



Article A Machine Learning-Based Assessment of Maize Silage Dry Matter Losses by Net-Bags Buried in Farm Bunker Silos

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Abstract: Estimating the dry matter losses (DML) of whole-plant maize (WPM) silage is a priority for sustainable dairy and beef farming. The study aimed to assess this loss of nutrients by using net-bags (n = 36) filled with freshly chopped WPM forage and buried in bunker silos of 12 Italian dairy farms for an ensiling period of 275 days on average. The proximate composition of harvested WPM was submitted to mixed and polynomial regression models and a machine learning classification tree to estimate its ability to predict the WPM silage losses. Dry matter (DM), silage density, and porosity were also assessed. The WPM harvested at over 345 (g kg⁻¹) and a DM density of less than 180 (kg of DM m⁻³) was related to DML values of over 7%. According to the results of the classification tree algorithm, the WPM harvested (g kg⁻¹ DM) at aNDF higher than 373 and watersoluble carbohydrates lower than 104 preserves for the DML of maize silage. It is likely that the combination of these chemical variables determines the optimal maturity stage of WPM at harvest, allowing a biomass density and a fermentative pattern that limits the DML, especially during the ensiling period.

Keywords: maize silage; porosity; density; dry matter loss; bunker silo; machine learning; classification tree analysis

1. Introduction

Whole-plant maize (WPM) silage is the main own farm fodder contributing to the formulation of total mixed ration for high-genetic-merit lactating dairy cows in the intensive farming systems of many European countries [1–3]. It is a high-yielding and flexible harvesting vegetable crop with a high nutritional value also for ruminant meat producers [4]. The main purpose of ensiling is to extend over time the use of WPM harvested at the optimum phenological stage to maximize the nutritional profile and limit the presence of harmful compounds such as mycotoxins [5]. However, the choice of the optimum ensiling plant and grain maturity is also related to the dry matter (DM) content and proximate composition of the freshly harvested maize because they affect the rate and extent of the main fermentation end-compounds both during the fermentative phase and the stable storage phase in the silo [5,6]. Furthermore, the choice of the maturity stage at harvest affects the DM losses (DML) across the silage-making process since they seem to be associated with the pre-ensiled DM content [6,7] alone or in interaction with the use of microbiological additives [8,9]. Bulk silage density, when expressed on a wet basis [10], DM silage density, and the porosity are reported to affect DML as well [9]. Porosity is positively related to the DM density and negatively to the bulk density [10,11]. Compacting adequately during the silo filling increases the bulk density and reduces the DML [8], and it favors suitable microbiological quality [12]. However, knowledge of the effect of the proximate composition of harvested WPM biomass on the DML that occurs during its



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). ensiling process and the following preservation phase is still a challenge, especially under field conditions. So far, various experimental approaches are proposed to assess the silage losses at the farm bunker silo scale, such as total-in versus total out mass flow of the silo, ash, or other nondegradable recovering biomarkers; the use of buried temperature loggers to measure heat evolution appears instead to be more correlated to the onset of aerobic deterioration [6,13]. A further experimental approach to simulate and predict the ensiling losses under on-farm conditions is the buried bag technique, which is accomplished using nylon mesh bags filled with freshly chopped forages and then buried in silos in order to allow gas and fluid exchanges with the surrounding silage; the net-bags are removed from the pile prior to air exposure, allowing the evaluation of DML [1,7]. However, rapid and accurate characterization of silage DML still remains a gap while exploring the best combination of whole-plant chemical constituents, which allows an optimal tuning up of the ensiling process. Such a gap can be tackled by using some machine learning techniques to define the best aggregate predictor able to achieve satisfactory predictive performance, such as recursive partitioning and decision tree analysis [14].

The aim of this study was to investigate the effect of the pre-ensiled chemical traits on the DML of a set of maize silage samples embedded as mini-bags inside the bunker silos in a cohort of Italian dairy farms. It also investigated the relationship between density and porosity, the incidence of DML, and the fermentative quality pattern of the WPM silages.

2. Materials and Methods

2.1. Experimental Design and Sampling Collection

The experimental trial was carried out in the lowland of the middle Veneto region $(45^{\circ}19' \text{ lat. N}, 11^{\circ}56' \text{ long. E; Northeast Italy})$. In the spring of 2020, 12 farms were selected to be representative of the intensive dairy farming system of the middle Po Valley (north of Italy) that uses whole-plant maize (WPM) silage as the main fodder in the total mixed ration of lactating dairy cows [15,16]. The maize crop consisted of a wide range of medium (FAO class 400–500, n = 11) and late (FAO class 600–700, n = 25) hybrids, processed under diverse agronomic management according to soil fertility and irrigation availability. The WPM was harvested in August at a targeted DM concentration of 28–36%, corresponding to the medium-early ripening phase (from half to two-thirds milk line stage) using multiple rows of self-propelled forage harvesters, and chopped at a theoretical cut length (TCL) of 15 \pm 0.6 mm. The fresh ground WPM biomasses were ensiled in horizontal concrete bunker silos with an averaged storage capacity of 920 ± 315 m³, mainly according to the length extension (40 \pm 12 m). The silage procedures were almost similar among dairy farmers as regards the filling rate of the silo, tractor weight used to apply pressure to increase the bulk density of harvested biomass, and the presence of lateral and longitudinal (black-on-white) silage wrap plastic films. Over the top of the silo were placed gravel sacks having an average weight of 180 ± 30 kg m² increasing self-compaction; no silage additives were used in this trial. Per each farm, three samples (around 8.0 kg each) of fresh ground (pre-ensiled) WPM were randomly collected across the crop areas and packed into net-bags (polyethylene, 0.5×0.35 m; 2 mm of mesh). The net-bags were buried in the middle part of the bunker silos at 2.0 m above the floor; thus, they can be considered embedded into the fermentative biomass as representative samples of the ensiling process (Figure 1). After the ensiling period, all the net-bags were retrieved to be weighted and then opened to collect WPM silage samples used to assay their proximate composition and fermentative profiles.



Figure 1. A net-bag buried in a bunker silo (**left**); the electrical probe (**right**) to collect a determined volume of whole-plant maize (WPM) silage used to calculate dry matter density and porosity.

2.2. Proximate Composition, Fermentative Profile, Density, Porosity, and Dry Matter Losses

The DM and proximate composition of the fresh WPM forage samples used to fill the net-bags were assayed on-farm by the use of a portable near-infrared (NIR) instrument (PoliSPEC^{NIR}; ITPhotonics, Fara Vicentino, Italy). At the end of the ensiling period, DM and fermentative profile of WPM silage of each net-bag were analyzed using a FOSS NIRSystem 5000 scanning monochromator (FOSS NIRSystem, Silver Pring, MD, USA). All samples were analyzed in triplicates, and NIR spectral data were averaged to predict the chemical variables using the calibration equation of our previous studies both on the portable [17] and bench-top [5] apparatus. The pH of silage samples was determined by using a pH meter (827 pH lab; Metrohm, Herisau, Switzerland). According to the farming operative conditions of maize silage in bunker silos of the Italian dairy farms, the net-bags were retrieved after 228 ± 64 days on average (median, 242 days). Before net-bags recovering from farm silos, bulk density was evaluated at three sampling points surrounding each net-bag, forcing an electrical powered cylindrical probe (2.3 cm of diameter) within the WPM silage mass by means of a pronged blade (Figure 1). The length of the collected cylindrical sample was recorded to calculate the volume accurately, and then the silage DM density (kg of DM m^{-3}) was determined using the DM content of the WPM silage sample from the buried net-bag. Porosity (Φ) [18] and dry matter (DM) losses (DML) of WPM maize silage were calculated following these equations:

$$\Phi = 1 - \rho S \times \{ [(1 - DM) / \rho W] + [(DM \times OM) / \rho OM] + [(DM \times (1 - OM)) / \rho ASH] \},$$
(1)

$$DML = [1 - (kg of DM of post-ensiled WPM / kg of DM of pre-ensiled WPM)] \times 100,$$
 (2)

where ρ S is silage density on the wet basis (g cm⁻³); ρ W is water density (1 g cm⁻³); DM and OM are dry and organic matter; ρ OM is organic matter density (1.6 g cm⁻³); ρ ASH is ash density (2.5 g cm⁻³); WPM is whole-plant maize.

The DM content (g kg⁻¹) also accounted for the presence of volatile carbon compounds, which might be lost in oven dissection [13,19]; thus, a correction was applied as DM corrected = $22.2 + 0.96 \times DM$ uncorrected [13].

2.3. Statistical Analysis and Machine Learning Algorithm

All the statistical analyses were performed using R-software (v4.0.2; R Core Team 2022). Data of replicates were averaged prior to statistical analysis. The chemical and physical traits of unprocessed and ensiled WPM and DML were normally distributed as assessed by the Shapiro–Wilk test. The association between DML and density, porosity, and DM_fresh was assessed with the Pearson correlation coefficient (r). A stepwise mixed regression model fitted by the restricted maximum likelihood (REML) criterion was performed to evaluate the most predictive pre-ensiled chemical variables to estimate the DML across the

ensiling period using the farm as a random effect in the nlme package. As the coefficient of determination of the model, a pseudo-R² was calculated adopting the MuMln package. Regression coefficients (b \pm standard error) were estimated. A polynomial regression model was also used to interpolate a 3D surface plot on DML, density, and DM_fresh data. According to the experimental outcomes of Köhler et al. 2019 [1], the DML values were split into three quantitative classes; considering that the average and standard deviation (s.d.) were $5 \pm 4\%$, the middle class (M-class) was centered on the mean, and the width of the class was equal to the s.d., thus between 3% and 7% (average minus or plus half s.d.). The three quantitative groups were: (i). DML < 3% (low DML, L-class); (ii). $3\% \leq DML \leq 7\%$ (medium DML, M-class); (iii). DML > 7% (high DML, H-class).

A machine learning classification tree analysis was performed to detect the most discriminating fresh WPM chemical features by using the experimental data set (n = 36). A recursive partitioning method was carried out within the rpart package; this package builds a decision tree having both the smallest number of classification branches and the lowest misclassification rate based on 10-folds cross-validation. The developed decision tree model was run in the original data set generating a confusion matrix to test the effectiveness of a correct assignment to the three actual DML classes by a set of qualitative statistical indicators [20].

3. Results

3.1. Chemical and Physical Traits of Harvested Biomass and Silage

The proximate composition and the structural and non-structural carbohydrates fractions of harvested fresh WPM forage are reported in Table 1. The data set highlighted a low variability of all variables, except for the content of water-soluble carbohydrates (WSC) that ranged between 22 and 112 g kg⁻¹ DM. The chemical traits and pH of WPM silage samples ensiled in concrete bunker silos showed a high-quality fermentative profile (Table 1). On average, the silages had a density of 214 kg DM m⁻³, but a wide variability was observed among individual silos (Table 1).

Table 1. Descriptive statistics for the chemical traits of green chopped whole-plant maize (WPM) and for dry matter (DM), fermentative profile (acids, ammonia, pH), porosity, DM density, and DM losses (DML) of post-ensiled WPM.

Variables Pre-Ensiled (g kg $^{-1}$ DM)	Mean	s.d.	IQR	Min	Median	Max
$DM_{fresh} (g kg^{-1})$	338	34	57	280	346	411
Crude protein	66.8	3.9	4.3	60.5	67.1	77.5
Ether extract	27.4	1.9	2.9	23.3	27.1	31.5
Ash	37.9	3.1	3.3	32.1	38.9	44.2
Starch	339	26	40	301	341	395
Water-soluble carbohydrates	70.1	32.3	64.8	22.1	62.5	113.2
aNDF	389	23	22	335	391	439
ADF	209	20	21	175	211	246
Lignin	25.5	5.7	7.5	16.0	26.5	38.1
Variables Post-Ensiled (g kg $^{-1}$ DM)						
$\overline{\text{DM}\left(\text{g kg}^{-1}\right)}$	336	31	51	281	335	395
Lactic acid	51.9	8.9	11.4	34.0	52.0	68.8
Acetic acid	26.5	6.8	7.8	5.9	25.4	39.9
Propionic acid	9.9	2.3	3.5	6.2	9.3	14.8
Butyric acid	0.87	0.12	0.19	0.70	0.85	1.10
NH_3 -N (g 100 g ⁻¹ total N)	7.8	0.7	0.8	6.3	7.9	9.5
pH	3.83	0.10	0.12	3.57	3.84	4.01
DM density (kg DM m ^{-3})	205	26	39	152	209	254
Porosity (decimals)	0.41	0.07	0.06	0.33	0.40	0.60
DM losses (%)	5.04	3.76	4.65	0.53	4.15	14.13

IQR, interquartile range (difference between the 75 and 25 percentile).

3.2. Dry Matter Losses (DML) of WPM Silage

The DML of WPM silage were $5.04 \pm 3.76\%$ on average; there was a large variability among individual net-bag, especially for samples with DML higher than the median value (Table 1). The DML were negatively correlated with DM density (r = -0.45; *p* = 0.006), whereas they were positively associated with porosity (r = 0.62; *p* < 0.001) and to DM_fresh (r = 0.37; *p* = 0.028). The mixed regression model (R² = 0.63) showed a positive association of DML (%) with DM_fresh (b = 0.044 ± 0.021 ; *p* < 0.05) and lignin (b = 1.00 ± 0.408 ; *p* < 0.05) and a negative association with ADF (b = -0.290 ± 0.111 ; *p* < 0.05).

As expected, porosity and density were negatively correlated (r = -0.54; p < 0.001). Moreover, due to multicollinearity, both of them cannot be present in the polynomial regression model as predictors of the DML occurring during the ensiling and preservation period of WPM. Therefore, the relationship between DML and two of the main predictive variables was assessed by choosing silage DM density and DM_fresh, as reported in Figure 1. The second-order regression polynomial model (adjusted $R^2 = 0.82$) estimated the following parameters: (i). silage density, first order $b = -0.448 \pm 0.243$, second order $b = 0.001 \pm 0.001$; (ii). DM_fresh, first order $b = 0.274 \pm 0.148$, second order $b = -0.0003 \pm 0.0002$. The visual interpretation of the 3D surface plot allowed us to establish the threshold value needed to limit a high level of DML (over 7%), which is 350 g kg⁻¹ of DM_fresh having a DM density higher than 180 kg DM m⁻³ before the feed-out phase (Figure 2).



Figure 2. Dry matter losses (DML) of whole-plant maize (WPM) silage (%) according to the fresh forage DM (DM_fresh) content (g kg⁻¹) and the DM density (kg DM m⁻³).

3.3. Modeling the DML of WPM Silage

To depict the range of variation of DML in the investigated bunker silos, three quantitative groups were defined as L-class (DML < 3%), M-class ($3\% \le DML \le 7\%$), and H-class (DML > 7%), and they accounted for 14, 15, and 7 samples of the original data set, respectively. According to these classification criteria, a decision tree algorithm was built on three selected chemical features of the fresh forage (Figure 3). The threshold of 345 g per kg of green chopped maize forage (DM_fresh) was able to discriminate between the L-group and the cluster M- and H-groups. The correct assignment for L-samples was 71%; meanwhile, for M- and H-samples was 63% and 26%, respectively. Moving to the left branch of the decision tree (47% of the original data set), a value of aNDF \geq 373 (g kg⁻¹ DM) discriminated the L-class (86%). Instead, the right branch of the decision tree showed that WSC < 104 (g kg⁻¹ DM) values could allow distinguishing M- from H-samples.



Figure 3. Dry matter losses (DML) of whole-plant maize (WPM) silage based on a decision tree algorithm. Data inside each node are (top to bottom): prevalent DML-class (increasing % of samples belonging to the class raising the intensity of color); fractions of the three DML classes within the node; percentage of original data set explained. 1—L-class, DML < 3%; 2—M-class, DML 3–7%; 3—H-class, DML > 7%. DM_fresh, NDF (as aNDF), and WSC refer to the pre-ensiled forage (see Table 1).

With regard to the evaluation of the algorithm performance, a confusion matrix highlighted a high level of sensitivity (Se) for the L- and M-classes (Se > 0.85), whereas the H-class had a fair level (Table 2). The specificity was highly satisfactory across the DML classes. The values of the balanced accuracy confirmed the suitable discriminative capability of the decision tree, especially for the prediction of a low level of DML (L-class) along the ensiling process of WPM (Table 2).

Table 2. The confusion matrix for the decision tree model applied to three dry matter losses (DML as %) quantitative classes (low, L vs. medium, M vs. high, H).

Prediction		Original Farm-Derived Data Set ($n = 36$)			
	Actual class	DML < 3.0	$3.0 \le DML \le 7.0$	DML > 7.0	
Predicted as	DML < 3.0 (L)	12	1	1	
Predicted as	$3.0 \leq \text{DML} \leq 7.0 \text{ (M)}$	2	13	1	
Predicted as	DML > 7.0 (H)	0	1	5	
Predictive Statistics					
Sensitivity		0.86	0.87	0.71	
Specificity		0.91	0.86	0.97	
Positive predictive value		0.86	0.81	0.83	
Negative predictive value		0.90	0.89	0.93	
Balanced accuracy		0.88	0.86	0.84	

4. Discussion

The study was planned in order to define a set of chemical and physical markers of fresh chopped WPM able to predict the DML that would occur during the ensiling fermentative process in operating farming conditions. Therefore, the experimental data set consisted of a total of 36 net-bags buried in many farm concrete bunker silos to simulate the ensiling process of WPM harvested at the expected stage of maturity that yields high nutrients contents (i.e., starch and CP) and optimal moisture to allow packing high-density forage. All silage samples from the net-bags were characterized by an optimal fermentation profile according to the literature [21,22], confirming that all farms performed the silage-making process properly by applying suitable management practices such as filling the silo within 48 h before sealing and ensuring an effective and uniform fresh forage compaction (i.e., use of tractors and covering with gravel sacks).

Although the trial was performed in a restricted agronomic area involving dairy farmers applying common harvesting and silage-making procedures, consistent with the literature [1,12], the range of DML measured in the trial was rather wide (1% to 14%) because of various physiological, physical, and chemical variables of the fresh material that could affect its ensiling pattern and the rate and extent of the main fermentation compounds production. In this study, both the mixed and polynomial regression models confirm a positive relationship between DM content of harvested WPM biomass and DML resulting from the ensiling process and fermented preservation. The WPM preservation through the ensiling is accomplished by avoiding the high level of DM of the green chopped WPM since it is reduced silage density and promotes aerobic deterioration [6]. When maize plants were ensiled at lower DM content (early maturity stage), the silage resulted in a low air circulation supporting intensive lactic acid-dominated fermentation pathways [23] and limiting the adverse effects due to an intense role of others than lactic acid bacteria (i.e., undesired fermentation generating CO_2) and/or the production of undesirable compounds from yeasts and molds [24,25]. As stated by Borreani et al. [6], the results of this study confirm that DML can be minimized by limiting porosity at 0.40 [11], and this seemed to be accomplished by increasing silage density over 200 kg DM m $^{-3}$. As the lower packing density at a recommended harvesting DM_fresh ranges between 300 and 400 g kg⁻¹ a threshold of 200 kg DM m⁻³ is recommended to ensure a low porosity of silage [10]. A higher porosity (over 0.40) of a lower density WPM silage could allow rapid oxygen movement inside the biomass, promoting microbial activity and silage damage [26,27]. A study by Gallo et al. [28] demonstrated that the lower density (measured by a mass penetration resistance) of the peripheral bunker silo zone significantly led to high air penetration, predisposing raising temperature and oxidative process, and an impaired acid profile consequently.

Several factors affect the DML, including DM at harvest and porosity, since there is evidence that the rate of oxygen movement through the silage is proportional to its density and porosity [10,29]. Moreover, porosity is proportional to DM content and negatively correlated to bulk density [18]. According to Woolford 1990 [30], the degree of anaerobiosis is the most crucial single factor influencing silage conservation. On the other hand, the considerable respiratory activity of plant and aerobic micro-organisms can cause DML as well. Borreani et al. 2018 [6] reported two formulas that negatively relate DML to the density and intensity of the packing procedures. However, Griswold et al. 2010 [31] confirmed a weak inverse relationship between DML and DM density but suggested that other factors play a role in DML. Moreover, the same authors proposed a response surface regression between DML, DM, and DM density where it appears that at lower densities $(100-200 \text{ kg DM m}^{-3})$, DML has a curvilinear trend with a minimum at about 350 g kg⁻¹ of DM, increasing up to 7% of DML at DM of 390 g kg^{-1} . In our study, the data ranges (min-max/median) were 0.33-0.60/0.40 for porosity, 152-254/209 (kg DM m⁻³) for density, 280-411/346 (g kg⁻¹) for DM_fresh, which corresponds to low density in the proposed response surface, and high porosity compared with those suggested by Holmes et al. 2007 [10]. These findings of our study are suggestive that aerobic activity has occurred

during the conservation period and may explain the positive relationship between DML and relatively high DM content.

The machine learning algorithm defined a threshold of 345 g per kg of harvested fresh WPM biomass to limit the DML to a very low level (L-class, DML < 3%). Although it would appear advantageous to ensile mature WPM because of the higher DM and starch yields, this forage is more difficult to pack, and the resulting silage often shows higher DML [1] and spoils rapidly when exposed to air [22]. However, the main outcome of the study is that the DM content alone of WMP harvested biomass could not explain the DML because lignin and WSC were additional predisposing factors, while aNDF seemed to be preventive [11,12]. It is well known that early phenological stages of harvest are associated with high aNDF and sugars contents; meanwhile, concentrations of DM and starch increase with increasing maturity [32]. A lower aNDF content in the advanced maturity stage of the maize plant is due to the rising incidence of grains, and greater starch concentration in developing caryopsis WSC are converted into polysaccharides [33]. Even though the H-class seems rarely to occur, 8% of the samples consistent with the algorithm decision tree represent the WPM harvesting target that should be avoided. Probably, harvesting biomass with high DM contents and WSC levels greater than 100 g kg⁻¹ DM might have predisposed to low DM density and excessive fermentative rate, two detrimental ensiling conditions, which resulted in a DML rate over the 7%.

However, botanical (i.e., hybrid and FAO classes) variability could affect the concentration of nutrients, especially WSC, across the phenological stages [17]. Indeed, the nutrients' variability is also associated with many agronomic factors and weather conditions (i.e., cool, rainfall) [11]. Therefore, before extending the suggested results to the maize silage population, validation is required by further on-farm investigation, testing the variability associated with crop and harvesting circumstances, and ensiling methods. The outcomes of this study suggest that a strengthened application of the decision tree analysis or similar machine learning techniques on chemical and physical data of fresh maize forage can be useful to explicate the probability of occurrence of a well-established DML threshold over the ensiling time. Although the complexity of the fermentation process makes it challenging to identify a clear explanation of drivers of the DML at the farm bunker silo scale [1], the buried net-bag method may be considered one of the most accurate to ascertain the role of the individual factors related to the chemical and physical characteristics of the fresh maize harvested biomass, even if the application of digital and automatic weighing systems could be made effective also the total-in versus total out method [1,13]. Quite apart from the method of assessment of silage DML, further investigations should consider increasing ensiling efficiency in terms of a careful assessment of the variables allowing an effective packing density of fresh forage and driving an optimal fermentative pattern along the ensiling process.

5. Conclusions

The current study suggests that the DM content of fresh WPM strongly affects the DML across the ensiling process due to the different silage densities achieved in the bunker silos. A maturity stage of WPM defined by a DM content of 345 g kg⁻¹ seemed the minimum threshold to achieve a bunker silo DM density driving an optimal anaerobic fermentation, which is at least 180 kg DM m⁻³. However, the optimal ensiling maize plant maturity to limit the DML seemed to be partially a linear DM-driven function, which is also related to a phenological stage allowing a rapid achievement of aerobic stability in the bunker silo. This phenological stage seemed to correspond to an aNDF content over 370 g kg⁻¹ DM. A prolonged harvesting time, reducing aNDF, and increasing lignin deposition may limit the effective packing of the biomass in the silo, thus decreasing the silage density along with the consequent adverse effects on the ensiling process due to aerobic deterioration. The study highlighted that using net-bags filled with freshly chopped forage and buried in the bunker silos can simulate the silage-making process in farming operating conditions, albeit in a miniaturized, controlled, and easy sampling fermentative environment. Therefore, this

technique can support an effective decision-making strategy for farmers and nutritionists to improve the fermentative and nutritional quality of maize silage used for lactating cows and beef cattle. However, the results of this study have to be integrated with further similar trials in the future to verify the accuracy and repeatability of the tested models.

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