



Occurrence of visible losses and relationship with corn silage management in dairy farms in the State of Paraná

Igor Quirrenbach de Carvalho¹, Clóves Cabreira Jobim^{1*} , Milene Puntel Osmari² and João Luiz Pratti Daniel¹

¹Departamento de Zootecnia, Universidade Estadual de Maringá, Avenida Colombo, 5790, Maringá, Paraná, Brasil. ²Departamento de Zootecnia e Desenvolvimento Rural, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brasil. *Author for correspondence. E-mail: ccjobim@uem.br

ABSTRACT. The aim of this study was to relate the occurrence of visible losses in silage (effluent, spoiled top-layer and during feedout) with silage-making practices, physical and chemical characteristics of silage, and milk composition in Brazilian dairy herds. One-hundred and eight silos from 95 farms, in the State of Paraná, were visited for data collection. Data were analyzed by Fisher's Exact and Pearson Correlation Test. Effluent loss was higher in silages with the lowest dry matter content. Using unwallied clamp (drive-over piles) silos, neglecting a protection over the plastic film, and unloading silage with a bucket increased the occurrence of top spoilage. Feedout losses were higher in farms where: the crop was harvested with self-propelled machines; the particle size was larger, and the silage density was lower. There was no relationship between visible losses and silage composition or milk composition, except for milk fat content that, unexpectedly, there was a positive correlation with spoiled silage in the top-layer. Silage losses are reduced by adopting good practices during silage production and feedout.

Keywords: aerobic deterioration; effluent; forage harvester; sealing; silo type.

Received on September 13, 2019.

Accepted on March 11, 2020.

Introduction

In Brazilian milk production systems, it is common to supply conserved feeds, especially corn silage, in order to improve the nutritional value of diets, reduce the effects of seasonality on pasture production, in addition to maintaining or increasing milk production. In the Central-Eastern region of the State of Paraná, the use of conserved forage becomes even more important, since it is a region of humid subtropical climate with intense winters and frequent frosts (Sistema de Tecnologia e Monitoramento Ambiental do Paraná [SIMEPAR], 2019), which makes it difficult to use only pastures to feed dairy cows.

Silage quality depends on crop management and environmental factors, which may alter nutrient recovery upon silage utilization. Under field conditions, silage dry matter (DM) losses during storage and feedout may exceed 300 g kg⁻¹ (Borreani, Tabacco, Schmidt, Holmes, & Muck, 2018). Beyond the occurrence of 'invisible' losses, which occur mainly by carbon dioxide formed during fermentation and nutrient oxidation, silage DM can be lost by effluent run-off and disposal of inedible silage. Harvesting crops with excessive moisture (DM <300 g kg⁻¹) may increase the effluent production, which decreases silage quality (Goeser et al., 2015) and pollutes the environment. Meanwhile, inefficient sealing, damage to the covering system, cracking of silo walls, water infiltration, and poor management of the silo face often increase aerobic deterioration (Borreani et al., 2018).

Few studies on silage losses have been carried out under field conditions. Additionally, the literature on silage management practices in Brazilian farms is scarce (Bernardes & Do Rêgo, 2014). Therefore, the aim of this study was to correlate the occurrence of visible silage losses (effluent, spoiled top-layer and during feedout) with silage making-practices, physical and chemical characteristics of silage, and milk composition in Brazilian farms.

Material and methods

A total of 108 silos from 95 farms were sampled in the Central-Eastern region of the State of Paraná, Brazil. Farms had 1.5 to 120 ha destined to silage production, 10 to 600 lactating dairy cows (~82% Holstein)

with average milk production from 17.7 to 42.2 kg d⁻¹ per animal. Together, all sampled farms cultivated 2,852 ha corn for silage and raised 10,061 lactating dairy cows.

During the visits, the follow information was recorded: silo type (bunker or drive-over), plastic film (black, white, black-on-white or black-on-grey) (all were polyethylene), plastic cover (none, soil or other materials) and silage unloading method (fork, bucket or defacer). Silage wet density (SWD, kg m⁻³) was measured at five points across the silo face: two samples at the top, two at the bottom and one at the center of the silo, in accordance with D'Amours and Savoie (2005). Samples were taken using a metal cylinder (20 cm length × 10 cm diameter) with a saw tooth cutting edge attached to a chainsaw to extract a core of silage (Krüger et al., 2017). The SWD was determined according to the equation: $SWD \text{ (kg m}^{-3}\text{)} = \text{sampled silage mass (kg) / volume collected (m}^3\text{)}$.

Silage temperature was measured in the same five points with a digital thermometer at 15 cm depth, before taking the samples. The occurrence of visible losses (effluent, spoiled top-layer and during feedout) was qualitatively judged by two people using the scores: none, low, medium and high.

After sample collection, a composite sample (± 4 kg) from each silo was prepared for chemical analysis, aerobic stability test, and determination of particle size distribution. For the aerobic stability test, an amount 2 kg of silage samples were placed in plastic buckets (20 L) and kept in a controlled environment at $25\pm 2^\circ\text{C}$ for 120h. The silage temperature was recorded every 24 hours and the aerobic stability was defined as the time elapsed until silage temperature increased by 2°C over the room temperature (Jobim, Nussio, Reis, & Schmidt, 2007).

Particle size distribution and mean particle size (MPS) were determined using a Penn State particle separator (Lammers et al., 1996). An aqueous extract (1 part of silage and 10 parts of distilled water) was prepared for measuring silage pH after 30 minutes of standing time using a pHmeter (Model TEC-11, Tecnal®, Piracicaba – SP, Brazil).

A sub-sample was dried at 55°C for 72h and ground in a Wiley mill (1-mm sieve) for analysis of DM (method number 934.01, Association Official Analytical Chemist [AOAC], 2005); crude protein (CP) by the Dumas combustion method (Nelson & Sommers, 1996); acid detergent fiber (ADFom) and neutral detergent fiber (aNDFom) according to Mertens (2002); *in vitro* dry matter digestibility (IVDMD) according to Tilley and Terry (1963); starch (Pereira & Rossi, 1995), and Zearalenone (mycotoxin) by liquid chromatography (Scott, 1997).

Bulk tank milk samples were sent to the Associação Paranaense dos Criadores da Raça Holandesa (Curitiba, Paraná, Brazil) for analysis of protein, fat, lactose and total solids by infrared analysis. The somatic cell count (SCC) was determined by flow cytometry.

The influence of independent data (silo and ensiling information) on the response variables (effluent, spoiled layer below the plastic film and losses during feedout) were tested by Fisher's Exact Test, using Chi-square analysis (χ^2) (Zar, 2010) and Pearson Correlation Test by software Statistical Analysis System (SAS, 2011), at 0.05 probability.

Results and Discussion

Most farmers (65%) used outsourced custom services for harvesting the corn crop and almost 55% of farmers used self-propelled harvesters (Table 1). In 73.15% farms, there was no silage loss as effluent, whereas 33% farms had top spoilage losses. Feedout losses had the highest frequency among the evaluated losses (97.2%), with a medium to high magnitude in most of the silos (53.7%) (Table 2).

The region where silages were sampled is an outstanding site of milk production in the State of Paraná, with many dairy cooperatives. Hence, the farmers can easily obtain funds to invest in their farms. However, investments on silage making were basically destined to improve silage storage, due to the high proportion of bunker silos. Smaller investments were observed for items related to planting, such as use of hybrid Bt, inoculants and fungicide. A quarter (25%) of farms have drive-over pile silos (Table 1), which have low building costs and mainly occurred in small farms with few animals and low milk production.

Data about feedout losses are from silage wastage and refusals by cattle. In addition, there are losses occurring in the silo during the feedout process. This is due to oxygen penetrating the silo face. This oxygen is used by aerobic spoilage microorganisms, reducing the carbohydrate content of the silage. In this way, the high frequency of feedout losses in the evaluated farms is the opposite of that verified in 88% Brazilian farms that provide spoiled silage for the animals. It may compromise animal health and milk production (Bernardes & Do Rêgo, 2014).

Table 1. Corn silage management practices on the sampled farms.

| Item | Frequency | |
|----------------------|-----------|----|
| | n | % |
| Silage harvesting | | |
| Own | 38 | 35 |
| Outsourced | 70 | 65 |
| Harvester type | | |
| Self-propelled | 59 | 55 |
| Pull-type | 49 | 45 |
| Silo type | | |
| Bunker | 81 | 75 |
| Drive-over pile | 27 | 25 |
| Plastic film color | | |
| Black | 53 | 49 |
| White | 11 | 10 |
| Black-on-white | 42 | 39 |
| Black-on-grey | 2 | 2 |
| Plastic cover | | |
| Soil | 92 | 85 |
| Other | 9 | 8 |
| None | 7 | 7 |
| Silage unload | | |
| Defacer ^a | 17 | 16 |
| Fork | 67 | 62 |
| Bucket ^b | 24 | 22 |

^aMilling type defacer. ^bFront-end bucket loader.

Data about presence of top layer spoilage (Table 2) may be considered high. The type of service had no influence on the visible losses ($p > 0.05$; Table 3). Around 75% silage produced was stored in bunker silos and 85% farmers used soil to cover the plastic film (Table 1), which reduced the occurrence of top spoilage (Table 2). The color of plastic film did not influence the visible losses in silages (Table 1 and Table 3).

Table 2. Occurrence of visible losses in corn silage.

| Item | None | | Low | | Medium | | High | |
|--------------------|--------|-------|-----|-------|--------|-------|------|-------|
| | n | % | n | % | n | % | n | % |
| | Losses | | | | | | | |
| Effluent | 79 | 73.15 | 17 | 15.74 | 6 | 5.56 | 6 | 5.56 |
| Top layer spoilage | 72 | 66.67 | 20 | 18.52 | 11 | 10.19 | 5 | 4.63 |
| During feedout | 3 | 2.78 | 47 | 43.52 | 44 | 40.74 | 14 | 12.96 |

Table 3. Relationship between silage management practices and visible losses in corn silage by Fisher's Exact Test.

| Item | Effluent | Top layer spoilage | During feedout |
|--------------------------------|--------------|--------------------|----------------|
| | $P > \chi^2$ | $P > \chi^2$ | $p > \chi^2$ |
| Harvesting (own or outsourced) | 0.83 | 0.37 | 0.35 |
| Harvester type | 0.96 | 0.36 | 0.02 |
| Silo type | 0.06 | 0.04 | 0.98 |
| Plastic film color | 0.92 | 0.74 | 0.61 |
| Film cover | 0.88 | <0.01 | 0.13 |
| Unloading method | 0.49 | 0.04 | 0.44 |

Harvesting with self-propelled machines increased the occurrence of feedout losses (Table 1), probably because self-propelled harvesters produce a large volume of harvested forage per hour, exceeding the packing capacity per layer in cases where the number and weight of tractors is not properly planned, decreasing the efficiency in silage compaction capacity. Consequently, the increase in silage porosity can favor microbial activity, increasing silage spoilage, which can be verified by the correlation between feedout losses and type of harvester (Table 3).

The spoiled layer below the plastic film was influenced by the silo type, plastic film covering (Borreani et al., 2018) and silage unloading method corroborating data in Table 3. At the same time, the greater the unloading rate, the lower losses (Borreani et al., 2018).

The use of bunker silos decreased the occurrence of losses from top spoilage in relation to drive-over pile silos. The higher losses in drive-over pile silos may be due to lower density compared to bunker silos, as described by Borreani et al. (2018).

High silage density decreases mass porosity, reduces air penetration during silage use and microbiological activity (Gallo et al., 2016; Borreani et al., 2018), decreasing losses from spoilage. Similarly, some management practices can be used to reduce silage losses, such as that observed when farmers cover the plastic films (e.g. tire walls, sand, woven anti-UV cover) (Amaral et al., 2014), or when fill the silo with adequate DM content ($\sim 300 \text{ g kg}^{-1}$) of the forage (Borreani et al., 2018).

Most silos were unloaded by fork (62%) (Table 1), which can indicate the lack of equipment (Bernardes & Do Rêgo, 2014), but reduced the occurrence of top spoilage ($p < 0.05$; Table 3). Silage unloaded by bucket may increase top spoilage in relation to the manual or defacer silage unloader. Probably, the irregular silage removal on the silo face when using bucket could causes cracks, making it more porous. It allows deep oxygen penetration into the ensiled mass, leading to deterioration and spoilage.

The occurrence of top spoilage had no relation with physical and chemical characteristics of the silage ($p > 0.05$), but there was a trend to be influenced by mean particle size, silo face temperature and aNDFom content ($p < 0.10$; Table 4). In this way, the proportion of short particles (retention between 1.18 - 8 mm) was inversely related with feedout losses ($p < 0.05$; Table 4). Therefore, harvesting with self-propelled machinery is not a synonym of well-chopped forage. In contrast, when silage particles are larger than 8 mm, they are able to promote chewing when fed by ruminants, as described by Zebeli et al. (2012).

Table 4. Pearson correlations between physical characteristics, chemical composition and visible losses in corn silages.

| Item | Effluent | | Top layer spoilage | | During feedout | |
|--|----------|-------|--------------------|-------|----------------|-------|
| | r | p > t | r | p > t | r | p > t |
| Physical characteristics | | | | | | |
| Dry matter, g kg^{-1} | -0.22 | 0.02 | -0.07 | 0.49 | 0.05 | 0.60 |
| Wet density, kg as fed m^{-3} | 0.09 | 0.34 | 0.06 | 0.53 | -0.21 | 0.03 |
| DM density, kg DM m^{-3} | -0.17 | 0.07 | -0.02 | 0.82 | -0.16 | 0.10 |
| Particle retention >19 mm, g kg^{-1} | -0.15 | 0.12 | 0.08 | 0.40 | 0.11 | 0.26 |
| Particle retention 8 - 19 mm, g kg^{-1} | 0.17 | 0.09 | 0.07 | 0.49 | 0.12 | 0.23 |
| Particle retention 1.18 - 8 mm, g kg^{-1} | -0.07 | 0.49 | -0.13 | 0.18 | -0.22 | 0.02 |
| Particle retention 0 - 1.18 mm, g kg^{-1} | -0.16 | 0.09 | -0.06 | 0.52 | 0.02 | 0.81 |
| Mean particle size, mm | 0.02 | 0.85 | 0.16 | 0.10 | 0.22 | 0.02 |
| Silo face temperature, °C | 0.12 | 0.22 | 0.17 | 0.08 | 0.31 | 0.001 |
| Aerobic stability, h | 0.004 | 0.97 | 0.11 | 0.27 | -0.12 | 0.22 |
| Chemical characteristics | | | | | | |
| pH | -0.25 | 0.01 | -0.09 | 0.37 | -0.001 | 1.00 |
| Crude protein, $\text{g kg}^{-1}\text{DM}$ | 0.19 | 0.05 | -0.07 | 0.44 | 0.12 | 0.22 |
| aNDFom, $\text{g kg}^{-1}\text{DM}$ | 0.02 | 0.84 | 0.17 | 0.07 | 0.02 | 0.84 |
| ADFom, $\text{g kg}^{-1}\text{DM}$ | -0.03 | 0.77 | 0.13 | 0.20 | 0.004 | 0.97 |
| Starch, $\text{g kg}^{-1}\text{DM}$ | -0.07 | 0.49 | -0.05 | 0.59 | -0.11 | 0.25 |
| IVDMD ^a , g kg^{-1} | -0.12 | 0.22 | 0.04 | 0.70 | -0.004 | 0.97 |
| Zearalenone, $\mu\text{g kg}^{-1}\text{DM}$ | -0.01 | 0.89 | -0.12 | 0.21 | -0.12 | 0.20 |

^a*In vitro* dry matter digestibility.

The presence of effluents showed a trend toward silo type ($p = 0.06$; Table 3). The effluent production was inversely related to silage DM content and silage pH ($p < 0.05$), corroborating Borreani et al. (2018), because the lower DM content of the silage, the greater the losses due to effluent production. However, silage DM density, particle retention on 8 - 19 mm, 0 - 1.18 mm and crude protein tended to be correlated with effluent loss (Table 4).

Silages with the highest effluent losses also had the lowest pH values, possibly because plants were harvested with higher sugar and moisture content, favoring the development of lactic acid bacteria, which is responsible for lactic acid production, with a consequent drop in silage pH, stimulating the fermentation (Senger et al., 2005). Nonetheless, effluent losses are undesirable, because they leach soluble carbohydrates, organic acids, minerals and soluble nitrogen compounds, decreasing the nutritional value of the silage, which was also reported by Senger et al. (2005).

The top spoilage losses had no relation to the physical and chemical silage characteristics ($p > 0.05$; Table 4). However, losses due to effluent production and DM content were inversely proportional, since the effluent volume is influenced by the DM content, compaction level, silo type and plant characteristics (Oliveira et al., 2010). At the same time, top spoilage losses can be minimized when silos are properly

sealed, with the placement of some material on the film cover, preferably soil, which would reduce de DM losses (Amaral et al., 2014; Bernardes & Do Rêgo, 2014).

In general, when farms have silages with high losses as effluents, a great amount of corn silage can be supplied to the animals, increasing the proportion of silage in the total diet offered. This practice can be an attempt to compensate for the quality losses, since silage materials with high moisture content result in losses of nitrogen compounds, carbohydrates and minerals through the effluent (Faria et al., 2010). In accordance to Holly et al. (2018), if not managed properly, silage runoff has the potential to be a major contributor to P and N losses from dairy farms and technologies or practices for abatement has strong potential for improved nutrient management.

The highest frequency of feedout losses was observed when the wet density and particle sizes <8.0 mm were the lowest ($p < 0.05$; Table 4), being still proportional to the average particle size and the silo face temperature. Large particle sizes reduce the compaction efficiency and the silage density, resulting in higher DM losses (D'Amours & Savoie, 2005; Gallo et al., 2016). The silage chemical composition was not related to the types of losses evaluated, except for the pH values, evidencing that nutrient losses are limited to the silo peripheral regions, mainly at the top and lateral layers, where compaction and sealing are not efficient and O_2 infiltration is greater (Borreani et al., 2018).

The milk fat content was the only variable (among milk composition) significantly correlated with silage losses, being higher in silages with the highest top spoilage losses ($p < 0.05$). Furthermore, milk protein and total solids showed a trend toward correlation with feedout losses ($p < 0.10$; Table 5).

Table 5. Pearson correlations between milk composition and visible losses in corn silages.

| Item | Effluent | | Top layer spoilage | | During feedout | |
|---|----------|-------|--------------------|-------|----------------|-------|
| | r | p > t | r | p > t | r | p > t |
| Fat, g kg ⁻¹ | 0.11 | 0.43 | 0.28 | 0.03 | 0.16 | 0.22 |
| Protein, g kg ⁻¹ | -0.15 | 0.25 | 0.03 | 0.80 | 0.25 | 0.05 |
| Lactose, g kg ⁻¹ | -0.17 | 0.24 | -0.13 | 0.38 | -0.06 | 0.67 |
| Total solids, g kg ⁻¹ | 0.11 | 0.46 | 0.21 | 0.15 | 0.25 | 0.09 |
| SCC ^a , cells mL ⁻¹ | 0.06 | 0.65 | 0.03 | 0.84 | -0.15 | 0.26 |

^aSomatic cell count.

When there were the highest top spoilage losses, the fat milk content was the highest, which can be related to the trend toward the largest particle size and highest aNDFom concentration on the spoiled silage layer (Grant, Colenbrander, & Mertens, 1990; Zebeli, Tafaj, Steingass, Metzler, & Drochner, 2006). In contrast, silages with very small particle sizes can reduce the milk fat content as well as the low NDF content of the diets (Zebeli et al., 2006).

Based on the findings, there are great opportunities to improve silage making in the State of Paraná, mainly by improving silage harvesting and unloading.

Conclusion

Most of visible losses occurred when the particle size was larger and silage density was lower. Lower silage DM content, silo type (drive-over pile), absence of plastic cover and use of defacer or bucket for silage unloading increased the extension of silage losses.

References

- Amaral, R. C., Santos, M. C., Daniel, J. L. P., Sá Neto, A., Bispo, A. W., Cabezas-Garcia, E. H., & Nussio, L. G. (2014). The influence of covering methods on the nutritive value of corn silage for lactating dairy cows. *Revista Brasileira de Zootecnia*, 43(9), 471-478. doi: 10.1590/S1516-35982014000900003
- Association Official Analytical Chemist [AOAC]. (2005). *Official Methods of Analysis* (18th ed.). Gaithersburg, MD: AOAC International.
- Bernardes, T. F., & Do Rêgo, A. C. (2014). Study on the practices of silage production and utilization on Brazilian dairy farms. *Journal of Dairy Science*, 97(3), 1852-1861. doi:10.3168/jds.2013-7181
- Borreani, G., Tabacco, E., Schmidt, R. J., Holmes, B. J., & Muck, R. E. (2018). Silage review: Factors affecting dry matter and quality losses in silages. *Journal of Dairy Science*, 101(5), 3952-3979. doi: 10.3168/jds.2017-13837

- D'Amours, L., & Savoie, P. (2005). Density profile of corn silage in bunker silos. *Canadian Biosystems Engineering*, 47, 2.21-2.28. doi: 10.13031/2013.17064
- Faria, D. J. G., Garcia, R., Tonucci, R. G., Tavares, V. B., Pereira, O. G., & Fonseca, D. M. (2010). Produção e composição do efluente da silagem de capim-elefante com casca de café. *Revista Brasileira de Zootecnia*, 39(3), 471-478. doi: 10.1590/S1516-35982010000300004
- Gallo, A., Bertuzzi, T., Giuberti, G., Moschini, M., Bruschi, S., Cerioli, C., & Masoero, F. (2016). New assessment based on the use of principal factor analysis to investigate corn silage quality from nutritional traits, fermentation end products and mycotoxins. *Journal of the Science of Food Agriculture*, 96(2), 437-448. doi: 10.1002/jsfa.7109
- Goeser, J. P., Heuer, C. R., & Crump, P. M. (2015). Forage fermentation product measures are related to dry matter loss through meta-analysis. *The Professional Animal Scientist*, 31(2), 137-145. doi: 10.15232/pas.2014-01356
- Grant, R. J., Colenbrander, V. F., & Mertens, D. R. (1990). Milk fat depression in dairy cows: role of silage particle size. *Journal of Dairy Science*, 73(7), 1834-1842. doi: 10.3168/jds.S0022-0302(90)78863-7
- Holly, M. A., Larson, R. A., Cooley, E. T., & Wunderlin, A. M. (2018). Silage storage runoff characterization: annual nutrient loading rate and first flush analysis of bunker silos. *Agriculture, Ecosystems and Environment*, 264(1), 85-93. doi: 10.1016/j.agee.2018.05.015
- Jobim, C.C., Nussio, L.G., Reis, R.A., & Schmidt, P. (2007). Avanços metodológicos na avaliação da qualidade da forragem conservada. *Revista Brasileira de Zootecnia*, 36(Supl. Esp.), 101-119. doi: 10.1590/S1516-35982007001000013Krüger, A. M.,
- Lammers, B. P., Buckmaster, D. R., & Heinrichs, A. J. (1996). A simple method for the analysis of particle size of forage and total mixed rations. *Journal of Dairy Science*, 79(5), 922-928. doi: 10.3168/jds.S0022-0302(96)76442-1
- Mertens, D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC International*, 85(6), 1217-1240.
- Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter. In C. A. Black (Ed.), *Methods of soil analysis, part 3: Chemical methods* (p. 961-1010). Madison, WI: Soil Science Society of America.
- Oliveira, L. B., Pires, A. J. V., Carvalho, G. G. P., Ribeiro, L. S. O., Almeida, V. V., & Peixoto, C. A. M. (2010). Perdas e valor nutritivo de silagens de milho, sorgo-sudão, sorgo forrageiro e girassol. *Revista Brasileira de Zootecnia*, 39(1), 61-67. doi: 10.1590/S1516-35982010000100008
- Pereira, J. R. A., & Rossi Junior, P. (1995). *Manual prático de avaliação nutricional de alimentos*. Piracicaba, SP: Fealq.
- Scott, P. M. (1997). Natural toxins. In P. Cunniff (Ed.), *Official methods of analysis* (16th ed). Gaithersburg, MD: AOAC International.
- Senger, C. C. D., Mühlbach, P. R. F., Sánchez, L. M. B., Netto, D. P., & Lima, L. D. (2005). Composição química e digestibilidade 'in vitro' de silagens de milho com distintos teores de umidade e níveis de compactação. *Ciência Rural*, 35(6), 1393-1399. doi: 10.1590/S0103-84782005000600026
- Sistema de Tecnologia e Monitoramento Ambiental do Paraná [SIMEPAR]. (2019). *Boletim climatológico inverno/2019*. Recovered from http://www.simepar.br/prognozweb/simepar/timeline/boletim_climatologico.
- Statistical Analysis Software [SAS]. (2011). *SAS/STAT User guide, Version 9.3*. Cary, NC: SAS Institute Inc.
- Tilley, J. A., & Terry, R. A. (1963). A two-stage technique of the *in vitro* digestion of forage crops. *Grass and Forage Science*, 18(2), 104-111. doi: 10.1111/j.1365-2494.1963.tb00335.x
- Zar, J. H. (2010). *Biostatistical Analysis* (5th ed). Upper Saddle River, NJ: Pearson Prentice-Hall.
- Zebeli, Q., Aschenbach, J. R., Tafaj, M., Boquhn, J., Ametaj, B. N., & Drochner, W. (2012). Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. *Journal of Dairy Science*, 95(3), 1041-1056. doi: 10.3168/jds.2011-4421
- Zebeli, Q., Tafaj, M., Steingass, H., Metzler, B., & Drochner, W. (2006). Effects of physically effective fiber on digestive processes and milk fat content in early lactating dairy cows fed total mixed rations. *Journal of Dairy Science*, 89(2), 651-668. doi:10.3168/jds.S0022-0302(06)72129-4