holarrhena antidysenterica)* traditionally known and used individually to manage diarrheal disorders. However, there are no scientific reports that demonstrate their anti-diarrheal activity in a combined form in a wet litter model in broilers.

Numerous models are available to evaluate wet litter problems in broiler chickens. One of them is that the addition of minerals in the diet at high doses can induce loose droppings/diarrhea in broilers by altering osmolarity and water reabsorption in the gastrointestinal tract (GIT), but the effect varies depending on individual minerals [18]. Magnesium, an indispensable positively charged ion in the diet of most animals [19], is commonly used as a laxative in humans [20,21] and as an osmoregulators in animal diarrhea models [22–25]. It has been reported that magnesium sulfate alters the luminal osmotic pressure, preventing the reabsorption of water from the digesta, and increasing the excreta moisture [25]. Similarly, higher levels of dietary magnesium oxide reduced the transit time of intestinal digestion in broilers [26].

Magnesium chloride ($MgCl_2$) is less toxic [27] and has the capacity to increase the excreta moisture content in comparison with other magnesium sources [28]. Therefore, it was used in the present study to increase the digesta moisture and reduce the water reabsorption instead of increasing water consumption. The objective of the study was to induce wet litter problems using a diet with $MgCl_2$ and evaluate the effect of PHF on growth performance, litter moisture, cecal moisture and consistency, and footpad lesion in Ross 308 broilers.

Materials and Methods

Polyherbal formulation

Stodi®, a registered PHF formulated by Natural Remedies Pvt. Ltd., Bengaluru, India. It contains rinds of *P. granatum*, aerial parts of *A. paniculata*, bark of *A. nilotica*, fruits of *T. bellirica*, and bark of *H. antidysenterica*.

Experimental setup and ethical approval

The experimental setup and the management of birds (Table 1) were followed as per Selvam et al. [29]. The present study was performed in compliances with guidelines laid down for the care and the use of animals, and protocol approval was provided by the Institutional Ethics Committee [No. AHS/PR/01/2018].

Experimental plan and feeding level

Twelve hundred Ross 308 chicks (1-day-old) were supplied by Kavi Protein and Feed Pvt. Ltd. (Bengaluru) and were allocated equally into five groups (six replicates/group; 40 birds/replicate), consisting of normal control (NC), negative control [NTC; treated with 1.7% magnesium chloride hexahydrate ($MgCl_2.6H_2O$)], and three treatment groups, T1, T2, and T3, where 750, 1,000, and 2,000 gm/ton of PHF, respectively, were supplemented. All groups were fed with a basal diet (Table 2) until day 7. However, the NTC and treatment groups were fed with a customized diet (1.7% $MgCl_2$) from day 8 to day 42.

Assessment of zootechnical parameters

The mortality of chicks was observed in each pen, once daily for 42 days. The chicks were weighed individually on days 1, 7, 21, 28, 35 and, finally, on 42nd day. The quantity of feed consumed by birds was determined on 7th, 21st, 28th, 35th, and 42nd days of the experiment by deducting the quantity of remaining feed over the total quantity of feed offered per pen, and feed efficiency (feed conversion ratio, FCR) was calculated by the ratio between the total quantity of feed consumed and the total body weight gain.

Footpad lesion

Footpad lesion was scored using a four-point grading scale [30] on days 35 and 42. It was allocated to one of four classes: score 0, normal footpad; score 1, <25% area covered by lesions; score 2, 25%–50% area covered by lesions; and score 3, >50% area covered by lesions. The ratio of cumulative lesion scores to that of 48 arbitrarily picked birds was considered as a mean footpad lesion score.

Cecal consistency

Cecal consistency (CC) was scored for six birds in each group on day 42. Briefly, the consistency was assigned to one of four classes: score 1, normal (semi-solid and well-formed); score 1, sticky droppings (gelatinous); score 2, mild diarrhea (fluid feces and not well formed); and score 3, severe diarrhea (watery and projectile).

Litter and cecal moisture content

Litter samples were taken from five different locations per pen (replicate) and pooled before measurement on days 21, 28, 35, and 42. The moisture content (%) was evaluated by weight loss on drying using a Hot Air Oven (FS-405 Model) purchased from Advantec Co., Ltd., (Saijyo, Japan). In summary, the pooled litter sample was weighed and allowed to dry at 105°C for 24 h. The dried sample was reweighed, and the weight difference (before and after drying) constitutes the moisture content. A similar procedure was used to evaluate the moisture content (%) in the cecal samples (n = 6/group) on day 42.

Statistical analysis

The statistical analysis was performed as per the procedures described by Selvam et al. [29], and $p < 0.05$ was considered as statistically significant.

Results

Zootechnical parameters of chickens

The initial live body weight in all the groups was not significantly different, indicating the uniform distribution of the chicks for each pen on day 1. The $MgCl_2$ diet group displayed a reduction in body weight gain on days 35 and 42. However, all the treatment groups (T1, T2, and T3) had significantly increased the body weight gain on days 21, 28, 35, and 42 (Table 3). Similarly, FCR was significantly worsened in the NTC compared to that in the NC group on days 21, 28, 35, and 42. Nevertheless, the groups treated with PHF showed a numerical improvement in the FCR on days 28, 35, and 42 (Table 4).

Footpad lesion score

Footpad lesion score was found to be high in the NTC group as compared to the NC group on days 35 and 42. However, the PHF-treated groups (750, 1,000, and 2,000 gm/ton) showed a numerical improvement in the footpad lesion score compared to that in the NTC group on days 35 and 42 (Table 5).

Litter moisture content

Litter moisture (gm/100 gm) content was significantly increased in the NTC group when compared to the NC group throughout the experimental period. However, litter moisture content (gm/100 gm) was numerically improved in the PHF-treated groups (750, 1,000, and 2,000 gm/ton) compared to that in the NTC group on days 35 and 42 (Table 6).

Cecal consistency score and cecal moisture content

CC score and moisture content (gm/100 gm) of NTC group were worsened significantly as compared to the NC group, whereas these parameters improved after PHF supplementation with the best response being observed in the 2,000 gm/ton of PHF group compared to that observed in the NTC group on day 42 (Table 7).

Discussion

In general, the functions of litter materials are to provide insulation to the bird by creating cushions on the hard floor and facilitate the evaporation of broiler excreta and spilled water by absorbing the moisture in the poultry house. However, any physiological disturbances due to the electrolyte imbalance, non-starch polysaccharides diets, and environmental change upsurge the water intake by stimulating the hypothalamus, which further dilutes the blood and suppresses the secretion of anti-diuretic hormone. This, in turn, urges the high urine output, and to compensate for

Table 1. Environmental conditions.

Day	Temperature at chick level (°C)	Relative Humidity (%)	Photoperiod
1–3	29–30	60–70	23 h light and 1 h darkness
4–7	28–30	50–60	
8–9	27–28	50–60	22 h light and 2 h darkness
10–11	27–28	50–60	21 h light and 3 h darkness
12–26	25–27	50–60	20 h light and 4 h darkness
> 27	24–26	50–60	

Table 2. Feed Composition (kg/ton).

Type of Feed	Pre-starter	Starter	Finisher
	(Day 1–10)	(Day 11–24)	(Day 25–42)
Corn local	550.00	595.40	580.00
Rice bran	24.50	-	45.24
Soya bean meal (46.5%)	340.00	290.00	274.00
Corn gluten meal (60%)	-	19.60	-
[a]Deoiled rice bran/$MgCl_2$	17.00	17.00	17.00
Limestone fine	11.00	9.00	8.00
Dicalcium phosphate	14.50	14.50	12.00
Salt common	2.30	2.50	2.80
Sodium bicarbonate	3.00	3.20	2.60
Blended oil (Veg)	21.00	33.00	44.00
L -Threonine 98.5%	1.10	1.00	0.80
L-Lysine	3.00	2.80	1.65
DL-Methionine 99%	3.40	2.90	2.60
Choline 60%	0.65	0.60	0.50
[b]Trace mineral mixture broiler	1.20	1.20	1.50
Vitamin C	-	0.25	0.25
Betaine	0.75	0.75	0.75
Toxin binders	1.00	1.00	1.00
Organic selenium	0.09	0.06	0.06
Sodium butyrate	0.50	0.25	0.25
[c]Premix broiler	5.00	5.00	5.00
Metabolizable Energy Kcal	3,000	3,125	3,150
Crude Protein %	21	20	18.5

[a]Deoiled rice bran (DORB) was replaced by Magnesium chloride hexahydrate ($MgCl_2.6H_2O$)
[b]Mineral premix supplied the following per kilogram: Fe, 40 gm; Cu, 10 gm; Mn, 100 gm; Zn, 100 gm; Se, 0.25 gm; and I, 1.5 gm.
[c]Premix broiler composition (5 kg) supplied the following per kilogram: antioxidants, 0.125 kg; emulsifier, 0.500 kg; Phytase, 0.100 kg; acidifier, 1.000 kg; liver tonic, 0.500 kg; [d]vitamin premix, 0.500 kg; DORB, 2.275 kg.
[d]Vitamin premix supplied the following per kilogram of vitamin premix: vitamin A, 25 MIU; vitamin D_3, 5 MIU; vitamin E, 24 IU; vitamin K, 3 gm; vitamin B_1, 3 gm; vitamin B_2, 10 gm; vitamin B_6, 4 gm; vitamin B_{12}, 0.015 gm; niacin, 30 gm; pantothenic acid, 20 gm; folic acid, 1 gm.

this loss, birds consume more water and excrete the watery droppings. As a result, the litter becomes damp, causing the development of FPD as well as worsening the production performance of chickens. Considering the wet droppings/wet litter as a useful early warning sign, the underlying cause must be identified and addressed as a matter of urgency to maintain the optimum health of birds.

Since the inclusion of excessive Mg leads to skeletal abnormalities in young birds [19], broiler chickens were raised on a diet with $MgCl_2$ (1.7%) from day 8 to day 42 in the current study. Birds raised on a diet with $MgCl_2$ for 35 days gained lesser body weight gain (1.7%) with higher feed intake (90 gm/unit of body weight) than birds in the NC group. These results are supported by several authors [28,31,32] who have observed the limited toxic effect of Mg on broiler performance. Similarly, CC and cecal and litter moisture content were worsened in chickens fed a diet with $MgCl_2$. This is consistent with the observations of [28], who reported that birds excreted the droppings with high moisture content when they were treated with three different Mg sources. Magnesium, a divalent cation, alters the osmolarity of the digesta [18] due to its poor absorbable character [21] and produces a laxative effect [21,33]. However, other authors did not observe any difference in the intestine even after increasing Mg from 0.15% to 0.80%, suggesting that the endocrine hormonal and neuronal effects were responsible for Mg-mediated diarrhea in broiler chickens. Nevertheless, the high concentration of

Table 3. Effect of PHF on body weight (gm) in Ross 308 broiler chickens ($MgCl_2$ induced wet litter).

Group	Day 1	Day 7	Day 21	Day 28	Day 35	Day 42
NC	46.87 ± 0.22	129.20 ± 1.19	^{a}726.62 ± 7.28	^{a}1,185.95 ± 12.73	^{a}1,829.75 ± 20.44	^{ab}2,494.75 ± 28.89
NTC	47.15 ± 0.24	135.08 ± 0.95	^{a}736.86 ± 6.56	^{a}1,188.28 ± 12.68	^{a}1,783.08 ± 19.73	^{a}2,452.91 ± 29.13
NTC + PHF (750 gm/ton)	46.72 ± 0.24	137.88 ± 1.15	^{b}771.53 ± 9.00	^{b}1,261.65 ± 14.30	^{b}1,886.60 ± 22.79	^{b}2,536.90 ± 31.51
NTC + PHF (1,000 gm/ton)	47.44 ± 0.22	139.40 ± 1.30	^{b}789.13 ± 7.77	^{b}1,282.73 ± 13.88	^{b}1,898.48 ± 22.37	^{b}2,534.35 ± 32.43
NTC + PHF (2,000 gm/ton)	46.86 ± 0.24	138.44 ± 1.14	^{b}773.58 ± 9.06	^{b}1,264.57 ± 13.48	^{b}1,888.78 ± 21.54	^{ab}2,525.14 ± 31.61

Values are expressed as mean ± standard error mean (SEM); n = 226–238; $^{a-b}$Means bearing different superscripts were significantly different ($p < 0.05$) by one-way analysis of variance (ANOVA) with location as a blocking factor followed by least significance difference (LSD) test using Statistical Package for the Social Sciences (SPSS).

Table 4. Effect of PHF on FCR in Ross 308 broiler chickens ($MgCl_2$ induced wet litter).

Group	Day 7	Day 21	Day 28	Day 35	Day 42
NC	0.859 ± 0.02	^{a}1.526 ± 0.01	^{a}1.682 ± 0.02	^{a}1.752 ± 0.01	^{a}1.812 ± 0.01
NTC	0.856 ± 0.01	^{b}1.579 ± 0.02	^{b}1.765 ± 0.02	^{b}1.848 ± 0.02	^{b}1.902 ± 0.02
NTC + PHF (750 gm/ton)	0.856 ± 0.02	^{b}1.577 ± 0.02	^{c}1.728 ± 0.01	^{b}1.819 ± 0.01	^{b}1.883 ± 0.01
NTC + PHF (1,000 gm/ton)	0.858 ± 0.01	^{b}1.586 ± 0.01	^{bc}1.744 ± 0.01	^{b}1.833 ± 0.01	^{b}1.891 ± 0.01
NTC + PHF (2,000 gm/ton)	0.857 ± 0.01	^{b}1.584 ± 0.02	^{bc}1.744 ± 0.01	^{b}1.834 ± 0.02	^{b}1.893 ± 0.02

Values are expressed as mean ± SEM; n = 6; $^{a-c}$Means bearing different superscripts were significantly different ($p < 0.05$) by One-way ANOVA with location as a blocking factor followed by LSD using SPSS.

Table 5. Effect of PHF on footpad lesion.

Group	Day 35	Day 42
NC	0.0	0.0
NTC	1.0	1.0
NTC + PHF (750 gm/ton)	0.9	0.6
NTC + PHF (1,000 gm/ton)	0.8	0.5
NTC + PHF (2,000 gm/ton)	0.8	0.8

Values are expressed as mean.

both Mg and Cl ions due to $MgCl_2$ inclusion, was excreted from the kidneys and disturbs the osmotic value after reaching the distal GIT [34]. This, in turn, prevents the water reabsorption and increases the digesta moisture, which is confirmed by high cecal moisture contents in the current study. It has also been reported that the high moisture content in the bedding material could significantly raise the incidence of FPD in broilers [35,36], and it was confirmed in the present study as well. The above findings revalidate the hypothesis that excess $MgCl_2$ can be added in the diet to develop the wet litter model, which is used to evaluate the effect of anti-diarrheal products or agents for the management of wet litter problems in broilers.

Birds supplemented independently with 750, 1,000, and 2,000 gm/ton of PHF showed an improvement in the body weight gain by about 3.4%, 3.3%, and 2.9%, respectively, with better FCR, namely, 19, 11, and 10 gm, respectively, lesser feed per unit body weight gain. In addition, the weight gain response to PHF (750, 1,000, and 2,000 gm/ton) was greater than that of the NC response. Our results are consistent with some previous studies, which showed that supplementing *A. paniculata* and *A. nilotica* in the diets of broilers increased the growth performance [29,37]. Moreover, PHF improve the cecal consistency and the cecal and litter moisture, which could be due to the presence of *P. granatum, A. paniculata, A. nilotica*, and *T. bellirica.* Moreover, *P. granatum* is used to treat stomach disorders and diarrheal cases [38] as its different concentrations of the extract ameliorated the diarrhea induced by castor oil by exerting the beneficial effects on GIT in *rats* [39]. *A. paniculata* has shown a highly significant anti-secretory activity [40] and an anti-diarrheal property against *E. coli* enterotoxins in guinea pigs and rabbit [41]. *A. nilotica* and *T. bellirica* extract reduced the diarrhea induced by castor oil in rats [42,43]. In addition, tannin is one of the chemical constituents present in each herb [44–48], which is used to treat acute diarrhea [49,50]. Recent studies also indicated that tannic acid improved the mucosal resistance [51] and can protect cells from oxidative damage [52,53]. Similarly, the inclusion of PHF was effective in normalizing the increased footpad lesion score caused by a diet with $MgCl_2$, which could be due to amoebicidal, anti-inflammatory, and analgesic properties of *H. antidysenterica* [54–56]. The findings of the previous literature on individual herbs related to diarrhea confirm that PHF under investigation has the potential to prevent/manage the gut health/wet litter problems.

Table 6. Effect of PHF on litter moisture content (gm/100 gm).

Group	Day 21	Day 28	Day 35	Day 42
NC	[a]35.83 ± 2.59	[a]33.13 ± 0.75	[a]30.63 ± 1.02	[a]29.10 ± 2.06
NTC	[b]44.53 ± 1.30	[b]42.39 ± 0.52	[b]42.80 ± 0.61	[b]43.02 ± 0.98
NTC + PHF (750 gm/ton)	[b]42.74 ± 1.46	[b]43.09 ± 0.83	[b]41.63 ± 2.46	[b]42.26 ± 3.41
NTC + PHF (1,000 gm/ton)	[b]42.45 ± 0.84	[b]42.42 ± 1.00	[b]40.13 ± 0.95	[b]40.40 ± 1.78
NTC + PHF (2,000 gm/ton)	[b]44.72 ± 1.50	[b]42.01 ± 0.84	[b]39.71 ± 1.76	[b]39.81 ± 2.16

Values are expressed as mean ± SEM; $n = 6$; [a–b]Means bearing different superscripts were significantly different ($p < 0.05$) by One-way ANOVA followed by LSD using SPSS.

Table 7. Effect of PHF on cecal consistency score and cecal moisture content.

Group	Cecal consistency score	Cecal moisture (gm/100 gm)
NC	[a]1.00 ± 0.00	[a]73.70 ± 2.93
NTC	[b]4.00 ± 0.00	[b]87.16 ± 0.67
NTC + PHF (750 gm/ton)	[bc]3.50 ± 0.22	[b]84.68 ± 2.60
NTC + PHF (1,000 gm/ton)	[c]3.00 ± 0.37	[b]83.26 ± 2.78
NTC + PHF (2,000 gm/ton)	[c]2.83 ± 0.48	[b]82.84 ± 2.19

Values are expressed as mean ± SEM; $n = 6$; [a-c]Means bearing different superscripts were significantly different ($p < 0.05$) by One-way ANOVA followed by LSD using SPSS.

Conclusion

In the present study, a wet litter broiler model was developed through the excessive feeding of $MgCl_2$ diet, which is characterized by the worsening of performance parameters and the emergence of problems associated with wet litter such as footpad lesion, high litter and cecal moisture, and poor cecal consistency. Supplementation with PHF ameliorated these problems and, therefore, it can be used as a natural alternative for the management of loose droppings and wet litter problems that arise during broiler production.

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Conflict of interests

The authors declare no conflict of interest.

Authors' contribution

Saravanakumar Marimuthu performed the work, compiled, analyzed and interpreted the data, and drafted the manuscript, Brindhalakshmi Balasubramanian performed the work and reviewed the manuscript, Ramasamy Selvam planned the study, analyzed and interpreted the data, and proof read and reviewed the manuscript, and Prashanth D'Souza planned the study and reviewed the manuscript.

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