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RESEARCH ARTICLE

EVALUATION OF REFUSED TEA AS AN ALTERNATIVE LITTER MATERIAL TO PADDY HUSK: EFFECTS ON BROILER PERFORMANCE THEIR BEHAVIOUR, LITTER PROPERTIES AND AMMONIA EMISSION

Belpagodagamage UD[1*], Atapattu NSBM[1], Wanniarachchi SD[2] and Seresinghe RT[1]

[1]Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka

[2]Department of Soil Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka

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ABSTRACT

The availability of paddy husk (PH) as a litter material for poultry production is decreasing. The objective of the present study was to determine the suitability of refused tea (RT), a waste generated during the processing of green leaves into black tea, as alternative litter material. Three experiments using completely randomized design were conducted for PH and RT each alone or as mixtures to determine the effects of the type of litter material on growth performance, behaviour of broilers, litter characteristics, and litter ammonia emission. In experiment one, 300 male broilers (Cobb–500) chicks were raised from day 5 to 21 on ten-floor pens ($1.5m^2$), each provided with either PH or RT as the litter material. Five and three PH and RT mixtures (v/v) were used in experiment two and three, respectively to raise broilers (n=300 in each experiment) from day 21 to 42. The type of litter material had no significant effect ($P>0.05$) on growth performances, hock burn damage score, bird's cleanliness score and dressing percentage. Compared to PH, RT litter reported a significantly ($P<0.05$) higher bulk density, N content and caked surface area and tend to have a higher moisture level ($p<0.10$). Compared to PH alone, RT alone or 1:1 PH:RT litter reduced the emission of ammonia by 53.6 and 34%, respectively. Percentage time spent on foraging was significantly ($P<0.05$) higher on PH than on RT. The time spent on and the frequency of inter-bird interactions were significantly ($P<0.05$) higher among the birds raised on RT, compared to those kept on PH. The study concluded that RT can be used as an alternative litter material for broilers. Other advantages of RT litter were the lower ammonia emission and higher litter N contents. Higher litter moisture content and incidence of caking were the disadvantages of RT as litter material.

Keywords: Broiler litter, Refused tea, Ammonia emission, Live weight

INTRODUCTION

Management of litter is one of the critical aspects of poultry production under a deep litter system. The quality of litter affects a range of economically and environmentally important parameters such as live weight, mortality, carcass quality, health and welfare of poultry and noxious gas emission from litter (Meluzzi *et al.* 2008). Due to the low cost and high availability, paddy husk (PH) is the most widely used litter material for the poultry industry in Sri Lanka (Atapattu *et al.* 2008). The use of PH as a fuel in bakery and clay-brick making industries and, tea factories have reduced the availability of PH as a poultry litter material. Consequently, expanding poultry industry needs alternative litter materials. Suitability of a wide variety of materials such as wood shavings (Khan *et al.* 2007), peanut hulls, pine shavings, recycling paper (Grimes *et al.* 2002), chopped grass (Davis *et al*. 2010), kenaf (Malone *et al.* 1990), sand (Billgilli *et al.* 2004), rice and wheat straw (Benabdeljelil and Ayachi, 1996), softwood chipping and fines leaves (Davis *et al.* 2010) as litter materials for poultry has been tested, with varying success.

*Corresponding author: udeshdharmasiri@yahoo.com

Under Sri Lankan conditions, processing 100 kg of fresh tea leaves into black tea results in 4 to 5 kg of refused tea (RT). Accordingly, based on national tea production data (Central bank report 2016), it is estimated that 55 millions kilograms of RT have been produced in Sri Laka in 2016. Refused tea has low bulk density thus favours litter aeration. Atapattu and Wickramasinghe (2007) showed that the growth performance of broilers from day 21-42 on RT-based litter was comparable with those reared on PH litter. However, no attempts have been made to determine the suitability of RT as a litter material over the whole production cycle of commercial broilers. Also, to determine the suitability of RT as a litter material, some other aspects such as its effects on animal behaviour and welfare should be evaluated. Therefore, three experiments were conducted to investigate the suitability of RT as a litter material for broilers, considering its effects on production, welfare and litter parameters.

MATERIALS AND METHODS

Three experiments were conducted. In Experiment one, four-day old male broiler chicks (Cobb- 500) (initial body weight=130±15g) were allocated into 10-floor pens (1.5m^2/pen). Five pens had RT as the litter material while PH was the litter material in the other five pens. Thirty chicks were assigned to each pen. Until assigned into pens, chicks were brooded as a single group. After allocating into pens, the heat was provided until day 10 using electrical bulbs arranged to each pen. Chicks were fed a commercial broiler starter diet ad libitum until day 21.

In Experiment two, 21-day-old (initial body weight=800±22g) male broilers chicks (Cobb-500) (n=300) were allocated into 25 floor pens (1.3m^2/pen). Resulting in a completely randomized design with five replicate pens per treatment. Five PH:RT combinations were used as the litters *ie.* 1). RT alone, 2) a mixture of (v/v) RT 75% + PH 25%, 3). RT 50% + PH 50%, 4) RT 25% + PH 75% and 5) PH alone. Each pen had a bell-shaped feeder and a drinker. Birds were fed a commercial broiler finisher diet until day 42.

Experiment 3 also followed a similar procedure as in experiment 1. However, only three litter combinations were used; 1). RT alone, 2) a mixture of RT 50% + PH 50%) and PH alone. The growth trial was done until day 42.

RT and PH were obtained from a local tea factory and a rice mill, respectively. RT tea and PH samples were analyzed for moisture content, bulk density, nitrogen and pH. In all experiments live weight, weight gain, mortality, feed conversion ratio (FCR), feed and water intake were used as the growth performance parameters. In experiment 2 and 3, two randomly selected birds from each pen were killed on day 41 and dissected to determine the internal organ weights, dressing percentage and to observe the presence of litter materials in the crop.

Birds were observed for hock burn damage and cleanliness (Experiment 2 & 3). A four-category scoring system was used to evaluate the cleanliness. Three randomly selected birds from each pen were observed for cleanliness and hock burn damages. Considering the cleanliness of legs, breast, abdomen, tail feathers and neck area birds were rated into four cleanliness categories; 1- appearance dirty; 2 - moderate dirty; 3 moderate clean; 4 - clean).

Hock burn damage was assessed as described by Kristensen *et al.* (2006) (Table 1).

In experiment 1, caked litter area was determined as a percentage of the total litter area. In experiment 2 and 3, litter cake formation and easiness for de-caking were estimated on day 28. De-caking easiness was assessed on five-point scoring system (Table 2).

For litter N, moisture and pH analysis (in experiment 2 and 3), avoiding the area around the feeder and drinker, five litter samples were taken from five randomly selected places of each pen. Five samples of a given pen were pooled and then analyzed for moisture (105^{0}C for 24 h; pH (Brake *et al*. 1992) and N (AOAC 1980).

Table 1: Scoring system used in hock burn damage assessment (Kristensen *et al.* 2006)

Hock burn damage levels	Score
No damages in both legs	2
One leg shows a moderate level and the other leg shows no damage	0.5
One leg shows a high level and the other leg shows no damage	0
Both legs show a moderate level of damage	-1
One leg shows a moderate level of damage and the other leg shows a high damage level	-1.5
Both legs show a high level of damage	-2

Table 2: Scoring system to determine the easiness of litter de-caking.

Easiness for de-caking	Score
Easiest	2
Easy	1
No easy nor difficult	0
Difficult	-1
Very difficult	-2

Litter NH_3 emissions were determined as described by Moore *et al.* (2008), with slight modifications. A fresh litter sample (250g) was drawn from each pen and put into conical flask. Flasks were equipped with air inflow and outflows. Samples were incubated at 30^0C for five hours. Air was continuously passed through each flask and NH_3 volatilized from litter samples in conical flask was trapped in 100 ml of 0.32 N H_3BO_4 solutions. The boric acid solution that trapped the NH_3 was titrated with 0.1 N HCl to determine the NH_3 emission. The emission rate was computed as mg of NH_3 emitted / kg of fresh litter/hr and g of NH_3/Animal Unit (AU)/hr. AU was defined as 500 kg of live weight (Coulfal 2006).

Scan sampling method (Mann1999) was used to record the behavioural data (Experiment 3). The behaviour of the birds was observed three hours per day (2-5 pm), in five consecutive days from day 35-39 using an ethogram. An observer was assigned to each pen and was asked to record the frequency of and the time spends on eight predetermined behaviours of an ethogram suggested by (Marchant *et al.* 2008) (Table 3). At the beginning of an observation session, an observer sat quietly about 2m away from the front of a pen, allowed 5 min for the chicks to habituate to the observer's presence, then started a stopwatch and recorded data.

Effects of litter material were statistically analyzed as a completely randomized design experiment. Growth performance parameters (live weight, weight gain, feed intake, feed conversion ratio), percentage of time spent on each behaviour and frequency of each activity observed/hr, carcass parameters and litter properties were analyzed using GLM procedure using Minitab inc. (Ver 11.12). Birds cleanliness scores, hock burn damage and easiness of de - caking data were analyzed using non-parametric (Kruskal Wallis) test. Effects were considered statistically significant when $p < 0.05$.

Table 3. Ethogram used in the behavioural study (adopted from Marchant *et al.* 2008)

Behaviour	Description
Eat	Head extended toward the feeder and appears to be injecting feed
Walking	Taking one or more steps
Standing	Standing with no apparent movement of legs
Laying	Performing no perceptible behaviour
Foraging	Scratch litter with beak and appears of eating
Feather picking	Self-manipulation of own feathers with beak
Bird's interaction	Two or more birds touching each other
Drinking	Head extended toward the drinker and appears to be drinking water

RESULT AND DISCUSSION

Table 4, 5 and 6 present the effects of RT, PH each alone or in combination on the growth performance of broilers from day 5-21 (Experiment 1) and 21-42 (Experiment 2 & 3).

While supporting earlier findings with relatively mature birds (Atapattu *et al*. 2008, Atapattu and Wickramasinghe, 2007), the present experiments revealed that the type of litter material had no direct impacts (p>0.05) on mortality rate even among the chicks from day 5-21 (Table 4 and 6), visible health conditions or physical damages,. This observation is important since some litter materials, for example, refined gypsum (Wyatt *et al*. 1992) and recycled paper chips (Lien *et al.* 1998) reported to have harmful effects.

The type of litter material had no significant effects (p>0.05) on growth performance parameters and litter moisture contents from day 5-21 (Table 4). A previous study by Atapattu *et al*. (2007) have reported that the use of RT as a litter material for broilers at the finisher stage (day 21-42) had no significant effects on growth performance. In line with those findings, broilers on RT litter (either alone or as a mixture with PH) resulted in similar growth performance parameters to those on PH. Monira *et al*. (2003) also reported that litter material had no significant effects on the growth performance of broilers. Results of the present study while confirming the results of the above study, showed that RT can be used as the litter material for young chicks as well, without having any adverse impacts on growth performance and mortality. RT and PH differ from each other both physically and chemically (Atapattu *et al.* 2008). It was expected that the use of RT and PH as a mixture would have complementary and supplementary effects, rather than using each material alone. However, the provision of such RT:PH mixture produced no beneficial effects. The comparable growth performance between the birds on RT and PH alone suggest that the use of RT and PH mixtures is not needed.

Hock burn damage has been identified as one of the major welfare issues of broiler production. Hock burn damage correlated with litter moisture, where high litter moisture levels reported to increase hock burn damages (Wyatt *et al*. 1992, Haslam 2007). There were no significant differences (p>0.05) in hock burn damage among the broilers reared on different litter materials in Experiment 2 & 3. Bird's cleanliness and dressing percentage were also not significantly (p>0.05) affected by the type of litter. Furthermore, there were no visible abnormalities of the carcass of the broilers raised on RT. Brake *et al.* (1992) and Atapattu and Wickramasinghe, (2007) have also reported that the type of litter material did not affect dressing percentage.

Table 4: Effects of litter type on growth performance and litter properties from day 5-21 (Experiment 1).

Parameter	Type of litter		SEM	P value
	PH	RT		
Feed intake (g/bird/day)	42	43	1.8	NS
Water intake (ml/bird/day)	212.8	121.0	3.2	NS
Mortality (%)	0.3	0	0.2	NS
Live weight on day 21 (g)	738	725	13	NS
Weight gain from 5d-21 d (g)	695	681	12	NS
FCR	1.41	1.38	0.2	NS
Ammonia emission (mg/kg litter/hr)	35.6	27.1	6.2	NS
Moisture (%)	48	51	3.8	NS
Caked litter (%)				
7th day	1.75	12	3.2	***
14th day	9	21	4.1	***
21st day	22.5	45	8.6	***

Note: SEM, mean standard error; PH, paddy husk; RT, refuse tea; NS, not significant; p>0.05; ***. P<0.001

Table 5: Effects of refused tea and paddy husk each alone or in combination as a litter material on broiler growth performance from day 21-42 and carcass parameters (Experiment 2).

Parameter	Treatments						
	0% RT	25% RT	50% RT	75% RT	100% RT	SEM	P value
Live weight (g) Day 21	778.8	807.4	822.3	786.6	814.6	8.82	NS
Live weight gain (g/bird) Day21- 42	1603.0	1593.6	1603.7	1650.5	1642.6	20.94	NS
Feed intake (g/bird/day) Day21- 42	140.4	136.3	136.6	133.3	131.5	1.72	NS
Feed conversion ratio Day 21- 42	1.6	1.6	1.6	1.6	1.6	0.02	NS
Water intake (ml/bird/d) Day 31- 42	394.3^{ba}	380.7^{b}	419.6^{a}	410.3^{ba}	419.7^{a}	0.57	*
Cleanliness score Day 41	3.00	3.00	2.4	3.4	2.6	0.21	NS

Note: SEM, mean standard error; values bearing different letter within a row are statistically different at *p <0.05; NS, not significant.

The results revealed that the type of litter material had significant effects (p<0.05) on water intake during day 31-42 (Table 5). However, such an effect was not observed in the other two experiments and the study of Atapattu and Wickramasinghe (2007). Reason/s for these discrepancies is/are not clear.

Litter characters and ammonia emission

Table 7 presents the litter characters and ammonia emission as affected by the type of litter material used. Though RT was found to be a more acidic material compared to PH (Table 8), in both experiments, litter pH levels were not significantly affected (p>0.05) by the type of litter material. As shown in previous studies (Coufal *et al.* 2006, Senaratna *et al.* 2007, Miles *et al.* 2011) irrespective of the litter material, litters gave basic pH levels. Basic pH levels and higher moisture levels are reported to increase the emission of ammonia (Moore *et al.* 1995).

RT had higher initial moisture content (12.6%) than PH (10.3%). An ideal litter material should not have too high a moisture level because it would increase the risk of pathogenic microbial growth (Baurhoo *et al.* 2007). Increased dustiness, due to too low litter moisture levels makes poultry more susceptible to respiratory diseases. In general irrespective of the litter material used, litter moisture levels were high, compared to the recommended litter moisture level of 30%. Even by day 34, the lowest recorded litter moisture level was above 40%. In experiment 2, significantly higher litter moisture (p<0.05) level was recorded in RT litter than in PH litter, on day 34. Litter moisture levels increased by day 40 but were not significantly affected (p>0.05) by the type of litter, in experiments 2&3. However, in both experiments, RT litter recorded higher moisture levels than PH.

Almeida *et al.* (2010) and Brake *et al.* (1992) noted that an ideal litter material should have both moisture absorption and releasing capacity. Moisture releasing capacity may be related to physiochemical properties and the air movements among the litter materials.

Bulk density and litter caking were also higher for RT litter than PH litter (experiments 1&2). This could be due mainly to the fibrous nature of RT. Higher bulk density can also be attributed to the formation of litter cakes. Higher moisture level, litter caking and bulk density seem to be interrelated properties. These aspects are identified as the disadvantageous properties of RT as litter material.

As observed in experiment 3, ammonia emission was tended to be (p=0.08) lower in RT-based litter than PH–based litter. The reductions of NH_3 emission when RT was used alone or in combination with PH at 1:1 ratio

Table 6: Effects of refused tea and paddy husk each alone or as a 1:1 mixture (v/v) as a litter material on broiler growth performance from day 21-42 and carcass parameters (Experiment 3).

	Treatments				
Parameter	**PH**	**RT**	**RT: PH (1:1)**	**SEM**	**P value**
Live weight (g/bird)					
Day 21	818.00	857.25	823.00	9.8	NS
Day 30	1298.00	1305.00	1276.75	8.6	NS
Day 42	2025.00	2001.25	2016.25	14.5	NS
Bird's cleanliness					
Day 41	2.75	2.50	3.50	0.49	NS
Water intake (ml/d/bird)					
Day 21-31	302.5	310.3	326.8	9.05	NS
Water: feed ratio					
Day 21-31	2.55	2.73	2.92	0.09	NS
Feed intake (g/bird)					
Day 22-42	130.8	126.8	129	2.50	NS
FCR 22-42	2.06	2.12	2.05	0.08	NS
Weight gain					
Day 22-42	1206	1144	1194	38.32	NS
Mortality (%)					
Day 22-42	5.1	7.1	1.0	1.3	NS
Dressing (%) (with giblet, de-skinned)					
Day 42	71.75	68.24	70.62	1.07	NS

Note: SEM, mean standard error; PH, paddy husk; RT, refuse tea; NS, not significant

(v/v), compared to PH litter were 53.6 and 34%, respectively. Lower ammonia emission from RT litter compared to PH has been reported earlier as well as by Atapattu *et al.* (2007). It has been well established that ammonia emission is increased under high litter pH and moisture conditions (Moore *et al.*1996). Management of litter moisture at the optimum level of 30% is particularly difficult when manual drinkers are used. Therefore, lower ammonia emission reported in this experiment is particularly important.

In experiments 2&3 litter N contents were significantly increased ($p<0.05$) when RT was the litter material. Wijesekara (2004) reported that N contains RT could be as high as 1.7%.

Table 7: Litter characters of broiler chicks reared on PH, RT, or mixture in Experiment 3

	Treatments				
Parameter	**PH**	**RT**	**RT: PH (1:1)**	**SEM**	**P value**
Litter moisture (%): Day 39	58.50	66.90	61.95	2.9	NS
Litter pH: Day39	9.72	9.525	9.575	0.06	NS
Litter N (%): Day 39	3.05[b]	3.92[a]	3.6[a]	0.10	***
Litter ammonia emission (g/hr/AU): Day 39	7.86	3.65	5.18	1.19	NS
Litter caking (%)					
Day 28	28.75	36.75	36.25	1.71	NS
Day 41	60.00[b]	70.00[a]	71.25[a]	2.17	***

PH - Paddy husk (100%), RT - Refused tea (100%) RT: PH (1: 1) SEM - stranded error mean, *** $P<0.001$

Table 8: Effect of five levels of refused tea mixture as a litter material on litter characteristics

Parameter	Treatments						
	0%RT	25%RT	50%RT	75%RT	100%RT	SEM	P value
Litter pH							
Initial	6.89[a]	6.77[b]	6.76[c]	6.72[d]	6.68[e]	0.01	***
Day 34	7.92	8.0	7.8	8.3	8.0	0.06	NS
Day 41	8.59	8.69	8.62	8.55	8.64	0.02	NS
Litter moisture %							
Initial[1]	12.6				10.3		
Day 34	47.0[b]	43.4[b]	57.8[a]	55.0[a]	57.8[a]	1.93	***
Day 41	50.3	54.5	59.3	60.6	59.3	1.40	NS
Bulk density (g/cm^3)							
Initial	0.108	0.105	0.089	0.076	0.063	0.00	NS
Day 41	0.16[c]	0.17[cb]	0.19[b]	0.20[b]	0.24[a]	0.00	***
Litter N							
Day 34	3.2[d]	3.0[d]	5.6[c]	6.5b[d]	7.6[a]	0.38	***
Day 41	3.6d[d]	3.9d[d]	5.6[c]	6.6[b]	7.8[a]	0.35	***
Litter caking %							
Day28	5[c]	16[bc]	27[ba]	36[a]	38[a]	0.03	***
Day 35	36[b]	42[ba]	56[a]	54[a]	59[a]	0.02	***
Day 41	51[b]	61[ba]	77[a]	72[a]	73[a]	0.03	**

Note: NS; not significant, *** P<0.001, [1]Moisture contents of 25, 50 and 75% RT treatments were not determined.

RT used in this experiment contained significantly higher initial N content (0.69%) than PH (0.39%). Higher litter N contents observed in RT litter can be due to a cumulative effect of higher initial N content of the RT and lower ammonia emission from RT litter. PH contains as high as 20% of silica and trace amounts of N, Phosphorus (P) and potassium (K) and thus is of low fertilizer value (Amanullah *et al*. 2010). Whereas, RT contains 1.6 % K and 0.3 % (Mangenya *et al*. 2014) and a trace amount of silica. Therefore, the presence of P and K in RT and higher N contents makes RT litter a better organic fertilizer than PH-based litter.

Nitrogen present in a litter is converted into ammonia by the enzyme urease produced by litter microbes and those present in the environment. Once formed, NH_3 is volatilized into air maintaining an equilibrium status between litter and the surrounding air. Lower ammonia emission from RT litter could be due to lower ammonia formation and/or volatilization from litter to the environment. Atapattu *et al*. (2007) suggested that tannins present in RT may inhibit the action of urease that converts excretory N into ammonia, Generally, it is accepted that ammonia emission is increased with increasing litter moisture level. However, recently, Miles *et al.* (2014) showed that there is a critical moisture level above which NH_3 formation is no longer increased but is reduced. Depending on the temperature, the critical moisture level reported varying between 37.4 and 51.1%. Therefore, it may be a possibility that higher moisture levels reported in RT litter fall within the above critical levels thus having a negative effect on ammonia emission. Meanwhile, higher litter cake formation in RT litter may also have reduced the volatilization of formed ammonia from litter thereby reducing further NH_3 formation.

Effects of the type of litter on behaviour

Effects of the type of litter on-time budget and frequency of the occurrence of different behaviours are given in (Table 9). Laying was the most obvious behaviour, both on PH (72% of the time budget) and RT (75% of the time budget). Also, laying was the most frequently

Table 9: Behaviour of broilers as affected by the type of litter materials

	% of the total time			Frequency(per hour)		
	Type of litter			Type of litter		
Parameters	**PH**	**RT**	**P-value**	**PH**	**RT**	**P-value**
Eating	5.3±0.8	5.9±0.5	NS	2.3±0.3	3.2±0.2	NS
Waking	2.8±0.4	2.9±0.3	NS	3.3±0.3	3.3±0.16	NS
Standing	9.0±0.8	7.1±0.5	*	6.2±0.3	5.5±0.3	NS
Laying	72.3±1	74.9±1	NS	8.0±0.9	8.4±0.6	NS
Foraging	3.4±0.5	1.8±0.4	**	2.4±0.4	1.9±0.3	NS
Feather picking	3.6±0.7	2.8±0.5	NS	3.7±0.5	3.3±0.3	NS
Bird's interaction	0.7±0.4	2.0±0.3	**	1.0±0.4	2.1±0.02	**
Drinking	2.6±0.4	2.2±0.3	NS	2.3±0.3	2.1±0.3	NS

Note: NS – Not significant. ** P<0.05

engaged activity on both PH (8 times/hr) and RT (8.4 times /hour). Shield *et al.* (2005), also reported that broilers spent more time on lying than on other behaviours. Time spent on foraging was significantly higher on PH (3.4%) than on RT (1.8%). Meanwhile, the time budget and the frequency of inter-bird interactions were significantly higher for the birds raised on RT, compared to those kept on PH. There are conflicting reports on the effects of litter materials on the behaviour of broiler chicken. Jong *et al*. (2016). Shields *et al*. (2005) found that the behaviour of the broilers kept on sand and wood-shaving litters were not significantly different. Meanwhile, Toghyani *et al*. (2010) reported behaviours are affected due to the litter materials used. In this experiment, some behaviours such as foraging and bird interactions were influenced by the type of litter materials. The frequency of inter bird interactions was also significantly higher ($p<0.05$) for the birds raised on RT, compared to those kept on PH. The more compact nature of the RT-based litter, compared to PH (Atapattu and Wickramasinghe, 2007) may be the reason for the reduced foraging activity of the birds kept on RT. It would be interesting to study whether the reduction of foraging behaviour resulted in an increase in the time spent on and the frequency of inter-bird interactions.

CONCLUSION

Broilers raised on refused tea from day 5-41 reported similar growth performance parameters, compared to those on conventionally used litter material; paddy husk. Furthermore, refused tea litter emitted a lesser amount of NH_3 resulting in higher N content in the litter. Birds on refused tea litter, compared to those on paddy husk spent more time interacting with each other while reducing time on foraging behaviour and standing. Higher incidence of litter caking and moisture level was identified as the disadvantages characters of refused tea-based litter. Based on growth performance, litter properties and birds behaviour, the study recommends refused tea as an alternative litter material to paddy husk.

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Author Contribution

BUD conducted the experiment and prepared the draft manuscript. NSBM conceptualized and designed the study and reviewed the manuscript. SDW and RTS assisted the design of experiment and the preparation of the manuscript.

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