

## EFFECT OF THE ADDITIVES MILK WHEY, *LACTOBACILLUS CASEI* AND *L. ACIDOPHILLUS* ON THE QUALITY OF SORGHUM SILAGE<sup>1</sup>

L. L. Schumcher<sup>3\*</sup>, J. Viégas<sup>2</sup>, S. N. Pereira<sup>2</sup>, T. J. Tonin<sup>2</sup>, L. T. Rocha<sup>2</sup>, R. F. Bermudes<sup>4</sup>, R. P. Carpes<sup>5</sup>

<sup>1</sup>Received: 04/07 /2019. Approved: 22/10/2019.

<sup>2</sup>Universidade Federal de Santa Maria, Programa de Pós Graduação em Zootecnia, Santa Maria, RS, BR.

<sup>3</sup>Universidade Federal de Santa Maria, Curso de Zootecnia, Santa Maria, RS, BR.

<sup>4</sup>Universidade Federal de Pelotas, Programa de Pós Graduação em Zootecnia, Pelotas, RS, BR.

<sup>5</sup>Universidade Federal de Santa Maria, Curso de Agronomia, Santa Maria, RS, BR.

\*E-mail: [larischumacher@hotmail.com](mailto:larischumacher@hotmail.com)

**ABSTRACT:**With the aim to evaluate the effects of the use of additives (milk whey and inoculant containing *Lactobacillus casei* and *Lactobacillus acidophilus*) in sorghum silage, an experiment was conducted in which the productive parameters, fermentative and the nutritional quality of silage were analyzed. The experimental design was completely randomized, testing different levels of inclusion of whey and live *Lactobacillus* strains based on forage green matter, constituting the following treatments: 1) Sorghum without inclusion of additives (CON); 2) Sorghum +  $5 \times 10^2$  cfu/g *L. casei* and *L. acidophilus* (LAC); 3) Sorghum  $5 \times 10^2$  cfu g<sup>-1</sup> whey (SL); 4) Sorghum +  $5 \times 10^2$  cfu/g-1 *L. casei* and *L. acidophilus* and  $5 \times 10^2$  cfu/g whey (LAC+SL). When *Lactobacillus* was included the mixture used was 50 ml of water and 10 grams of *Lactobacillus*. To measure silage fermentation, pH, dry matter recovery, gas and effluent losses, ammonia nitrogen, buffer power and density were estimated. The addition of whey and *Lactobacillus* inoculant in sorghum silage did not significantly change the bromatological values of silage when compared to the original material. The inclusion of additives increased ( $P < 0.05$ ) the effluent losses during fermentation. The use of whey presents a viable alternative for producers, as it does not compromise the fermentation and chemical composition of sorghum silage and assists in the reduction of dairy residues in nature.

**KEY WORDS:** silage, fermentation, grass, by-product.

### EFEITO DA ADIÇÃO DE ADITIVOS: SORO DO LEITE, *LACTOBACILLUS CASEI* E *ACIDOPHILLUS* SOBRE A QUALIDADE DA SILAGEM DE SORGO

**RESUMO:** Com o objetivo de avaliar os efeitos do uso de aditivos (soro de leite e inoculante contendo *Lactobacillus casei* e *L. acidophilus*) em ensilagem de sorgo, foi conduzindo um experimento em que se analisou os parâmetros produtivos, fermentativos e a qualidade nutricional das silagens. O delineamento experimental foi o inteiramente casualizado, sendo testados diferentes níveis de inclusão de soro de leite e cepas de *Lactobacillus* vivos com base na matéria verde da forragem, constituindo os seguintes tratamentos: 1) Sorgo sem inclusão de aditivos (CON); 2) Sorgo +  $5 \times 10^2$  ufc g<sup>-1</sup> *L. casei* e *L. acidophilus* (LAC); 3) Sorgo  $5 \times 10^2$  ufc g<sup>-1</sup> soro de leite (SL); 4) Sorgo +  $5 \times 10^2$  ufc g<sup>-1</sup> *L. casei* e *L. acidophilus* e  $5 \times 10^2$  ufc g<sup>-1</sup> de soro de leite (LAC+ SL). Quando houve inclusão de *Lactobacillus* a mistura utilizada foi 50 ml de água e 10 gramas de *Lactobacillus*. Para mensurar a fermentação da silagem, estimou-se o pH, recuperação de matéria seca, perdas de gases e efluentes, nitrogênio amoniacal, poder tampão e densidade. A adição de soro de leite e do inoculante com *Lactobacillus* na ensilagem de sorgo, não modificou significativamente os valores bromatológicos da silagem, quando comparada ao material original. A inclusão dos aditivos aumentou ( $P < 0,05$ ) as perdas por efluentes durante a fermentação. A utilização do soro de leite apresenta uma alternativa viável para os produtores, pois não compromete a fermentação e a composição química da silagem de sorgo e auxilia na diminuição de resíduos lácteos na natureza.

Palavras-chave: silagem, fermentação, capim, subproduto

## INTRODUCTION

To minimize the disappearance of digestible nutrients and boost lactic fermentation, the use of chemical additives or microbiological inoculants is recommended (ZOPOLLATO et al., 2009). Within this category, according to Muck, 2010, the *Lactobacillus acidophilus* inoculants are part of the group of obligatory homofermentative bacteria, where they are capable of converting the carbon of hexoses into lactic acid. *Lactobacillus casei*, on the other hand, are gram-positive facultative heterofermentative bacteria that induce phosphorquelatase, producing acetic acid and lactic acid as final products (SILVA et al., 2011).

Another product that may be able to favor the production of organic acids and lactic acid in anaerobic environment is whey. This material is a residual by-product of dairy agribusiness, resulting from the processing of milk in derived products.

Whey retains 55% of milk nutrients, containing in its composition lactose, soluble proteins, salts, lactic acid and other nutrients in smaller amounts such as vitamin B (BARBOSA et al., 2010).

It has low commercial value when fresh, and its excess can cause serious problems of environmental pollution, Silva et al. (2018) states that whey is approximately one hundred times more polluting than domestic sewage because it has a high biological oxygen demand and a significant rate of organic matter (MAGALHÃES et al., 2011).

Thus, one of the alternatives for the reuse of whey from the dairy industry residues and reducing the environmental impact is the use in grass silage as a source of lactic bacteria, which may boost the fermentation profile of the silo (SANTOS et al., 2006).

Thus, the objective of this paper was to evaluate the effects of the inclusion of whey, *Lactobacillus casei* and *Lactobacillus acidophilus* on the fermentation and nutritional quality of sorghum silage.

## MATERIAL AND METHODS

The experiment was conducted by the Additives in Animal Production Study Group (GEAPA), and bromatological analyzes were performed at the Ruminant Bromatology and Nutrition Laboratory (LABRUMEN) of the Federal University of Santa Maria located in the Central Depression region in Rio Grande do Sul.

The experimental period was from December 2012 to August 2013. Grass sowing was performed manually on December 17, 2012 with sowing density according to recommendations for the Convert™ SS318 (Brevant) sorghum hybrid with conventional tillage. Potassium and phosphoric fertilizations were made according to the recommendations expressed by the Fertilization and Liming Manual (ROLAS, 2004). The urea based nitrogen fertilization was 120 kg/ha, divided in two applications.

Three plants were harvested from each experimental sample for manual separation of structural components: stem, leaf, senescent material and panicle. The samples of the structural components were dried in a forced ventilation oven at 55 ° C for 96 hours to obtain the estimate of the partially dry matter and to determine the crop production and morphological components, shown in table 1.

Plants were harvested for the ensiling process on May 1, 2013, after 134 days of cultivation, when the hybrid presented 29.8% of dry matter. The plants were manually cut at 0.20 m from the ground and processed in a forage mill to obtain an average particle size of 2 cm. After mincing, 8 Kg of material were weighed and tested for different levels of inclusion of whey and live lactobacillus strains based on forage green matter, which constituted the following treatments: 1) Sorghum without additives (CON); 2) Sorghum +  $5 \times 10^2$  cfu/g *L. casei* and *L. acidophilus* (LAC); 3) Sorghum +  $5 \times 10^2$  cfu/g whey (SL); 4) Sorghum +  $5 \times 10^2$  cfu/g *L. casei* and *L. acidophilus* and  $5 \times 10^2$

**Table 1.** Structural composition values of sorghum plants, Convert™ SS318 hybrid.

	BOTANICAL COMPOSITION (%)				
	Leaf	Colm	Panicle	Senescent Material	DM Production Kg/ha
Dry Matter	11.99	53.11	32.09	2.81	20.674

cfu/g whey (LAC + SL). When *Lactobacillus* was included, the mixture used was 50 ml of water and 10 grams of *Lactobacillus* sprayed on the material to be ensiled.

The material was manually compacted in 20 liter buckets and then sealed in micro silos with 4 plastic bags to protect against air ingress and light. The sequence of the plastic bags followed this order: the first bag with holes in the bottom was intended for the discharge of effluents and accommodation of the ensiled material; the second bag contained washed and kiln-dried sand for effluent absorption; the third bag was used for better sealing of the others, and the fourth bag was dark for light protection. The experimental silos contained 8 kg of silage and 2 kg of sand.

Buffering capacity (BC) was analyzed using the original material described by Playne and McDonald (1966), in which 0.015 kg of forage sample was diluted in 250 ml of distilled water and titrated to pH 3.0 with HCl (0.1 N) and then titrated with NaOH.

The silos were opened after 90 days of fermentation. First, the bag, sand and silage set were weighed, then each component separately in order to determine the effluent loss, gas and DM recovery. The equations cited by Jobim et al. (2007) were used:

$$E = (Pab - Pen) / (MVfe) \times 1000$$

E = Effluent production (kg/t of green mass);  
Pab = Weight of the set (silo+sand+cloth+canvas) upon opening (kg);  
Pen = Weight of the set (silo+sand+cloth+canvas) in ensilage (kg);  
MVfe = Green mass of ensiled forage (kg).

$$G = [(PCen - Pen) * MSen] - [(PCab - Pen) * MSab] \times 100 [(PCen - Pen) * MSen]$$

G = Loss by gas in % of DM;  
PCen = Weight of full silo upon ensilage (kg);  
Pen = Weight of the set (silo+lid+sand+canvas+cloth) upon ensilage (kg);  
MSen = Content of DM of forage upon ensilage (%);  
PCab = Weight of full silo upon opening (kg);  
MSab = DM content of forage upon opening (%).

$$RMS = [(MFs \times MSs) / (MFf \times MSf)] \times 100$$

RDM = recovery of DM;  
MFs: forage mass of silage, composed by the weight of the bag + silage;  
MFf = forage mass prior to silo, composed by the weight of the bag + forage;  
MSs = content of silage DM;  
MSf = content of forage DM.

The remaining material was homogenized and a subsample of silage was removed to determine the pH in a digital potentiometer (SILVA and QUEIROZ, 2002). With the aid of a press, the extract was removed for determination of ammonia nitrogen (N-NH<sub>3</sub>) by the Weatherburn colorimetry method (1967).

Subsequently, another sample of silage was taken and submitted to a forced air oven with an average temperature of 55°C until the constant weight was reached. Later, the sample was milled in a Willey type mill with 1 mm sieve (chemical analysis) and 2 mm sieve (digestibility estimate). The evaluation was carried out with dry matter (DM) in a greenhouse at 105°C for a minimum of 8 hours, organic matter (OM), mineral matter (MM) by incineration in 550°C muffle for 4 hours and crude protein (CP) by micro Kjeldahl method according to AOAC (1995).

Neutral detergent fiber (NDF) levels were obtained using thermostable  $\alpha$ -amylase (Termamyl 120L, Novozymes Latin America, LTDA) with autoclave, according to the technique described by Senger et al. (2008). The acid detergent fiber (ADF) content and lignin content (ADL) were achieved according to the methodology of Van Soest et al. (1991), with LDA being extracted with the use of sulfuric acid 72%. Hemicellulose (HEM) and cellulose contents were obtained by differences between the levels (NDF and ADF) and (ADF and lignin), respectively.

In situ dry matter degradability was estimated according to Mehrez and Orskov (1997) methodology with 48 hours of incubation for all treatments. 1.0 g of sample in 41 $\mu$  porosity polyamide bags was weighed. Subsequently, inoculum of fistulated cattle was collected, which were adapted with silage. After the incubation period, the bags were removed, washed and underwent a neutral detergent (NDF) analysis process. They were

dried in an oven at 105°C for 8 hours and then burned in a muffle furnace at 500°C for 24 h to evaluate the percentage of residual mineral matter and calculate the percentage of missing organic OM. The energy content (TDN) was estimated through the degradability of organic matter and the NDF value proposed by Cappelle et al. (2001), by the equation:

$$\text{NDT} = 3.71095 - 0.129014\text{FDN} + 1.02278 \times \text{DMO}$$

The experimental design was completely randomized with four treatments and four replications per treatment (4x4). The experimental model  $y_{ij} = \mu + t_i + e_{ij}$  was used, where  $y_{ij}$  = value observed in the experimental unit that received treatment  $i$ , repetition  $j$ ;  $\mu$  = overall effect of the mean;  $t_i$  = effect of treatment  $i$ ;  $e_{ij}$  = random error (residue). Data were subjected to analysis of variance and when there was difference the means were compared by Tukey test at 5% probability of error. Statistical analyzes were conducted using the SAS®Viya® and SAS Studio 5.1 statistical package.

## RESULTS AND DISCUSSION

The treatment with the highest effluent losses was when whey associated with *Lactobacillus* was added ( $P < 0.05$ ), since whey has high moisture contents. According to Ferreira et al. (2013), silages with high effluent production may have negative impacts on silage quality,

given the nature of lost products. This causes loss of nutrients and soluble compounds through the leaching mechanism.

Santos et al. (2006), when performing an experiment on the effect of adding cheese whey to elephant grass silage, also found increased effluent losses in elephant grass silage treatment + 5% whey (36.13 Kg / Ton). In the work of Cajarville et al. (2012), a 30% linear increase ( $P < 0.01$ ) in effluent production was observed when whey was added to silage.

Despite the variations found in PE, dry matter recovery, gas losses and density were not influenced by the addition of additives. From these results, it can be stated that there was no enterobacterial fermentation. These microorganisms are competitors of acidolactic bacteria for soluble products, forming gases inside the silo and thus causing losses of dry matter and energy.

Results found in the present study regarding gas losses, with an average of 2.84% in DM, were low compared to the silage of Anjos et al. (2018), who observed losses of 11.08% in dry matter.

Kung et al. (2018) describe that the combination of homofermentative and heterofermentative bacteria provide adequate lactic fermentation and good dry matter recovery, in addition to an increase in acetic acid concentrations produced by heterofermentative bacteria ( $\text{glucose} + \text{ADP} + \text{Pi} = \text{lactate} + \text{acetate} + \text{CO}_2 + \text{H}_2\text{O}$ ).

**Table 2.** Evaluation of fermentative characteristics and nutritional losses of sorghum silages treated or not with whey and / or *Lactobacillus*

VARIABLES	TREATMENTS				MEANS	SD (%)	P>F
	CON	LAC	SL	LAC+SL			
LE (kg/Ton of DM)	8.34 <sup>c</sup>	9.73 <sup>ab</sup>	9.08 <sup>bc</sup>	10.62 <sup>a</sup>	9.44	0.63	0.002
LG (% DM)	2.53	2.83	2.82	3.19	2.84	0.32	0.09
RDM (% DM)	88.39	85.07	90.84	90.61	88.73	3.97	0.22
Density (Kg/m <sup>3</sup> )	632.00	619.10	631.50	576.80	614.85	52.80	0.43
pH (%)	3.96	3.97	3.96	3.95	3.96	0.009	0.28
N-NH <sub>3</sub> *	8.00	8.00	9.00	9.00	8.50	0.03	0.94
Buffer Power**	9.69	9.59	9.5	8.82	9.40	0.94	0.57

Means followed by distinct uppercase letters in the same column and distinct lowercase letters in the same row differ ( $P < 0.05$ ) by the Tukey test.

CON=control; LAC= *Lactobacillus casei* and *acidophilus*; SL= Whey; MIST= *Lactobacillus* + Whey; SD= Standard Deviation; LE= Loss by effluents; LG= Loss by Gases; RDM= Recovery of Dry Matter;

\*% of Ammoniacal Nitrogen in relation to total nitrogen; \*\*eq mg HCL/100g MS

Density with an average volume of 700 kg / m<sup>3</sup> with 30-40% DM is recommended by Borreani (2018), who proposes that high density silages present high compaction, low porosity, resulting in lower DM losses. Even though the average volume of 614.85 kg / m<sup>3</sup> is below the recommended value, the dry matter recovery value is present in the literature.

For pH and N-NH<sub>3</sub> values, no differences (P > 0.05) were observed between treatments. The pH and N-NH<sub>3</sub> are criteria, as the organic acids used in the literature as qualitative evaluation parameters of silages, and the silages of the present study are classified as good quality.

For Mc Donald et al. (1991) the ideal pH for silages is in the range of 3.8 to 4.2. Analyzing the results found, the silages of the present study apparently had a rapid drop in pH after silo closure, resulting in lower nutrient losses, higher conversion of plant soluble carbohydrates to organic acids (Muck, 2010) and growth inhibition of undesirable microorganisms such as clostridia and yeast (MACÊDO et al., 2017).

When Repetto et al. (2011) determined the effects of the use of fresh whey as an additive at different levels (20, 50 and 100 g / kg) on alfalfa silages, they reported that the use of whey led to a linear reduction in pH content.

Tabacco et al. (2011), when incorporating *Lactobacillus buchneri* or *Lactobacillus plantarum* in sorghum silage, obtained pH levels of 3.95 and 3.78 respectively. These values are similar to the ones found in the present study.

Ammoniacal N is indicative of protein deamination during the fermentation phase (OLIVEIRA et al., 2010). The values found in the present study are lower than the ones found in the literature, in which concentrations below 10-15% of ammoniacal N, compared to total-N, result in an adequate fermentation process. In the study by Thomas et al. (2013), they added mixtures of *Lactobacillus buchneri*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactobacillus plantarum* and *Enterococcus faecium* in sorghum silage reaching 2.67% N-NH<sub>3</sub> content in relation to total nitrogen.

Buffer power reflects the ability to resist changes in pH, as determined by buffering substances, which are represented in plants by inorganic bases and organic salts (SILVA et al., 2011). In the present research, the buffering power showed no difference between the treatments, presenting an average of 9.40%. Silva et al. (2010), when using a combination of lyophilized *Lactobacilli casei* and *Streptococcus faecalis* in their treatments did not promote statistical difference in the buffer power values in the evaluated silages.

**Table 3.** Bromatological composition of sorghum silage with the addition (%DM) of  $5 \times 10^2$  cfu/g of whey and inoculated with *L. casei* and *L. acidophilus*.

VARIÁVES	TREATMENTS							
	FM	CON	LAC	SL	LAC+SL	Means	DP	P>F
DM (%)	29.81	29.89	29.3	29.54	28.02	29.31	1.44	0.32
CP	7.52	7.35	7.17	7.41	7.82	7.45	0.39	0.17
MM	5.17	4.55	4.83	4.43	4.51	4.70	0.19	0.06
OM	94.82	95.44	95.41	95.63	95.48	95.36	0.21	0.51
NDF	43.50	47.31	46.08	44.64	48.39	45.98	2.74	0.29
ADF	18.80	25.24	24.81	23.33	25.93	23.62	1.87	0.29
HEM	24.69	22.97	21.25	21.3	22.45	22.53	1.11	0.33
LDA	5.57	1.33	1.17	1.24	2.20	2.30	0.77	0.25
DOM(NDT)	60.56	63.64	59.11	58.92	59.81	60.41	4.31	0.4
Apa. Dig. OM	63.87	66.68	62.11	61.66	62.65	63.39	4.49	0.4

MF = Fresh Material; CON= control; LAC= *L. casei* e *L. acidophilus*; SL= whey; LAC+SL= Whey and *L. casei* and *L. acidophilus*; SD = Standard Deviation; DM= Dry matter; CP = Crude Protein; MM= Mineral Matter; MO= Organic Matter; FDN= Neutral Fiber Detergent; FDA= Acid Detergent Fiber; HEM= Hemicellulose; LDA= Lignin; DOM= Digestibilidade Of Organic Matter; Apa. Dig. OM= Aparent Digestibility of Organic Matter.

The inclusion of whey or *L. casei* and *L. acidophilus* strains did not significantly change the dry matter content compared to the control (Table 3).

Adequate levels of DM are required for good lactic fermentation to occur. Oliveira et al. (2011) and Macêdo et al. (2017) recommend cutting sorghum for silage with 28 to 40% DM, ie, stage in which the grains are in the farinaceous point, a situation observed in the present work. Santos et al. (2006) added 5% whey to elephant grass silage and found a 0.57% decrease in DM content compared to control silage.

Regarding CP content, no significant differences were observed between treatments, since the added products have low protein in their composition. According to Van Soest (1994), the ruminal flora requirements are at least 7% CP. The materials analyzed in the research presented adequate CP levels to meet the microbial growth demand.

The inclusion of additives in silage did not significantly affect the contents of OM and MM. Britos et al. (2007) also did not detect differences from the levels of OM when they added three cheese whey levels (2, 5 and 10) to a silage obtained from a mixture of grasses and legumes.

With respect to silage fiber fraction, the NDF, ADF, HEM and LDA contents of the silages did not respond significantly to the different inclusions of whey or *Lactobacillus* strains. The average contents of 45.98%, 23.62%, 22.53% and 2.30% respectively of NDF, ADF, HEM and LDA are lower than those found in the literature.

These fractional contents of the silage fibrous components are considered excellent and demonstrate that when the tested additives are included in the silage, there is no change in the fibrous constituents. Thus, it indicates that the use of forage and the compromise of consumption cannot be affected.

Macedo et al. (2012), when evaluating the fermentative profile and bromatological composition of sorghum silages in response to nitrogen fertilization, found values higher than those of the present study, with averages of 65.45% for NDF, 51.33% for ADF, 16, 15% for hemicellulose and 9.72% for lignin.

The DOM values and apparent digestibility of organic matter showed no significant

difference between treatments, presenting an average of 60.41% and 63.39% respectively. Stella et al. (2016) considered in their work that for a silage to be considered of good quality, it must have 64 to 70% of TDN. Considering this estimate, silages were close to ideal (60.41%). However, it is noteworthy that the TDN values were estimated by equation, which does not present an absolute accuracy.

## CONCLUSION

The addition of whey and / or *Lactobacillus casei* and *L. acidophilus* makes it possible to obtain good quality sorghum silage, as it does not compromise bromatological composition, nutritional values and digestibility in relation to the control treatment. However, there was an increase in effluent losses, which may cause leaching of soluble compounds.

## REFERENCES

- ANJOS, G.V.S.; GONÇALVES, L.C.; RODRIGUES, J.A.S.; KELLER, K.M.; COELHO, M.M.; MICHEL, P.H.F.; OTTONI, D.; JAYME, D.G. Effect of re-ensiling on the quality of sorghum silage. **Journal of Dairy Science**, v.101, p.1-8, 2018. <https://doi.org/10.3168/jds.2017-13687>
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. **Official methods of analysis**. 16. ed. Washington, D.C.: AOAC, 1995. 2000p.
- BARBOSA, A.S.; ARAUJO, A.S.; FLORENCIO, I.M.; BEZERRA, R.R.A.; FLORENTINO, E.R. Estudo cinético da fermentação do soro de queijo de coalho para produção de aguardente. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v.5, p.237-254, 2010. <https://www.gvaa.com.br/revista/index.php/RVADS/article/view/329/329>
- BORREANI, G.; TABACCO, E.; SCHMIDT, R.J.; HOLMES, B.J.; MUCK, R. E. Silage review: Factors affecting dry matter and quality losses in silages. **Journal Dairy Science**, v.101, p.3952-3979, 2018. <https://doi.org/10.3168/jds.2017-13837>
- BRITOS, A.; REPETTO, J. L.; GARCIARENA, D.; CAJARVILLE, C. Efecto del suero de queso como aditivo de ensilajes de pastura sobre la conservación, los azúcares solubles y la producción de gas in vitro. **Revista**

- Agrociencia**, v.11, p.72-77, 2007. <http://www.fagro.edu.uy/agrociencia/index.php/directorio/article/view/194/134>
- CAJARVILLE, C.; BRITOS, A.; GARCIARENA, D.; REPETTO, J.L. Temperate Forages ensiled with molasses or fresh cheese whey: Effects On conservation quality, effluent losses and ruminal degradation. **Animal Feed Science and Technology**, v.171, p.14-19, 2012. <https://doi.org/10.1016/j.anifeedsci.2011.09.016>
- CAPPELLE, E. R.; VALADARES FILHO, S.C.; SILVA, J.F.C.; CECON, P.R. Estimativas do valor energético partir de características químicas e bromatológicas dos alimentos. **Revista Brasileira de Zootecnia**, v. 30, p.1837-1856, 2001. <https://doi.org/10.1590/s1516-35982001000700022>
- FERREIRA, D.J.; LANA, R.P.; ZANINE, A.M.; SANTOS, E.M.; VELOSO, C.M.; RIBEIRO, G.A. Silage fermentation and chemical composition of elephant grass inoculated with rumen strains of *Streptococcus bovis*. **Animal Feed Science and Technology**, v.183, p.22-28, 2013. <http://dx.doi.org/10.1016/j.anifeedsci.2013.04.020>
- JOBIM, C.C.; NUSSIO, L.G.; REIS R. A.; SCHMIDT, P. Avanços metodológicos na avaliação da qualidade da forragem conservada. **Revista Brasileira de Zootecnia**, v.36, p.101-119, 2007. <http://dx.doi.org/10.1590/S1516-35982007001000013>
- KUNG, J.R.L.; SHAVER, R.D.; GRANT, R.J.; SCHMIDT, R.J. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. **Journal of Dairy Science**, v. 101, p. 4020-4033, 2018. <https://doi.org/10.3168/jds.2017-13909>
- MACÊDO, A.S.J.; SANTOS, E.M.; OLIVEIRA, J.S.; PERAZZO, A.F. Microbiologia de silagens: Revisão de Literatura. **Revista Eletrônica de Veterinária**, v.18. p.1-11, 2017.
- MACEDO, C.H.O.; ANDRADE, A.P.; SANTOS, E.M.; SILVA, D.S.; SILVA, T.C.; EDVAN, R.L. Perfil fermentativo e composição bromatológica de silagens de sorgo em função da adubação nitrogenada. **Revista Brasileira de Saúde e Produção Animal**, v.13, p.371-382. 2012. <http://revistas.ufba.br/index.php/rbspa/article/view/2135>
- MAGALHÃES, J.A.; RODRIGUES, B. H.N.; NEWTON DE LUCENA COSTA, N.L.C.; TOWNSEND, C.R.; PEREIRA, R.G.A.; PEIXOTO, M.J.A.; FERREIRA COSTA, M.R.G.F. Silagem mista de capim-elefante e leucena: proteína bruta e minerais. **Publicações em Medicina Veterinária e Zootecnia**, Londrina, v. 5, ed. 178, 2011. <http://www.pubvet.com.br/artigo/1998/silagem-mista-de-capim-elefante-e-leucena-proteiacutena-bruta-e-minerais>
- McDONALD, P.; HENDERSON, A.R.; HERON, S.J.E. **The biochemistry of silage**. 2ª ed. Mallow: Chalcombe Publications, 1991. 340p.
- MEHREZ, A. Z.; ORSKOV, E. R. A study of the artificial fibre bag technique for determining the digestibility of feed in the rumen. **Journal of Agricultural Science**, v. 88, p. 645-650, 1977. <https://doi.org/10.1017/s0021859600037321>
- MUCK, R.E. Silage microbiology and its control through additives. **Revista Brasileira de Zootecnia**, v. 39, p. 183-191, 2010. <http://dx.doi.org/10.1590/S1516-35982010001300021>
- OLIVEIRA, L.B.; PIRES, A.J.V.; CARVALHO, G.G.P.; RIBEIRO, L.S.O.; ALMEIDA, V.V.; PEIXOTO, C.A.M. Perdas e valor nutritivo de silagens de milho, sorgo-sudão, sorgo forrageiro e girassol. **Revista Brasileira de Zootecnia**, v. 39, p. 61-67, 2010. <http://www.scielo.br/pdf/rbz/v39n1/08.pdf>
- OLIVEIRA, M.R.; NEUMANN, M.; OLIBONI, R.; GOBETTI, S.T.C.; FARIA, M.V. Uso de aditivos biológicos na ensilagem de forrageiras. **Ambiência - Revista do Setor de Ciências Agrárias e Ambientais**, v.7, p. 589-601, 2011. <https://doi.org/10.5777/ambiencia.2011.03.01rb>
- PLAYNE, M.J.; MCDONALD, P. The buffering constituents of herbage and of silage. **Journal of the Science of Food and Agriculture**, v.17, p. 264-268, 1996.
- REPETTO, J.L.; ECHARRI, V.; AGUERRE, M.; CAJARVILLE, C. Use of fresh cheese whey as an additive for Lucerne silages: Effects on chemical composition, conservation quality and ruminal degradation of cell walls. **Animal Feed Science and Technology**, v. 170, p.160-164, 2011. <https://doi.org/10.1016/j.anifeedsci.2011.09.004>
- REDE OFICIAL DE LABORATÓRIOS DE ANÁLISE DE SOLO E DE TECIDO

- VEGETAL - ROLAS. **Manual de adubação e calagem para os estados do Rio Grande do Sul e Santa Catarina**. 10.ed. Sociedade Brasileira de Ciência do Solo, 2004. 400 p.
- SANTOS, E.; ZANINE, A.; FERREIRA, D.; OLIVEIRA, J.; PEREIRA, O.; ALMEIDA, J. Efeito da adição do soro de queijo sobre a composição bromatológica, fermentação, perdas e recuperação de matéria seca em silagem de capim-elefante. **Ciência Animal Brasileira**, v.7, p.235-239, 2006. <https://www.revistas.ufg.br/vet/article/view/416>
- SENGER, C.C.D.; KOZLOSKI, G.V.; SANCHEZ, L.M.B.; MESQUITA, F.R.; ALVES, T.P.; CASTAGNINO D.S. Short communication: Evaluation of autoclave procedures for fibre analysis in forage and concentrate feedstuffs. **Animal Feed Science and Technology**, v. 146, p.169-174, 2008. <https://doi.org/10.1016/j.anifeedsci.2007.12.008>
- SILVA, D. J.; QUEIROZ, A.C. **Análise de Alimentos: Métodos químicos e biológicos**. 3 ed. Viçosa: UFV, Imprensa Universitária, Viçosa, MG, 2002. 165p.
- SILVA, R.R.; SIQUEIRA, E.Q.; NOGUEIRA, I.S.; Impactos ambientais de efluentes de laticínios em curso d'água na Bacia do Rio Pomba. **Engenharia Sanitária Ambiental**, v.23, p. 217-228, 2018. <https://doi.org/10.1590/S1413-41522018138062>
- SILVA, J.M.; CARNAÚBA, J.C.; SILVA, I.O.; ANDRADE, D.E.G.T; MIRANDA, E.C.; AMORIN, E.P.R. Influência de inoculante bacteriano-enzimático sobre a microbiota e qualidade nutricional de silagens de grãos úmidos de milho. **Ciência Animal Brasileira**, v. 11, p. 62-72, 2010. <https://doi.org/10.5216/cab.v11i1.4916>
- SILVA, T.C.; SILVA, M.V.; FERREIRA, E.G.; PEREIRA, O.G.; FERREIRA, C.L.L.F. Papel da fermentação láctica na produção de silagem. **Publicações em Medicina Veterinária e Zootecnia**, v. 5, p. 1-17, 2011. <http://www.pubvet.com.br/uploads/f2638ee5f0404a6b92f3de75abf1a222.pdf>
- STATISTICAL ANALYSIS SYSTEM - SAS. **Statistical analysis system user's guide**. Cary: Statistics Cary SAS Institute, 2001.
- STELLA, L.A.; PERIPOLLI, V.; PRATES, E.R.; BARCELLOS, J.O.J. Composição química das silagens de milho e sorgo com inclusão de planta inteira de soja. **Boletim da Indústria Animal**, v.73, p. 73-79, 2016. <http://dx.doi.org/10.17523/bia.v73n1p73>
- TABACCO, E.; RIGHI, F.; QUARANTELLI, A.; BORREANI, G. Dry matter and nutritional losses during aerobic deterioration of corn and sorghum silages as influenced by different lactic acid bacteria inocula. **Journal of Dairy Science**, v.94, p.1409-1419, 2011. <https://doi.org/10.3168/jds.2010-3538>
- THOMAS, M.E.; FOSTER, J. L.; MCCUITION, K. C.; REDMON, L. A.; JESSUP, R. W. Nutritive value, fermentation characteristics, and in situ disappearance kinetics of sorghum silage treated with inoculants. **Journal of Dairy Science**, v. 96, p.1-12, 2013. <https://doi.org/10.3168/jds.2013-6635>
- VAN SOEST, P.J. **Nutritional ecology of the ruminant**. 2ed. Ithaca, NY: Cornell University Press, 1994. 476p.
- VAN SOEST, P.J.; ROBERTSON, J.B.; LEWIS, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v.74, p.3583-3597, 1991. [https://doi.org/10.3168/jds.s0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.s0022-0302(91)78551-2)
- WEATHERBURN, M.W. Phenol-hypochlorite reaction for determination of ammonia. **Analytical Chemistry**, v. 39, p. 971-974, 1967.
- ZOPOLLATTO, M.; NUSSIO, L.G.; MARI, L.J.; SCHMIDT, P.; DUARTE, A.P.; MOURÃO, G.B. Alterações na composição morfológica em função do estágio de maturação em cultivares de milho para produção de silagem. **Revista Brasileira de Zootecnia**, v.38, p.452-461, 2009. <http://dx.doi.org/10.1590/S1516-35982009000300008>