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RESEARCH ARTICLE

# Use of spent brewer's yeasts as an additive of pasture silages and its effects on nutritional quality and pH

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## ABSTRACT

Livestock systems at Region Aysén del General Carlos Ibáñez del Campo depend on forage conservation during the spring-summer season, generally with low nutritional quality to animal requirements. To reduce nutrient loss, additives are commonly used, being spent brewery yeast a potential additive. Therefore, a study was carried out with the inclusion of two spent brewer's yeast (Lager and Ale) as an additive in pasture mini silos compared to two commercial additives (SiloSolve MC™ and Josilac grass™) and mini silo without additive. It was observed that spent brewer's yeast has a negative effect on crude protein and metabolizable energy, contents that decreased to 9.8% and 2.5%, respectively, compared to control. However, Ale yeast declined faster and maintained lower pH levels below 4.1. Spent brewer's yeast do not improve nutritional quality, however decreased pH levels of pasture silage. Therefore, it should be noted that the effect of spent brewer's yeasts has been insufficiently evaluated. Thus, is still a lack of trials within large scale of silage conservation and its effects on animal feed performance.

**Key words:** Additive, Brewer's yeasts, fermentation, *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, silages.

## INTRODUCTION

Livestock systems at Region Aysén del General Carlos Ibáñez del Campo depend on pastures with seasonal effects on their growth and quality. During spring-summer this pasture expresses the greatest growth, generating an excess of forage, which is used to forage conservation like hay, haylage or silage. These conserved forages are used during winter season when environmental conditions do not allow the pasture growth, due to low temperatures, it can last between 3 and 6 mo (May to October). In addition, on winter and beginning of spring the calving period begins, therefore, nutritional requirements of animals increase significantly. However, there is limited information on the nutritional quality on forage conservation in the region. Daza et al. (2021) mention that the nutritional quality of conserved forages (hay and silage), especially in the metabolizable energy (ME) content, do not meet animal nutritional requirements for calving period, finding ME levels between 1.66 and 2.31 Mcal $kg^{-1}$ DM and crude protein (CP) between 9.7% and 13.2%. The foregoing can be attributed to the time of forage harvest, weather conditions, machinery access, because of nutrient losses by effluents on conservation with a weak silage fermentation process. To reduce nutrient losses by effluents and improve fermentative quality of silages, the industry has different biological additives, which are used to reduce pH and ammonia N, also to avoid butyric fermentation and stimulate lactic acid fermentation (Kung et al., 2003). Within silage additives, it has recently been mentioned that yeasts could benefit conservation process, inhibiting detrimental microorganisms (Muck et al., 2018). Those yeasts of *Saccharomyces cerevisiae* (Ale), that were used in previous studies as additives in rice (Ok et al., 2006), king grass (Sofyan et al., 2011) and corn (Duniere et al., 2015) silages. However, *S. carlsbergensis* (Lager) yeasts have not been evaluated for silage conservation, despite that they have been valued as a lactic acid substrate for fermentation (Puligundla et al., 2020) and contain a high level of water-soluble carbohydrates (WSC). Both types of yeasts are generated as liquid waste in brewing industry, having little acceptability and becoming an environmental contamination problem (Zaror, 1993). Due to the above, it is necessary to study the use of brewer's yeast residues in animal feeding processes including silage conservation.

Thus, the aim of the present study was to evaluate the inclusion of spent brewers' yeast as an additive in pasture mini silos compared to commercial additives.

## MATERIALS AND METHODS

**Mini silo preparation**

Prior to the study, four liquid samples of each Lager and Ale type of brewer's yeast (BY) were obtained to determine the water-soluble carbohydrates (WSC) content. Brewer's yeasts were applicated in an inactive way, using the WSC content to determine the dose application. The WSC content for BY was 24.26% for Lager and 32.79% for Ale type, so to apply the same amount of WSC for each dry BY, different grams were applied for fresh forage. Two commercial silage additives were assayed, SiloSolve MC™ (ANASAC, Santiago, Chile) and Josilac grass™ (JOSERA, Weilbach, Germany), which handle different inoculation doses based on lactic acid bacteria (SiloSolve MC™ 100 g 50 $t^{-1}$ fresh forage and Josilac grass™ 150 g 50 $t^{-1}$ fresh forage). As the experience was carried out in mini silos of approximately 1 kg, each additive was weighed at laboratory to obtain the exactly amounts for 20 kg fresh forage (Table 1).

**Table 1.** Application doses at fresh forage for each additive.

| Additives | For 50 t fresh forage | For 20 kg fresh forage |
|---|---|---|
| | g | mg |
| Lager BY (*Saccharomyces carlsbergensis*) | 150 | 60 |
| Ale BY (*S. cerevisiae*) | 100 | 40 |
| SiloSolve MC™ (ANASAC) | 100 | 40 |
| Josilac grass™ (JOSERA) | 150 | 60 |

With additives doses established, the study started on 8 March 2022, selecting a pasture on the humid zone of Villa Mañihuales sector (45°11'56.60" S, 72°11' 8.77" W), Region Aysén del General Carlos Ibáñez del Campo (from this point on Aysén Region), Chile.

For the study, 100 kg fresh pasture were harvested at a height of 5 cm. Total pasture harvested was divided into five buckets, each with 20 kg for five treatments: Silage without additives (control), silage with Lager BY, silage with Ale BY, silage with SiloSolve MC™, and silage with Josilac grass™. The experimental design used was a randomized complete block design, with five treatments and three mini silos samples at four post conservation times (5×3×4).

In the case of treatments with dry BY and commercial additives, they were applied with a 500 mL sprayer, diluting the additive in 100 mL water at 26.6 °C, as recommended on the label of commercial products. This process was carried out in such a way that the products to be applied were as homogeneous as possible between 20 kg, applying the product and mixing at the same time. Subsequently, 12 mini silos were pressed for each treatment in 1 L plastic bottles. During the process, five samples of the liquid (effluent) that the fresh forage pasture lose were obtained to determine the initial pH of the ensiled material. At the end of the pressing of each mini silo, they were sealed with film paper and the respective lid of the bottle, thus vacuum sealing each mini silo and avoiding the oxygen inlet.

**Productive and nutritional quality characteristics of conserved pasture**

Three samples of fresh forage pasture were taken at ground level with a 0.5 $m^2$ frame to evaluate DM herbage allowance, botanical composition and nutritional quality. Samples were taken to the Instituto de Investigaciones Agropecuarias (INIA) Tamel Aike soil laboratory (Coyhaique, Chile), to determine the species present at the pasture and for DM with a forced-air oven at 60 °C for 48 h and ground through a 1 mm screen to determine crude protein (CP), ash, neutral (NDF) and acid detergent fiber (ADF), D value and metabolizable energy (ME) contents by infrared reflectance spectroscopy (NIRS) at Animal Nutrition laboratory of the Austral University of Chile (Table 2).

**Table 2.** Characteristics of fresh pasture. D value: Digestibility value; SD: standard deviation.

| Item | Mean ± SD |
|---|---|
| Herbage allowance, kg DM $ha^{-1}$ (measured at ground level) | 4695 ± 619 |
| Botanical composition | |
| Ryegrass, % | 37.0 ± 10.5 |
| Red clover, % | 53.6 ± 12.8 |
| Weeds, % | 3.58 ± 1.5 |
| Other grasses, % | 5.69 ± 2.5 |
| Nutritional quality | |
| DM, % | 15.2 ± 0.3 |
| Ash, % | 7.7 ± 0.3 |
| Crude protein, % | 12.6 ± 1.0 |
| Neutral detergent fiber, % | 46.9 ± 2.0 |
| Acid detergent fiber, % | 28.4 ± 2.0 |
| D value, % | 63.4 ± 0.4 |
| Metabolizable energy, Mcal $kg^{-1}$ DM | 2.42 ± 0.03 |

**Mini silos analysis**

Mini silos were kept in dark boxes that were ventilated to maintain controlled temperature of 22 ± 1 °C and weight control until standardize their weight (Table 3). In addition, three mini silos for each treatment were opened at 30, 60, 90 and 120 d post conservation. For pH, 10 g ground fresh material with 20 mL distilled water were mixed and shaken, then, the mixture left to rest for 15 min and pH was measured with a pH meter (Orion 3 Star pH Benchtop, Thermo Fisher Scientific, Waltham, Massachusetts, USA). For nutritional quality, three mini silos were taken from 90 and 120 d post conservation. The samples were dried in a forced-air oven at 60 °C for 48 h for DM and ground through a 1 mm screen to determine CP, ash, NDF, ADF and ME by NIRS.

**Statistical analysis**

All data (pH and nutritional quality) were analyzed by ANOVA using Tukey test at a significance of 95%. This was done to compare the fermentative and nutritive variables between the pasture, additive type and days post conservation.

**Table 3.** Mini silo weight control. Weight 1: 8 Mar 2022; Weight 2: 1 Apr 2022; Weight 3: 8 Apr 2022; Weight 4: 18 Apr 2022; Weight 5: 27 Apr 2022; Weight 6: 5 May 2022; SD: standard deviation.

| | Treatments | | | | |
|---|---|---|---|---|---|
| Weight number | Control | Lager | Ale | SiloSolve MC™ | Josilac grass™ |
| Weight 1 | 836.4 | 831.9 | 828.6 | 833.2 | 820.7 |
| SD | 27.3 | 28.5 | 32.0 | 27.2 | 30.2 |
| Weight 2 | 761.7 | 751.6 | 754.5 | 748.9 | 739.7 |
| SD | 29.6 | 30.2 | 28.1 | 24.1 | 30.7 |
| Weight 3 | 760.0 | 749.7 | 752.9 | 748.2 | 726.6 |
| SD | 29.5 | 30.1 | 27.7 | 24.1 | 23.0 |
| Weight 4 | 764.4 | 744.6 | 750.9 | 745.5 | 726.6 |
| SD | 32.4 | 31.4 | 29.3 | 25.3 | 23.0 |
| Weight 5 | 763.8 | 743.8 | 750.5 | 744.7 | 726.0 |
| SD | 32.2 | 31.3 | 29.1 | 25.1 | 22.8 |
| Weight 6 | 763.4 | 743.2 | 750.0 | 744.4 | 725.8 |
| SD | 32.1 | 31.2 | 29.1 | 25.1 | 22.9 |

## RESULTS AND DISCUSSION

National and international information on the use of spent BY as silage additives is limited. This information has focused on the effect of *Saccharomyces cerevisiae* yeasts, and the effects of *S. carlsbergensis* (Lager) have not been reported. In addition, at Chile it is the first experience in the use of spent brewers' yeasts on nutritional and fermentative quality of pasture silage.

Previous studies mentioned that yeasts should not interfere with silage process and negatively impact the nutritional and fermentative quality of forage (Savage et al., 2014; Duniere et al., 2015). During this study, the fermentative quality of mini silos (Figure 1) treated with Ale BY (*S. cerevisiae*) decreased more rapidly pH level, and on day 90 post conservation pH stabilized close to 4.0, compared to the other treatments over 4.1. This can be attributed to high WSC contents on dry Ale BY, which favors lactic acid fermentation. In addition, the low relationship between C and N of BY favor their use as additives in fermentation bioprocesses (Mathias et al., 2015). As a consequence of this, Lager BY was not able to a lower pH level in the same way as the Ale BY, since BY have metabolic differences that are reflected in the pH reduction, similar effects were reported by Jang et al. (2011).

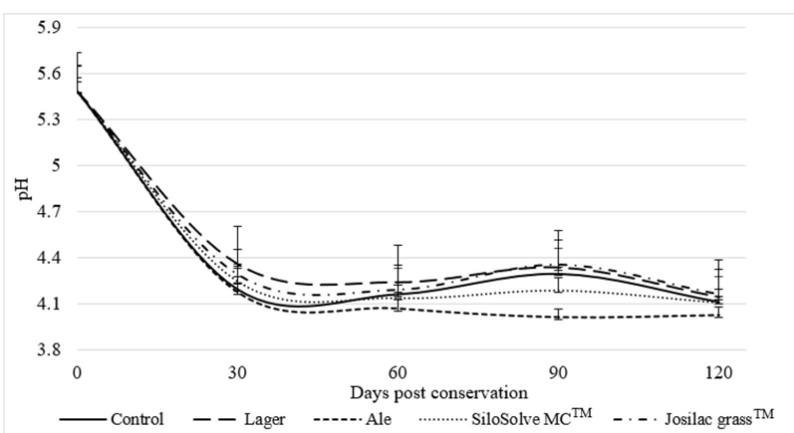


**Figure 1.** Fermentative quality (pH) variation of pasture mini silos with different additives types.

These results can be compared with the previous studies carried out with Ale BY (Duniere et al., 2015; Xu et al., 2019), where authors report nonsignificant differences between treatment without additive and with the application of yeast for 90 and 118 d post storage, respectively. However, Duniere et al. (2015) and Tang et al. (2024) mentions that *S. cerevisiae* yeasts could assimilate WSC and lactate quickly than other types of yeasts evaluated.

On the other hand, in the current study, pH decrease at 0 and 30 d post conservation was 74%, which is similar to reported by previous authors using *S. cerevisiae* as additive (Ok et al., 2006; Duniere et al., 2015; Xu et al., 2019).

The results for nutritional quality changes for pasture, compared to mini silos per treatment at 120 d post conservation, are shown in Table 4. Increases in DM and fibers (NDF and ADF) were observed for additive's treatments, compared to pasture. Crude protein (CP) for control, Lager BY and Josilac grass™, increased, regard to the pasture, contrary for Ale BY and SiloSolve MC™. This can be attributed to changes in DM and possible effluent losses at the time of pressing. Regarding to nutritional quality between silage treatments, significant differences were observed in CP, having control treatment the highest value, compared to all-other treatments. Similarly, metabolizable energy (ME) achieved highest concentration in control, similar results at Josilac grass™ treatment; contrary to NDF and ADF, where control decreased concentrations. All treatments decreased pH to the original pasture, which was expected within the process.

**Table 4.** Nutritional and fermentative quality of pasture mini silos during 120 d post conservation with different additives types. CP: Crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ME: metabolizable energy; SD: standard deviation; SE: standard error of the mean.

| | | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Item | Pasture | Control | Lager | Ale | SiloSolve MC™ | Josilac grass™ | SE | P-value |
| DM, % | 15.25 | 17.98 | 18.96 | 18.72 | 18.32 | 17.98 | 0.77 | 0.85 |
| Ash, % | 7.77 | 8.48 | 7.91 | 7.86 | 7.80 | 8.19 | 0.23 | 0.27 |
| CP, % | 12.64 | 16.33[a] | 14.43[b] | 11.25[c] | 12.16[c] | 14.72[b] | 0.46 | < 0.01 |
| NDF, % | 46.99 | 49.00[c] | 51.65[abc] | 54.69[a] | 53.94[ab] | 51.15[bc] | 1.02 | 0.01 |
| ADF, % | 28.44 | 35.02[c] | 37.26[ab] | 38.66[a] | 38.84[a] | 36.50[bc] | 0.55 | < 0.01 |
| ME, Mcal $kg^{-1}$ DM | 2.42 | 2.31[a] | 2.22[b] | 2.17[bc] | 2.13[c] | 2.25[ab] | 0.02 | < 0.01 |
| pH | 5.49 | 4.11[b] | 4.14[ab] | 4.02[c] | 4.11[b] | 4.16[a] | 0.01 | < 0.01 |

For CP significant differences ($p < 0.01$) were observed between treatments and post conservation days (Figure 2). Control treatment showed stable CP content on 90 and 120 d post conservation, compared with additives treatments, which decreased CP between days 90 and 120 post conservation.

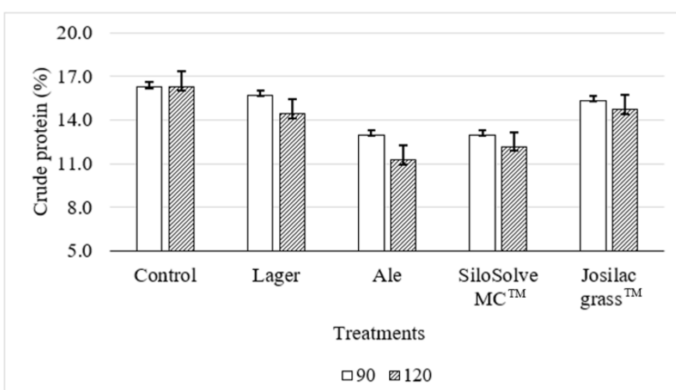


**Figure 2.** Crude protein variation of pasture mini silos during 90 and 120 d post conservation with different additives types.

As for ME, control treatment increases approximately 0.5 Mcal kg$^{-1}$ DM at 120 d post conservation, compared to 90 d post conservation (Figure 3).

Pasture was harvested directly and with accumulated precipitation during morning (around 30 mm) with DM content close to 15%. Previously, it has been reported that harvesting with rain could generate around 5% changes in silage DM (Borreani et al., 2017). This is reflected with the results obtained in the present study, where the mini silos increased their DM content by approximately 2.7%. Nonsignificant differences were presented between treatments, similar to those obtained by other authors (Nair et al., 2019). Similar increases were observed for CP, NDF and ADF content for control, Lager BY and Josilac grass™ treatments. However, CP content decreased with Ale BY and SiloSolve MC™ treatments, with significant differences between treatments at 120 d post conservation. This differs from the results in other studies (Duniere et al., 2015; Xu et al., 2019; Kung et al., 2020), where CP increased for silages with yeasts additives, but similar with Nair et al. (2019), who reported up to 5% CP loss with respect to the original forage. Regarding the increase in NDF and ADF contents in all treatments, compared to the pasture, the results coincide with other authors for silages treated with different strains of *Saccharomyces* (Duniere et al., 2015). The ME content decreased in the treatments with Lager and Ale BYs, which is attributed to the increase in the ADF content. It is important to mention that WSC contents were not evaluated, which could improve the results of the study.

Finally, between 90 and 120 d post conservation, CP changes were observed for Lager and Ale BY silages.

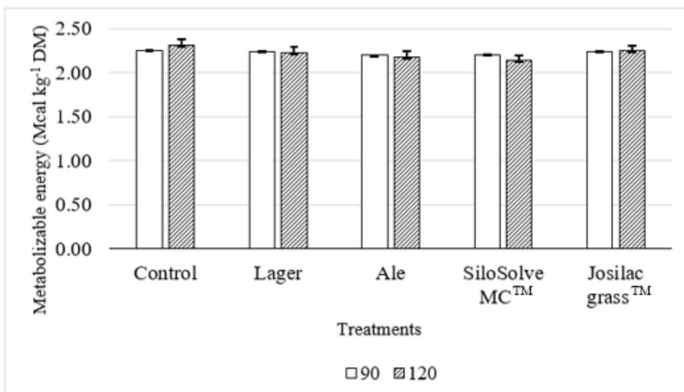


**Figure 3.** Metabolizable energy variation of pasture mini silos during 90 and 120 d post conservation with different additives types.

## CONCLUSIONS

Spent brewer's yeast (BY) do not improve nutritional quality of pasture silage. However, Ale BY decreased and maintained low pH levels, which would make better conserved process. Therefore, there is still a lack of trials within this same field to apply brewer's yeast in forage conservation at field level or other uses in direct animal feed.

**Author contribution**

Conceptualization: J.D., R.M., J.L.U. Data curation: J.D., V.G., R.M. Formal analysis: J.D. Funding acquisition: J.D., V.G., C.R. Investigation: J.D., V.G., C.R., J.R., C.S., F.M. Methodology: J.D., A.C., V.S. Project administration: J.D. Resources: J.D. Supervision: J.D. Writing-original draft preparation: J.D., V.G. Writing-review and editing: J.D., V.G., C.R., R.M. All authors have read and agreed to the published version of the manuscript.

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