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Research Article

Ensiling Caged Layer Droppings with Grass and Rice Straw: Nutritional and Preservation Effects

MST. Taslima Easmin[1], Syed Walik Akib[1], Md. Nadiruzzaman Limon[1], Md Al Amin[2], S.M. Ariful Islam[1] A.K.M. Ahsan Kabir[1] and Md. Rokibul Islam Khan[1]✉

[1]Department of Animal Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

[2]Department of Science and Technology, Graduate School of Medicine, Science and Technology, Shinshu University, Kami-ina, Nagano 399-4598, Japan

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Correspondence
Md. Rokibul Islam Khan
✉: rikhanbau@yahoo.com

ABSTRACT

The research was conducted to evaluate the potential of ensiling caged layer droppings (CLD) with roadside green grass (RGG) and rice straw (RS) (*Oryza sativa L.*) in different amounts to assess the nutritional value of ensilage as well as preservation quality. Four different treatments T_0 (0% CLD+ 45% RGG + 50% RS +5% molasses), T_1 (10% CLD+ 35% RGG + 50% RS +5% molasses), T_2 (20% CLD+ 25% RGG + 50% RS +5% molasses) and T_3 (30% CLD+ 15% RGG + 50% RS +5% molasses) were prepared to evaluate the organoleptic quality (color, smell, texture, fungal growth), pH, nutritional composition (dry matter, crude protein, crude fiber, ether-extract, ash), *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) at 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th} days of ensiling. All parameters of different treatments changed significantly ($p<0.05$) under different ensiling periods. Organoleptic parameters (color, smell, texture, fungal growth) were satisfactory regarding increased CLD and ensiling period. pH was significantly ($p<0.05$) dropped under 5 after 28 days of ensiling. Among all treatments, T_3 (30%) had significantly ($p<0.05$) the highest crude protein (13.10%) but significantly ($p<0.05$) the lowest DM (57.47%), CF (16.33%), EE (1.91%) respectively. After 28 days of ensiling only CP significantly ($p<0.05$) increased from 11.58% to 13.11% but DM, CF, EE and Ash decreased significantly ($p<0.05$). *In vitro* organic matter digestibility and Metabolizable Energy content significantly ($p<0.05$) increased in T_3 after 28 days of ensiling. Considering all the physical and chemical parameters, T_3 will be the potential source of cattle feed. However, feeding trials are needed to justify the present findings.

Introduction

Feed insufficiency is a serious concern for the livestock industry stakeholders in Bangladesh. Ruminants mainly live on naturally grown grasses, agricultural residues, and cereal byproducts with low nutrient content and nutritional value (Maity *et al.,* 2020). Most animals remain malnourished as their primary feed sources contain a minimal amount of essential nutrients (Huque & Sarker, 2014). According to Haque *et al.* (2007), available roughage and concentrate can only cover 50% and 10% of the total requirements. Moreover, the feed cost of ruminants is too high (Habib *et al.,* 2024). In this aspect, nutritionists have recently become very interested in agro-industrial wastes such as poultry droppings, cow dung, slaughterhouse by-products, biogas slurry, and so on due to their economic and nutritional potentialities in animal feeding (Razak *et al.,* 2024). Using agro-industrial wastes as ruminant feed can reduce feed costs and minimize environmental pollution (Alimi *et al.,* 2024).

Rice straw (RS) is commonly available as Bangladesh is the 3rd largest rice producer in the world (World Population Review, 2022). RS is used as the main animal feed in Bangladesh. It is low in metabolizable energy (ME) and crude protein (CP) but high in silica and lignin content, which contributes to low digestibility (Aquino *et al.,* 2020; Sarker *et al.,* 2018). Rice straw's digestibility and nutritional content can be improved through different types of treatments such as urea-molasses, ammonia, or microbial treatments (Davy *et al.,* 2024; Kwaido *et al.,* 2024). Another way to improve the protein content of RS is to treat it with poultry droppings (Sharmin *et al.,* 2020).

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Roadside green grass (RGG), such as Durba and Kawn are easily available in Bangladesh and contains approximately 10-15% protein (Silalahi *et al.,* 2023). RGG contains approximately 55-78% moisture, 7.27% to 11.13% CP, and 25-31% Crude fiber (CF), which is essential for the growth and lactation of ruminants (Kumbhar *et al.,* 2003). Moreover, it improves the nitrogen deficiency for rumen microbes and increases ruminants' feed intake (Tiwari *et al.,* 1990).

Approximately 150,000 poultry farms exist in Bangladesh (Alam *et al.,* 2019). The annual production of poultry droppings in Bangladesh is 13.46 MT (Alam *et al.,* 2019). Due to a lack of suitable disposal systems, poultry droppings create environmental and health hazards. Poultry droppings break down immediately after excretion and produce ammonia (Matsumura *et al.,* 2021). Excessive amounts of ammonia can harm the health and productivity of chickens, farm workers, and the environment. But it can be a good source of protein for ruminant feed after preparation of ensilage (Islam *et al.,* 2023). The chemical composition of poultry droppings includes a moisture content of 9.62%, dry matter of 90.38%, ash of 28.83%, crude protein of 21.34%, fat content of 2.61%, crude fiber of 16.09%, and carbohydrates of 21.53% (Usman *et al.,* 2019).

The ensiling process of poultry droppings reduces the pathogens and also enhances the nutritional quality (Ibeawuchi and Echumba, 2021). Ensilage prepared with poultry droppings, rice straw, and green grass can be an alternative nutrient-based ruminant feed source. It can also be a cost-effective feed in Bangladesh. Research has been done on the preparation of ruminant feed (Sultana *et al.* 2020) and *in-vivo* experiments utilizing poultry droppings (Elemam *et al.,* 2009; Khan *et al.,* 2016). The current experiment was conducted considering the above evidence to improve the quality of rice straw ensiling with poultry droppings.

Materials and Methods

Experimental site and materials

The experiment was conducted in two phases: ensilage preparation and laboratory analysis. Ensilage preparation and associated operations were carried out at Goat, Sheep and Horse Farm, Department of Animal Science, Bangladesh Agricultural University (BAU), Mymensingh. Laboratory analysis was conducted at the Animal Science Lab and Animal Nutrition Lab at BAU. Ensilage preparation and laboratory analysis were conducted from 1st December to 29th May 2023.

Caged layer droppings were collected from the Poultry Farm at BAU. Polythene sheets were placed under the cages of layer birds and proper care was taken so that the layer droppings would not be mixed with sand, feathers and other debris materials. Roadside grass and rice straw were collected from the Goat, Sheep and Horse farm at BAU. Molasses, polythene sheets and airtight plastic containers (8 L) were purchased from nearby local markets.

Preparation of ensilage

Ensilage preparation and related operations were conducted on the Goat, Sheep and Horse Farm at BAU. At first, RS and RGG were chopped by 3-4 cm in length. After that, ensilage was prepared by mixing previously chopped RS and RGG with freshly collected Caged layer droppings (CLD) and molasses according to this treatment formula:

T_0 = 0% CLD + 45% RGG + 5% Molasses + 50% RS
T_1 = 10% CLD + 35% RGG + 5% Molasses + 50% RS
T_2 = 20% CLD + 25% RGG + 5% Molasses + 50% RS
T_3 = 30% CLD + 15% RGG + 5% Molasses + 50% RS

To ensure proper mixing, CLD and molasses were added first, then RS and RGG. RS, RGG, CLD, and molasses were mixed and stored in airtight plastic containers marked with the treatments. Finally, plastic containers were kept at room temperature (24-28 °C) for 28 days to ensure effective ensiling.

Physio organoleptic test and chemical analysis

Samples of ensilage were collected on the 0th, 7th, 14th, 21st and 28th days. Texture, color, smell and fungal growth were observed on each sampling day. Organoleptic tests with non-parametric analysis were conducted using a scoring procedure that involved observing and evaluating color, texture, and smell. In the experiment, there were four panelists to evaluate the parameters.

(a) Color assessment

Five different colors were observed during assessment: straw, light brown, brown, dark brown, and chocolate

(b) Smell assessment

Five different types of smells were found during the assessment-

Straw- Dry, woody, earthy type of smell

Pleasant smell- Sweet, slightly sour or vinegar-like smell.

Moderate good- Sour but with a slight ammonia or earthy note type smell

Acceptable smell- Mildly sour or neutral, with no offensive smell

Good- A balance of mild acidic, slightly sweet, and faint straw or grass type smell

(c) Texture assessment

Hard- Dry, coarse, and difficult to compress by hands

Moderate hard- Slightly moist, firm but compressible by hands.

Soft- wet, soggy and mushy

Two samples were taken from each treatment. One sample was dried in an oven at 55°C for 72 hours for chemical analysis and the other one was for dry matter (DM) content calculation. A digital pH meter was used to determine the pH of the ensilage by adding 5g of sample to 50 ml of distilled water. Proximate analysis for dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and ash were carried out following the methods of AOAC (AOAC, 2004). *In vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) content of ensilage were determined following the method and formula described by Menke and Steingass (1988)-

IVOMD = 16.49+0.9024 GP+0.0492CP+0.0387TA
ME = 2.20+0.1357GP+0.0057CP+0.0002859EE2

IVOMD = *In vitro* organic matter digestibility (%); ME = Metabolizable energy (MJ/kg DM); GP = Gas production expressed in ml per 200 mg DM; CP = Crude protein (g/kg DM); TA = Total ash (g/kg DM); EE = Ether extract (g/Kg DM)

Statistical Analysis

The experiment was conducted using a 4x5 factorial design, with three replicates for each treatment. SAS Statistical Discovery Software, NC, USA, was used to analyze the data statistically, and Duncan's Multiple Range Test (DMRT) was used to identify the differences between the treatment means.

Results and Discussion

Organoleptic Parameters

The physical properties of ensilage of different treatments (T_0, T_1, T_2, and T_3) at the different ensiling times (0th, 7th, 14th, 21st and 28th days) were shown in Table 1. After the 28th day of ensiling, the colors of T_3 turned into a chocolate color from light brown, whereas T_0 was brown. The change of color was due to the fermentation process that helped microbes (lactic acid bacteria) to produce organic acid (lactic acid) and also promoted the breakdown of chlorophyll of green grass to produce a dark color (Panna *et al.,* 2019; Sharmin *et al.,* 2020). Among other treatments, T_3 had a pleasant smell at 28 days of ensiling which was acceptable by cattle. Controlled T_0 remained hard in texture but T_1, T_2, and T_3 were soft. After 28 days of ensiling T_2, and T_3 were very soft in texture. Fungal growth was observed only in T_0 after 28 days of ensiling but other treatments were free from fungal growth. These findings were supported by Panna *et al.* (2019) and Khatun *et al.* (2015).

Table 1. Organoleptic Parameters of different treatments of ensilage

Characteristics	Observation (Day)	Treatment			
		T_0	T_1	T_2	T_3
Color	0th	Straw color	Straw color	Light brown	Brown
	7th	Light brown	Light brown	Brown	Brown
	14th	Light brown	Brown	Brown	Dark brown
	21st	Brown	Brown	Dark brown	Dark brown
	28th	Brown	Brown	Chocolate	Chocolate
Smell	0th	Straw	Straw	Bad	Bad
	7th	Straw	Straw	Good	Bad
	14th	Straw	Moderate	Good	Good
	21st	Straw	Moderate	Acceptable	Pleasant
	28th	Straw	Moderate	Good odor	Pleasant
Texture	0th	Hard	Hard	Hard	Hard
	7th	Hard	Hard	Hard	Hard
	14th	Hard	Moderate soft	Moderate soft	Soft
	21st	Hard	Soft	Soft	Very soft
	28th	Hard	Soft	Very soft	Very soft
Fungal Growth	0th	Absent	Absent	Absent	Absent
	7th	Absent	Absent	Absent	Absent
	14th	Absent	Absent	Absent	Absent
	21st	Absent	Absent	Absent	Absent
	28th	Present	Absent	Absent	Absent

pH

Figure 1 showed that the pH of different treatments in different ensiling periods changed with increasing time. The pH value decreased in all treatments as the CLD level increased. T_3 (30% CLD) had the lowest pH (3.8) followed by T_2, T_1 and T_0. After 28 days of ensiling, the pH values of all treatments decreased and were below 5, indicating good fermentation.

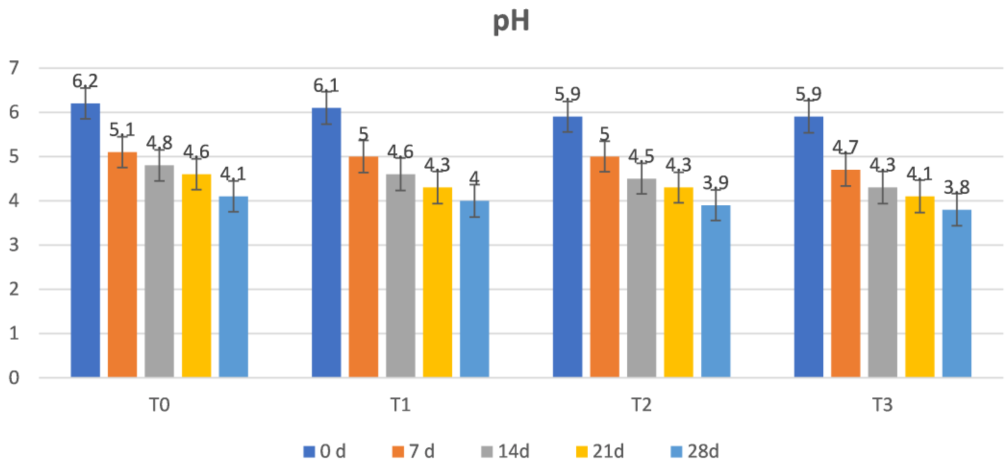

Figure 1 pH of different treatments.

T_0 = 0% CLD + 45%RSG + 5% molasses + 50% RS, T_1 = 10% CLD + 35% RSG + 5% molasses + 50% RS, T_2 = 20% CLD + 25% RSG + 5% molasses + 50% RS, T_3 = 30% CLD + 15% RSG + 5% molasses + 50% RS

The acidic pH of the ensilage was mainly caused by the growth and activities of microbial communities and biodiversity during the ensiling period (Islam *et al.,* 2023). Lactic acid bacteria (LAB) produced lactic acid during the ensiling time and were mainly responsible for the decline in pH (Carvalho *et al.,* 2021). The addition of molasses to ensilage contributed to the production of lactic acid and was further responsible for the reduction of the pH. Molasses also improved the overall ensilage quality (Zhao *et al.,* 2019). Mu et al. reported that silage treated with molasses exhibited lower pH and higher concentrations of lactic acid (Mu *et al.,* 2021). Yuan et al. also observed the same result (Yuan *et al.,* 2016). Mson & Sangodoyin showed that poultry droppings combined with forages such as rice straw and green grass enhanced nutrient content and created an acidic environment (Mson & Sangodoyin, 1995).

Chemical composition of ensilage

Dry matter

The Dry matter (DM) content of different treatments decreased significantly ($P < 0.05$) with the increased amount of CLD and ensiling period (Table 2). T_0 (0% CLD) had the highest DM content, followed by T_1 (10% CLD), T_2 (20% CLD) and T_3 (30% CLD). DM content decreased by 2.75% with the increase in CLD. After 28 days of ensiling, DM content decreased by 6.23%. (Table 3).

Table 2. Effect of treatments on various parameters of ensilage

Treatment	Dry Matter (%)	Crude Protein (%)	Crude Fiber (%)	Ether Extract (%)	Ash (%)	IVOMD (%)
T_0	60.22^{d} ±0.18	11.00^{a} ±0.02	21.12^{d} ±0.05	2.39^{d} ±0.13	13.53^{d} ±0.23	53.16^{a} ±0.06
T_1	58.91^{c} ±0.16	11.10^{b} ±0.06	19.99^{c} ±0.08	2.26^{c} ±0.05	12.84^{c} ±0.13	54.25^{b} ±0.06
T_2	58.10^{b} ±0.07	12.23^{c} ±0.05	17.79^{b} ±0.04	2.15^{b} ±0.02	11.92^{b} ±0.12	55.15^{c} ±0.11
T_3	57.47^{a} ±0.06	14.50^{d} ±0.03	16.33^{a} ±0.10	1.91^{a} ±0.03	11.24^{a} ±0.09	55.57^{d} ±0.12
Level of Significance						
T	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
D	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
T*D	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

**Means with different superscripts within a column are significantly different (P<0.05)*

T_0 = 0% CLD + 45% RSG + 5% molasses + 50% RS, T_1 = 10% CLD + 35% RSG + 5% molasses + 50% RS, T_2 = 20% CLD + 25% RSG + 5% molasses + 50% RS, T_3 = 30% CLD + 15% RSG + 5% molasses + 50% RS; $^{a, b, c, d}$ values in the same column with different superscripts differ significantly (p< 0.05).

Table 3. Effect of treatments and ensiling time on dry matter of ensilage

Ensiling (Days)	Treatment					Level of significance		
	T_0	T_1	T_2	T_3	Mean	T	D	T*D
0	63.75± 0.33	63.02±0.17	61.48±0.23	59.85±0.10	62.02^{e}±0.21	<.0001	<.0001	<.0001
7	61.25±0.38	61.13±0.25	59.27±0.10	58.52±0.04	60.04^{d}±0.19			
14	59.79±0.12	59.47±0.11	57.84±0.02	56.94±0.12	58.51^{c}±0.09			
21	58.43±0.07	55.89±0.22	56.30±0.01	57.42±0.02	57.20^{b}±0.08			
28	57.87±0.01	55.03±0.06	55.65±0.02	54.64±0.01	55.79^{a}±0.03			
Mean	60.22^{d}±0.18	58.91^{c}±0.16	58.10^{b}±0.07	57.47^{a}±0.06				

*Means with different superscripts within row and column are significantly different (P<0.05)
T_0 = 0% CLD + 45%RSG + 5% molasses + 50% RS, T_1 = 10% CLD + 35% RSG + 5% molasses + 50% RS, T_2 = 20% CLD + 25% RSG + 5% molasses + 50% RS, T_3 = 30% CLD + 15% RSG + 5% molasses + 50% RS; $^{a, b, c, d}$ values in the same row with different superscripts differ significantly (p< 0.05).

The ensiling process promoted the growth and activity of lactic acid bacteria (LAB), which broke down sugar and produced organic acids. These microbial activities led to the reduction of DM content as volatile fatty acids (VFAs) were produced and some organic matter was lost in the form of gases (Feng *et al.,* 2020). Moreover, the high moisture content of poultry droppings (Higgins *et al.,* 2021) might dilute the total DM content when combined with drier materials such as rice straw and grass (Islam *et al.,* 2018). The decreasing trend of DM content of ensilage prepared from caged layer droppings was supported by Jamee et al. (Jamee *et al.,* 2020) and Islam et al. (Islam *et al.,* 2018). Khatun et al. reported an increasing trend of DM content where the ensilage was prepared from oat forage (*Avena sativa*) along with poultry droppings (Khatun *et al.,* 2015).

Crude Protein

Table 2 showed that the Crude protein (CP) content of different treatments changed significantly (p<0.05). T_3 (30% CLD) had the highest and T_0 (0% CLD) had the lowest level of CP. The addition of CLD from 0% (T_0) to 30% (T_3) resulted in a significant (p<0.05) improvement of CP content from 11% to 14.50%. 28 days ensiling period also increased CP significantly (p<0.05) from 11.58% to 13.10% (Table 4).

Table 4. Effect of treatments and ensiling time on the Crude Protein of ensilage

Ensiling (Days)	Treatment					Level of significance		
	T_0	T_1	T_2	T_3	Mean	T	D	T*D
0	10.00±0.02	10.20±0.03	12.15±0.06	14.00±0.02	11.58^{a} ±0.02	<.0001	<.0001	<.0001
7	10.12±0.01	10.57±0.04	12.24±0.02	14.10±0.03	11.75^{b}±0.05			
14	10.89±0.02	11.04±0.15	12.28±0.03	14.38±0.02	12.14^{c}±0.06			
21	11.15±0.00	11.76±0.02	12.34±0.11	14.87±0.09	12.53^{d}±0.04			
28	11.57±0.03	12.00±0.06	12.30±0.01	15.00±0.01	13.10^{e}±0.05			
Mean	11.00^{a}±0.02	11.10^{b}±0.06	12.23^{c}±0.05	14.50^{d}±0.03				

**Means with different superscripts within row and column are significantly different (P<0.05)*
T_0 = 0% CLD + 45%RSG + 5% molasses + 50% RS, T_1 = 10% CLD + 35% RSG + 5% molasses + 50% RS, T_2 = 20% CLD + 25% RSG + 5% molasses + 50% RS, T_3 = 30% CLD + 15% RSG + 5% molasses + 50% RS; $^{a, b,c, d}$ values in the same row with different superscripts differ significantly (p< 0.05).

Poultry droppings contain high amounts of Nitrogen, which when treated with carbon-rich material such as rice straw, promotes microbial activity that enhances protein synthesis during fermentation (Ngele *et al.,* 2009). The ensiling process also contributed to the breakdown of fibrous materials which also increased the CP content. Chun et al. (Ng *et al.,* 2020); Haque et al. (Haque *et al.,* 2022) and Khatun et al. (Khatun *et al.,* 2015) reported the same increasing trend of CP content while ensilage is prepared from CLD.

Crude Fiber

The Crude fiber (CF) content of different treatments changed significantly (P < 0.05) with the increased amount of CLD and ensiling period (Table 2). T_0 (0% CLD) had the highest CF content, followed by T_1 (10% CLD), T_2 (20% CLD) and T_3 (30% CLD). CF content decreased by 4.79% with the increase in CLD from 0% to 30%. After 28 days of ensiling, CF content decreased by 3.84% (Table 5).

Table 5. Effect of treatments and ensiling time on crude fiber of ensilage

Ensiling (Days)	Treatment					Level of significance		
	T_0	T_1	T_2	T_3	Mean	T	D	T*D
0	23.48 ±0.02	21.98±0.06	19.63±0.12	18.06±0.33	20.78^{e} ±0.13	<.0001	<.0001	<.0001
7	22.72 ±0.01	20.10±0.11	18.98±0.03	17.26±0.04	19.76^{d} ±0.05			
14	20.51±0.02	19.88±0.12	18.13±0.03	16.94±0.06	18.82^{c} ±0.06			
21	19.79±0.07	19.10±0.09	16.67±0.14	15.22±0.06	17.69^{b} ±0.09			
28	19.10±0.11	18.91±0.02	15.58±0.08	14.17±0.03	16.94^{a} ±0.06			
Mean	21.12^{d}±0.05	19.99^{c}±0.08	17.79^{b}±0.04	16.33^{a}±0.10				

**Means with different superscripts within row and column are significantly different (P<0.05).*
T_0 = 0% CLD + 45%RSG + 5% molasses + 50% RS, T_1 = 10% CLD + 35% RSG + 5% molasses + 50% RS, T_2 = 20% CLD + 25% RSG + 5% molasses + 50% RS, T_3 = 30% CLD + 15% RSG + 5% molasses + 50% RS; $^{a, b,c, d}$ values in the same row with different superscripts differ significantly (p< 0.05).

The reduction of CF content may be due to the addition of CLD and molasses, which promoted microbial growth and broke down fibrous components (Islam *et al.,* 2018; Panna *et al.,* 2019). Li et al. reported that the activities of microbes and molasses promoted the degradation of fiber such as cellulose and hemicellulose and improved the fermentation process and nutritional quality of ensilage (Li *et al.,* 2022). Khatun *et al.* (2013) and Islam *et al.* (2023) also reported a similar trend in the reduction of CF content.

Ether Extract

Table 2 showed that the Ether Extract (EE) content of different treatments in different ensiling times changed significantly (p<0.05). T_3 (30% CLD) had the lowest and T_0 (0% CLD) had the highest level of EE. The addition of CLD from 0% (T0) to 30% (T3) resulted in a significant (p<0.05) decline of EE content from 2.39% to 1.91%. After 28 days of ensiling period, EE also decreased significantly (p<0.05) from 2.44% to 1.94% (Table 6).

Table 6. Effect of treatments and ensiling time on ether extract of ensilage

Ensiling (Days)	Treatment					Level of significance		
	T_0	T_1	T_2	T_3	Mean	T	D	T*D
0	2.61±0.12	2.54±0.02	2.40±0.00	2.21±0.01	2.44^{e}±0.04	<.0001	<.0001	<.0001
7	2.54±0.18	2.49±0.00	2.27±0.02	2.04±0.03	2.34^{d}±0.06			
14	2.4±0.26	2.32±0.12	2.15±0.02	1.91±0.02	2.20^{c}±0.11			
21	2.23±0.00	2.17±0.01	2.01±0.03	1.83±0.01	2.06^{b}±0.01			
28	2.19±0.11	2.02±0.08	1.89±0.04	1.65±0.09	1.94^{a}±0.08			
Mean	2.39^{d}±0.13	2.26^{c}±0.05	2.15^{b}±0.02	1.91^{a}±0.03				

**Means with different superscripts within row and column are significantly different (P<0.05).*
T_0 = 0% CLD + 45%RSG + 5% molasses + 50% RS, T_1 = 10% CLD + 35% RSG + 5% molasses + 50% RS, T_2 = 20% CLD + 25% RSG + 5% molasses + 50% RS, T_3 = 30% CLD + 15% RSG + 5% molasses + 50% RS; $^{a, b,c, d}$ values in the same row with different superscripts differ significantly (p< 0.05).

Islam et al. and Panna et al. reported that the decline in EE content may be due to the ensiling process which enhanced microbial activities and led to the breakdown of lipids (Islam *et al.,* 2018); (Panna *et al.,* 2019). During the ensiling process, pH decreased which also resulted in a negative trend in lignin solubility contributing to lower EE content of ensilage (Khatun *et al.,* 2015). Moreover, the addition of high-fibrous materials like rice straw and green grass was responsible for the decline in EE as these components contained lower lipid content (Yuan *et al.,* 2016).

Ash

The Ash content of different treatments changed significantly (P < 0.05) with the increased amount of CLD and ensiling period (Table 2). The addition of CLD from 0% (T_0) to 30% (T_3) resulted in a significant (p<0.05) decrease in Ash content from 13.53% to 11.24%. After 28 days of ensiling, a falling trend was also observed in Ash from 13.07% to 11.77% (Table 7).

Table 7. Effect of treatments and ensiling time on the ash of ensilage

Ensiling (Days)	Treatment					Level of significance		
	T_0	T_1	T_2	T_3	Mean	T	D	T*D
0	14.11±0.05	13.57±0.14	12.78±0.12	11.85±0.03	13.07^{e}±0.08	<.0001	<.0001	<.0001
7	13.78±0.27	13.10±0.15	12.16±0.05	11.64±0.03	12.67^{d}±0.13			
14	13.59±0.27	12.67±0.24	11.96±0.18	11.17±0.02	12.34^{c}±0.18			
21	13.28±0.19	12.59±0.06	11.52±0.21	10.89±0.33	12.07^{b}±0.19			
28	12.91±0.12	12.31±0.07	11.21±0.04	10.65±0.02	11.77^{a}±0.06			
Mean	13.53^{d}±0.23	12.84^{c}±0.13	11.92^{b}±0.12	11.24^{a}±0.09				

**Means with different superscripts within row and column are significantly different (P<0.05).*

T_0 = 0% CLD + 45%RSG + 5% molasses + 50% RS, T_1 = 10% CLD + 35% RSG + 5% molasses + 50% RS, T_2 = 20% CLD + 25% RSG + 5% molasses + 50% RS, T_3 = 30% CLD + 15% RSG + 5% molasses + 50% RS; $^{a, b,c, d}$ values in the same row with different superscripts differ significantly (p< 0.05)

The ensiling process enhanced the growth and activities of microbes which promoted the breakdown of fibrous materials, resulting in a reduction of ash content as organic matter is converted into microbial biomass (Islam *et al.,* 2018). Al Rokayan *et al.* (1988) and Flachowsky and Hennig (1990) found that increasing the proportion of broiler droppings led to a linear increase in ash levels which contrasts with these findings.

In vitro Organic Matter Digestibility of (IVOMD) Ensilage

Table 2 showed that *In vitro* organic matter digestibility content increased significantly (p<0.05) based on the level of CLD and different ensiling periods. T_3 produced the highest *in vitro* OMD content (55.57%), whereas T_0 had the lowest (53.16%). The ensiling period also increased the IVOMD content from 54.26% to 55.13% (Table 8)

Table 8. Effect of treatments and ensiling time on organic matter digestibility (IVOMD) (%) of ensilage

Ensiling (Days)	Treatment					Level of significance		
	T_0	T_1	T_2	T_3	Mean	T	D	T*D
0	52.50±0.05	53.27±0.19	54.60±0.03	56.69±0.16	54.26^{b}±0.11	<.0001	<.0001	<.0001
7	52.86±0.03	53.57±0.06	54.76±0.03	54.90±0.27	54.02^{a}±0.09			
14	53.23±0.04	54.70±0.02	54.91±0.26	55.12±0.07	54.49^{c}±0.09			
21	53.53±0.05	54.82±0.01	55.40±0.14	55.39±0.03	54.78^{d}±0.05			
28	53.72±0.12	54.91±0.05	56.10±0.12	55.78±0.08	55.13^{e}±0.09			
Mean	53.16^{a}±0.06	54.25^{b}±0.06	55.15^{c}±0.11	55.57^{d}±0.12				

**Means with different superscripts within row and column are significantly different (P<0.05)*

T_0 = 0% CLD + 45%RSG + 5% molasses + 50% RS, T_1 = 10% CLD + 35% RSG + 5% molasses + 50% RS, T_2 = 20% CLD + 25% RSG + 5% molasses + 50% RS, T_3 = 30% CLD + 15% RSG + 5% molasses + 50% RS; $^{a, b,c, d}$ values in the same row with different superscripts differ significantly (p< 0.05)

The addition of CLD and molasses initiated fermentation, which decreased pH and promoted the growth of microbes. Microbes were responsible for the breakdown of nutrients which increased the organic matter digestibility (Panna *et al.*, 2019; Islam *et al.,* 2018). The increasing trend of *In vitro* organic matter digestibility content was supported by Islam *et al.* (2018); Sarker *et al.* (2018) and Khatun *et al.* (2015).

Metabolizable Energy content of ensilage

Figure 2 illustrated the ensilage's metabolizable energy. Significant differences (p<0.05) were observed in the metabolizable energy (ME) content (MJ/Kg DM) of ensilage between the treatments. The ensilage with 30% CLD (T_3) produced the highest ME 9.22 MJ/Kg and T_0 had the lowest ME 8.368 MJ/Kg after 28 days of ensiling. Sharmin *et al.* reported the same findings (Sharmin *et al.,*2020).

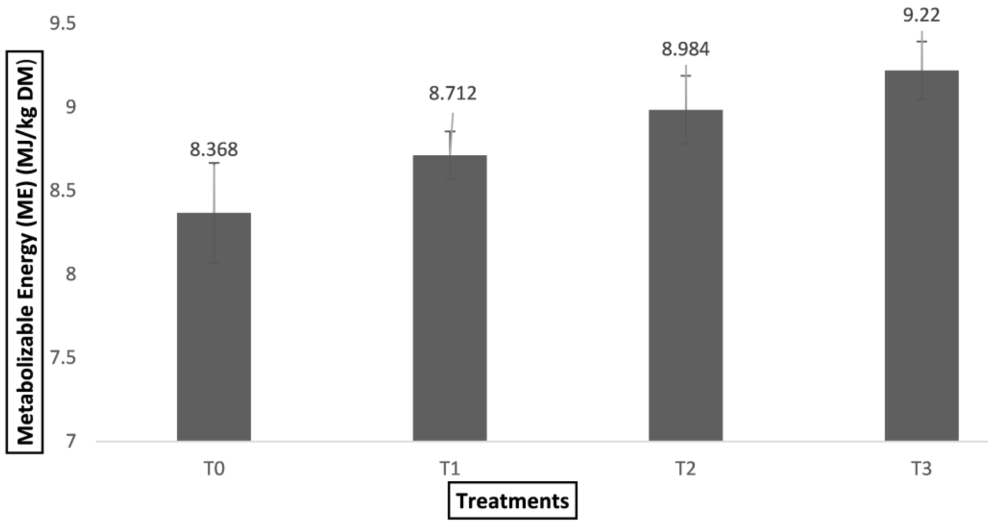

Figure 2 Metabolizable Energy (MJ/kg DM) of different treatments.

T_0 = 0% CLD + 45%RSG + 5% molasses + 50% RS, T_1 = 10% CLD + 35% RSG + 5% molasses + 50% RS, T_2 = 20% CLD + 25% RSG + 5% molasses + 50% RS, T_3 = 30% CLD + 15% RSG + 5% molasses + 50% RS;

Conclusion

Among all treatments, T_3 (30% CLD+ 15% RGG+ 5% molasses + 50% RS) produced a fungus-free, pleasant-smelling, soft-textured ensilage after 28 days of ensiling. Also, T_3 had the highest CP, ME, and *in vitro* organic matter digestibility (IVOMD) content. The findings demonstrated that the addition of caged layer droppings significantly improves the nutritional value of ensilage and could be an efficient option to preserve and convert CLD into a palatable and beneficial feed for cattle. It also improved the quality of RS and can be a nutrient-based alternative source of feed for ruminants. Therefore, considering all the physical and chemical parameters, T_3 can be regarded as the best experimental output. Heavy metal analyses and *In vivo* animal trials weren't done in this experiment, so further investigations are required to justify the present findings.

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