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Glyphosate in oat desiccation for haylage harvest at different wilting times

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ABSTRACT. The objective was to evaluate the ruminal disappearance rate of dry matter and NDF of black oat haylage subjected to mechanical and chemical dehydration and six wilting periods in the field, as follows: 0, 3, 6, 9, 12 and 15 days. Forage haylages were ensiled and stored in PVC mini-silos for 60 days until opening for in situ evaluations. The pre-set ruminal incubation times were: 0, 6, 12, 24, 36 and 48 hours. Regardless of the dehydration time, forage subjected to mechanical dehydration showed ruminal disappearance rates of dry matter and NDF, thus DISDM-48h and DISNDF-48h were superior for this method compared to the chemical method. There was a reduction in DISDM-48h and DISNDF-48h with advancing time of wilting in the field for haylage dehydrated by the chemical method. In general, the chemical dehydration method seems to be more related to better haylage making practices than nutritional factors, where the haylage obtained by mechanical dehydration is superior.

Keywords: black oat; *Avena strigosa* Schreb; dehydration; NDF digestibility; silage.

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Introduction

The crop of black oat (*Avena strigosa* Schreb) is a forage of use opportunities, with high yield capacity and nutritional value (David et al., 2010), and with excellent potential for harvesting and producing haylage (Meinerz et al., 2011). When harvested at the pre-flowering stage, oats have a representative content of crude protein in chemical composition, in addition to having a high moisture content, which can lead to buffering of the ensiled mass, making it difficult to lower the pH to levels considered ideal for proper preservation (Horst et al., 2017). This could lead to secondary fermentations and development of undesirable microorganisms (Paris, Zamarchi, Pavinato, & Martin, 2015).

Bernardes and Rêgo (2014) highlight that the conventional (mechanical) method of forage dehydration for haylage consists of harvesting the forage followed by periodic turning to accelerate the water loss process. This practice can generate high contamination with microorganisms undesirable to the fermentation, present in the soil, which, together with minerals in the soil, can contribute to haylage buffering (Horst et al., 2018). Therefore, some authors have instigated the use of other forage dehydration methods for harvesting and producing haylage, such as chemical dehydration using glyphosate (Carneiro et al., 2017; Bueno et al., 2018). Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate- (EPSP) synthase, responsible for catalyzing the synthesis of shikimic acid, which acts in the conversion of carbohydrates derived from glycolysis and the pentose-phosphate route into aromatic amino acids (phenylalanine, tyrosine and tryptophan), which are precursors of lignin, alkaloids, flavonoids and benzoic acids, in addition to being important for protein synthesis and cell division in meristematic regions (Zobiole et al., 2010).

According to Bueno et al. (2018), the use of the chemical dehydration method, where the forage is harvested and immediately ensiled, allows to achieve an adequate moisture content without the volume losses seen in the conventional method and perhaps enabling better preservation of forage nutrients. However, it is supposed that changes in the composition of the plant cell wall can occur and impair the digestibility of the food produced by this method.

Knowing that the dry matter content of the forage at the time of ensiling is a critical point to ensure adequate fermentation, and that although some authors have already suggested the appropriate times for this (Horst et al., 2017; Carneiro et al., 2017), the dehydration time necessary to reach such levels, as well as the alterations that each one generates in the digestibility of pre-dried silage are still rarely found in the literature. Thus, the objective was to evaluate the rate of in situ ruminal disappearance of dry matter and neutral detergent fiber from pre-dried black oat silage subjected to mechanical and chemical dehydration under six times of wilting in the field.

Knowing that the dry matter content of the forage at the time of ensiling is a critical point to ensure adequate fermentation, and that although some authors have already suggested the appropriate times for this (Horst et al., 2017; Carneiro et al., 2017), the dehydration time necessary to reach such levels, as well as the alterations caused in the digestibility of haylage are still rarely found in the literature. Thus, the objective was to evaluate the rate of in situ ruminal disappearance of dry matter and neutral detergent fiber from black oat haylage subjected to mechanical and chemical dehydration and six wilting periods in the field.

Material and methods

The experiment was carried out in the municipality of Guarapuava, state of Paraná, Brazil, located in the subtropical zone of the state, at coordinates 25º23'02" S and 51º29'43" W and 1,026 m altitude. According to Köppen's classification, the region is classified as Cfb (Mesothermal Humid Subtropical), with mild summers and moderate winters, with no defined dry season, and severe frosts. The average annual rainfall is 1,944 mm, average annual minimum temperature of 12.7°C, average annual maximum temperature of 23.5°C and relative humidity of 77.9%.

The black oat (*Avena strigosa* Schreb) EMBRAPA 139 cultivar crop was planted according to agricultural zoning for the region. Sowing was carried out with row spacing of 17 cm, 4 cm sowing depth and density of 400 seeds m^{-2}, determining a population of 220 thousand plants ha^{-1}. The experimental area was 867 m^2, divided into five blocks, with 12 plots each, thus totaling 60 plots of 14.5 m^2 (2.9 m × 5.0 m).

For basal fertilization, 08-30-20 (N-P_2O_5-K_2O) commercial fertilizer was used, at a dose of 300 kg ha^{-1}, respecting the recommendations of the Manual of Fertilization and Liming for the State of Paraná (Pauletti et al., 2017). Nitrogen fertilizer was applied 30 days after emergence (DAE) as topdressing with urea (46% N), at a dose of 120 kg N ha^{-1}.

The experimental design used was a 2 × 6 factorial randomized block design, with two methods of forage dehydration: mechanical and chemical, and six wilting periods in the field for harvest: 0, 3, 6, 9, 12 and 15 days. Each treatment contained four replicates (blocks). The mechanical method consisted of harvesting the plant with sickles, respecting the wilting period in the field with periodic turning of the harvested forage until the harvest time. The chemical method consisted of the application of glyphosate (Roundup Transorb®) at a dose of 0.5 L ha^{-1}, and after respecting the respective wilting periods in the field, the cut for immediate ensiling was carried out. Roundup Transorb® is registered under number 04299 at the Ministry of Agriculture, Livestock and Supply (MAPA) for use in black oats haylage in the dose used.

Figure 1 illustrates the meteorological data during the experimental period, which corresponded to the time of dehydration in the field, that is, between the beginning of dehydration (harvest or herbicide application) and harvesting for ensiling.

Evaluations began when the plants reached the pre-flowering phenological stage (88 DAE), when they had an average height of 80 cm and an average density of 946 thousand tillers ha^{-1}. For all plots of the mechanical treatment (conventional), plants were harvested at 8 cm from the ground with a hand sickle. For the chemical treatment plots, herbicide was applied using an electric backpack sprayer, with a bar composed of four spray nozzles, regulated to a flow rate of 200 L ha^{-1}. The application took place at 17h00min on day 0, with solar radiation, 35% relative humidity and a temperature of 21ºC.

Forage dry matter contents at ensiling are listed in Table 1. The chemical composition of haylages were described by Carneiro et al. (2017).

Forage harvested at different pre-set wilting periods was chopped in a stationary forage harvester Nogueira®, EM 6400, to a theoretical particle size of 20 mm. The chopped material was stored in PVC mini-silos measuring 10 cm in diameter and 45 cm in length. Forage was compacted with a manual press to reach a specific density of 235 kg DM m^{-3}. After 60 storage days, silos were opened and 300 g samples were collected and pre-dried in a forced air oven at 55ºC for 72 hours.

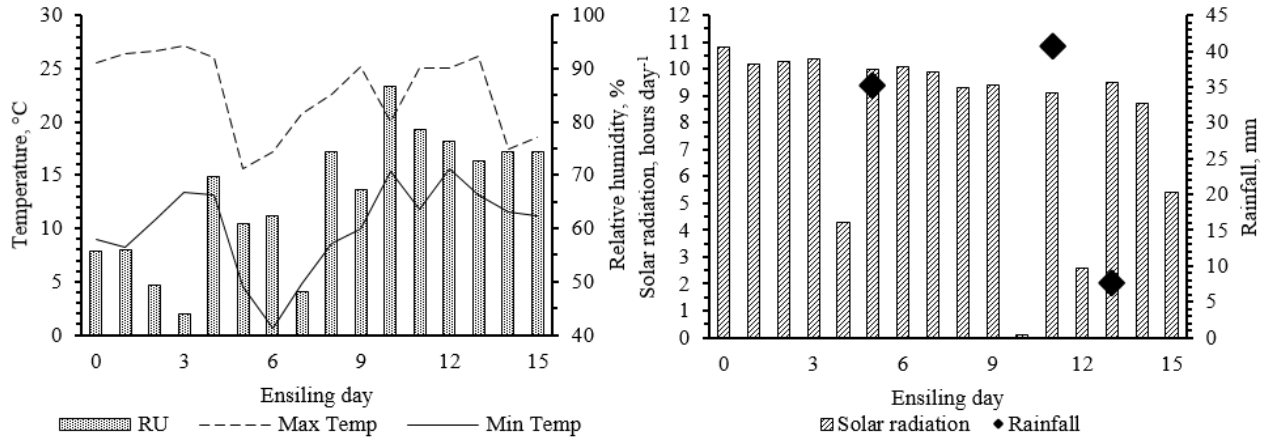

Figure 1. Data on rainfall (%), maximum temperature (solid line) and minimum (dotted line), solar radiation (hours day^{-1}) and relative air humidity (%), during the forage dehydration period in the field.
Source: IAPAR Meteorological Station at CEDETEG/UNICENTRO, Guarapuava, state of Paraná.

Table 1. Dry matter of black oat forage subjected to mechanical and chemical dehydrations and different wilting periods in the field.

Method	Wilting period in the field, days						Equation	R^2
	0	3	6	9	12	15		
Mechanical	16.21	31.24	34.50	41.94	44.77	48.35	mY = 20.49 + 2.05x	0.87
Chemical	13.95	17.86	17.94	20.48	21.57	27.16	qY = 13.75 + 0.79x	0.85

The in situ ruminal disappearance rate of dry matter and neutral detergent fiber were performed using nylon bags measuring 12 cm × 8 cm and with 50 µm pores, containing 5 g material, ground to 1 mm, for subsequent incubation in the rumen (Nocek, 1988). The incubation times used for evaluation were: 0, 6, 12, 24, 36 and 48 hours, where the 0-hour time represented the soluble fraction of dry matter. For this purpose, two 48-month-old steers with an average live body of 650 kg and with permanent ruminal fistula were used. Samples of silages and residues from ruminal incubation were sent for analysis of neutral detergent fiber (NDF) following the methodology proposed by Soest, Robertson and Lewis (1991). For the in situ ruminal digestibility data, values of 24 and 48 hours were used following the same methodologies.

Data were tested by Shapiro-Wilk and Bartlett tests, in order to check the assumptions of normality and homogeneity of variance, respectively. Once these assumptions were met, the F-test was applied by Analysis of Variance (ANOVA) at a 5% confidence probability level, in the Statistical Analysis Sofware (SAS, 1993). Data referring to the ruminal disappearance rate were subjected to regression analysis by the Regression procedure (PROC REG) at the same confidence level and in the same software.

Results and discussion

With advancing time of wilting in the field, there was a reduction in DISDM-48h of the haylage dehydrated by the chemical method, and no significant changes were detected between the wilting periods in the field for the haylage subjected to the mechanical method (Figure 2). The action of glyphosate alters the selective permeability of plant cells and reduces the plant photosynthetic capacity, that is, the plant remains metabolically active while losing water, which causes an energy expenditure subtracted from its own reserves (Bueno et al., 2018). Plant cell death after glyphosate application is slow, taking days to weeks, proportionally reducing digestibility due to this gradual decline in reserve substrates (Orcaray, Zulet, Zabalza, & Royuela, 2012).

Conversely, dehydration with the conventional (mechanical) method proved to be more efficient in resisting the reduction of haylage ruminal digestibility, because the reduction in water activity caused by sunlight inactivates plant and bacterial enzymes, therefore, there is energy savings and a greater amount of substrate kept in the plant (Zobiole et al., 2010). Regardless of the time of wilting in the field, the use of glyphosate as a dehydration method resulted in a reduction in haylage digestibility, with a more drastic accentuation from the ninth day onwards ($R^2 = 0.73$).

As demonstrated by Carneiro et al. (2017), from the ninth day of wilting in the field, there was an increase in the haylage NDF content for both treatments, justifying another possible reason for the drop in digestibility

from that moment on. The drop in DISDM-48h of the haylage dehydrated by the mechanical method and collected at 6, 12 and 15 days wilting, although not significant, may be due to rainfall events prior to these moments of harvesting and ensiling (Figure 1). This was not observed when there was chemical dehydration, demonstrating an advantage over the conventional method. Weather conditions faced during the wilting period in the field, that is, maximum temperatures below 25°C, RH above 60% and intense dew in the mornings caused the slow rate of dehydration observed in Table 1. It is also worth noting that the forage dehydrated by the chemical method did not reach the minimum content of 40% dry matter (Horst et al., 2017) in any of the wilting periods, a value that is considered necessary to avoid the development of undesirable microorganisms and nutrient losses by effluents in haylage. Even not differing significantly, on day 0, the forage dehydrated by the mechanical method was below this threshold, and with lower dry matter digestibility between the wilting periods evaluated.

After 15 days of wilting, a more expressive drop in DISDM-48h was found, which may be due to cell plasmolysis, causing the cell to lose its selective permeability (Zobiole et al., 2010). This process usually occurs when the plant dry matter content exceeds 45% DM, and deserves special attention, as the loss of selective permeability generates rehydration from dew or rain.

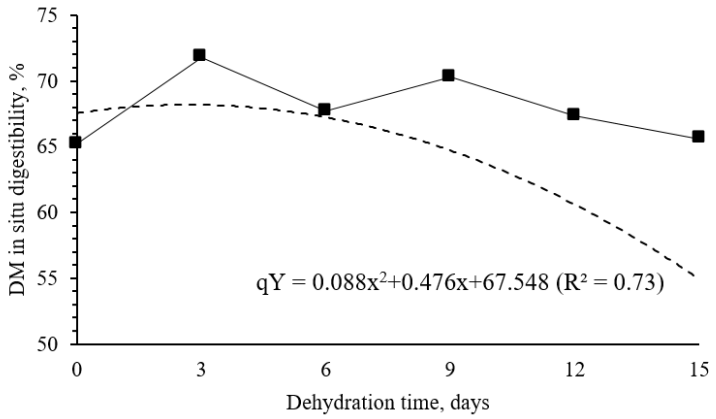

Figure 2. DISDM-48h of haylage dehydrated by mechanical (solid line) and chemical (dotted line; qY) method, according to the different times of wilting in the field.

The ruminal disappearance rate of dry matter in haylage ensiled on day 0 did not differ between the dehydration methods, proving that there were no immediate changes after harvesting or desiccation (Figure 3a). After three days of wilting (Figure 3b) it was noticed that at zero time of ruminal incubation, haylage subjected to the mechanical method of dehydration presented a higher value (intercept), suggesting that it retained more its soluble compounds. After six days, there is always superiority at this same point for haylage dehydrated by the chemical method (Figure 3c). According to Bueno et al. (2018), the rapid and immediate loss of water in newly harvested forages can carry soluble nutrients, reducing their concentration in the plant. Under the effect of glyphosate, this loss occurs more slowly for reasons of energy consumption to maintain metabolism.

After three days of dehydration in the field (Figure 3b), both treatments started from the same concentration of soluble nutrients (intercept), however, the degradation rate was higher for the mechanical treatment (0.63 versus 0.53% hour^{-1}), resulting in 71.8 and 68.9% digestibility with 48 hours incubation for haylage of the mechanical and chemical treatment, respectively.

With the forage wilted for six days in the field (Figure 3c), haylage of the mechanical treatment showed higher digestibility and higher degradation rate (0.58% against 0.47% hour^{-1}). After nine days of wilting (Figure 3d), haylage of the chemical treatment continued to show a lower degradation rate per hour (0.38 against 0.70% hour^{-1}), which resulted in a lower digestibility after 48 hours, where the chemical treatment presented a value of 61.4% against 70.3% of the haylage dehydrated by the mechanical method.

In the same way, after 12 days of wilting in the field (Figure 3e), haylage of the chemical treatment showed a lower ruminal disappearance rate (0.44% against 0.66% hour^{-1}). And just as in the previous time, the same occurred after three more days of wilting, where with 15 days of wilting (Figure 3f), the degradation rate per hour was lower for the chemical method (0.26% against 0.55 % hour^{-1}). Even with lower digestibility values,

our data obtained with the chemical method are still superior to those highlighted by Horst et al. (2017), with an average of 29% using the same black oat cultivar (EMBRAPA 139) in a similar situation. In turn, Meschede, Carbonari, Velini, Trindade and Gomes (2011) used glyphosate at doses of 360 and 720 g ha^{-1} in *Brachiaria decumbens*, and harvested 30 days after application, the authors described an increase in the levels of ADF (40.8% and 45.8%, respectively) and lignin (13.3 and 24.2%, respectively) when compared to fresh forage, indicating that lignification may be one of the main factors responsible for the decrease in digestibility, increasing as the wilting time extends.

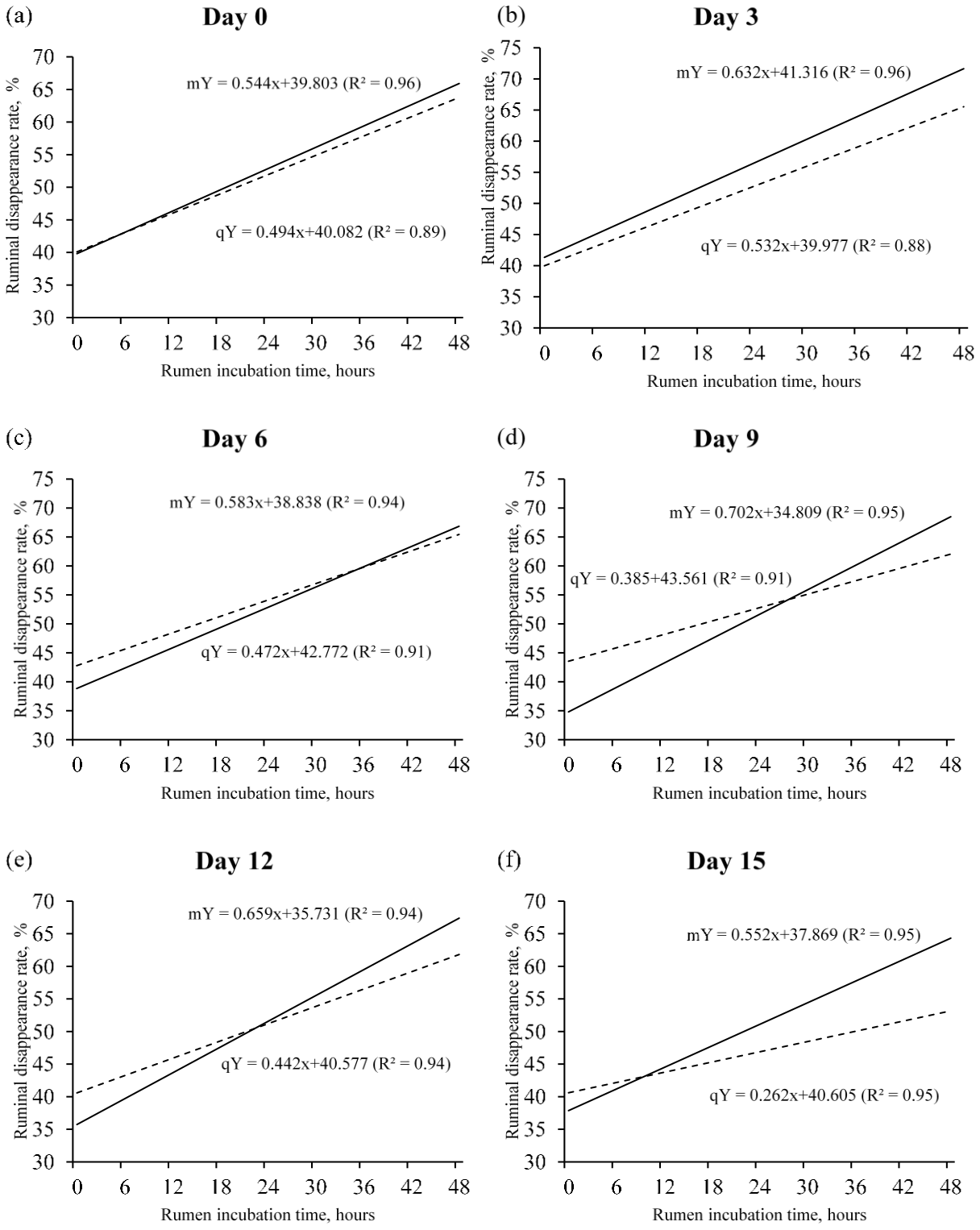

Figure 3. Ruminal disappearance rate of haylage DM subjected to mechanical (solid line; mY) and chemical (dotted line; qY) methods of dehydration according to the different times of wilting in the field.

Souza et al. (2006) described lower effective dry matter degradability of maniçoba grass silage (*Manihot epruinosa*) wilted in the sun for 4 to 5 hours, when compared to fresh grass, and related this difference to the loss of soluble compounds, either during drying in the sun or during the fermentation process. Our findings may be due to the same losses, but for different reasons, as explained above.

The pattern in the DISNDF-48h (Figure 4) was similar to that observed for DISDM-48h. There was no difference between the wilting periods when dehydration was promoted by the mechanical method. When the forage was subjected to chemical dehydration, a decrease in NDF digestibility was found, with greater expression from the sixth day of wilting. Carneiro et al. (2017) described higher contents of ADF for haylage dehydrated by the chemical method, with no difference in NDF content between the two methods. This indicates that hemicellulose, the most digestible fiber fraction, was more present in the mechanical method, favoring the higher NDF digestibility of this treatment. According to Bueno et al. (2018), even after the application of glyphosate, the plant does not completely interrupt its metabolism. Glyphosate works in the shikimic acid cycle, the pathway responsible for generating metabolites necessary for the formation of lignin. However, the dose seems not to have been sufficient to stop this deposition in a short time, therefore, there may have been continuity in lignin deposition and a consequent drop in NDF digestibility of haylage subjected to this type of dehydration. On the other hand, under mechanical action, the plant metabolic activity is interrupted, with no change in fiber composition from the moment of harvest (Meschede et al., 2011).

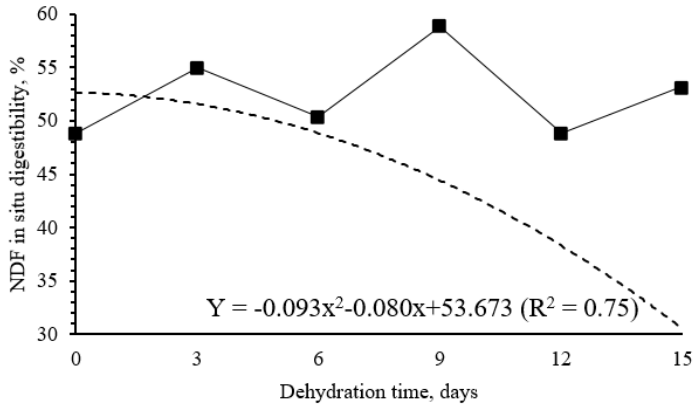

Figure 4. DISNDF-48h of haylage dehydrated by mechanical (solid line) and chemical (dotted line; qY) methods according to the different times of wilting in the field.

Figure 5 shows that immediately after harvesting or applying glyphosate (day 0; g), ruminal disappearance rate was close between haylages subjected to mechanical and chemical dehydration methods (0.72 and 0.69% hour^{-1}, respectively), with greater DISNDF-48h for haylage subjected to the mechanical dehydration method.

In all other periods of wilting in the field, ruminal disappearance rate of NDF was higher for haylage subjected to the mechanical dehydration method (Figure 5), with values of 0.63% with three days (h); 0.58% with six (i); 0.70% with nine (j); 0.65% with twelve (k) and 0.55% with fifteen days (l), while for the haylage subjected to the chemical dehydration method, values were 0.53% with three days (h); 0.47% with six (i); 0.38% with nine (j); 0.44% with twelve (k) and 0.26% with fifteen days (l).

Filya (2003) reported for wheat silage at pre-flowering and wilted for six hours in the field, DISNDF-48h of 41.8%, similar to that found in the present study for haylage on day 0. With 15 days of wilting (l), haylage subjected to the mechanical method of dehydration showed a ruminal disappearance rate of NDF of 52%, against 44% for haylage subjected to the chemical method, which is the greatest amplitude observed.

Conclusion

Regardless of the wilting period in the field, forage subjected to the mechanical dehydration method provides a better ruminal disappearance rate of dry matter. DISNDF was also superior for haylage dehydrated by the mechanical method and reduced with advancing time of wilting in the field when dehydrated by the chemical method.

In general, the chemical dehydration method seems to be more related to better haylage making practices than nutritional factors, in which haylage obtained by mechanical dehydration is superior.

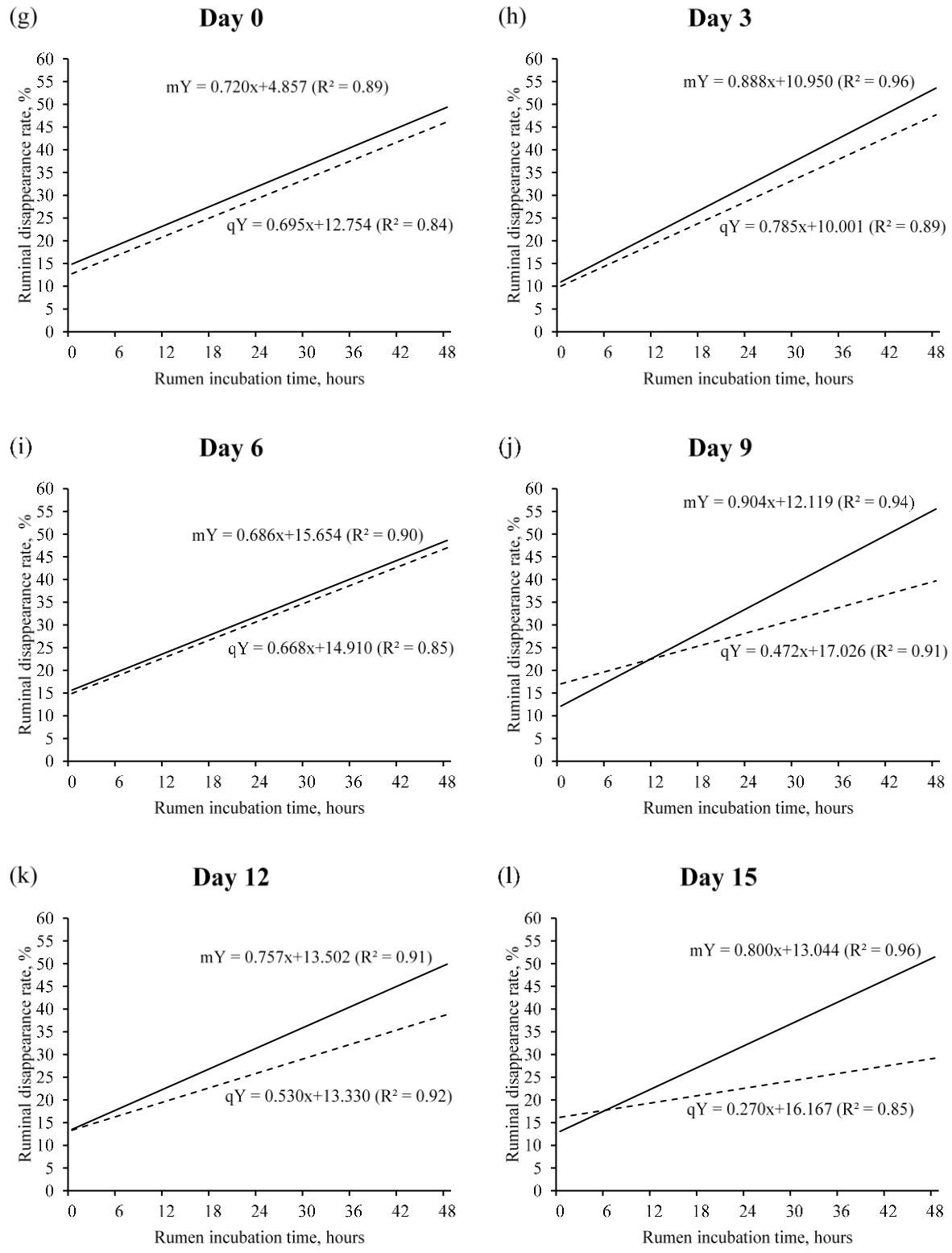

Figure 5. Ruminal disappearance rate of haylage NDF subjected to mechanical (solid line; mY) and chemical (dotted line; qY) methods according to the different times of wilting in the field.

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