

# Milk production and quality of Brown Swiss cows supplemented with beer-derived hydrolysate

## *Producción y calidad de leche de vacas Brown Swiss suplementadas con hidrolizado de cerveza*

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### Abstract

Currently, the search for low-cost food supplements (additives) that increase productive yields, improve ruminal efficiency and physiology in cattle, is of vital importance for dairy production. **Objective:** To evaluate the effect of supplementing Brown Swiss cows with brewer's yeast enzymatic hydrolysate on milk production and quality. **Materials and methods:** An experiment was developed in the province of Manabí, Ecuador, during the months of September to December 2015, with a duration of 120 days. Twelve animals were used in a Cross over design. Four treatments were evaluated: (1) grazing + concentrate, (2) grazing + concentrate + 100 ml/L of hydrolysate, (3) grazing + concentrate + 150 mL/L of hydrolysate, and (4) grazing + concentrate + 200ml/L of hydrolyzed). The animals grazed *Megathyrsus maximus* in 0.40 ha paddocks. The following parameters were determined: dry matter, protein, fiber in neutral and acid detergent, phosphorus, calcium, cellulose, hemicellulose and cellular content of the grass. Milk production was evaluated every 15 days, to determine the quality of the milk, the

protein, fat, total solids and acidity contents were measured. **Results:** Milk production showed an increase of more than 1 L when hydrolysate was used in the ration. In T3, the percentage of protein and fat in the older milk was 4.34% and 4.87%, respectively, showing differences compared to the other treatments. The opposite was observed for total solids, with the highest percentage recorded in T2 (13.70%). The density and acidity variables did not reflect differences between the treatments. **Conclusions:** Supplementation with beer enzymatic hydrolysate had a positive effect on milk production and quality, particularly when 150 and 200 ml/L were included in the animals' diet.

**Keywords:** Supplementation, milk quality, production performance, milk fat, enzymatic hydrolysate.

## Resumen

En la actualidad, la búsqueda de suplementos alimentarios (aditivos) de bajo costo, que incrementen los rendimientos productivos, mejoren la eficiencia y fisiología ruminal en el ganado vacuno, es de vital importancia para la producción lechera. **Objetivo:** Evaluar el efecto de suplementar vacas Brown Swiss con hidrolizado enzimático de levadura cervecera sobre la producción y la calidad de la leche. **Materiales y métodos:** Se desarrolló un experimento en la provincia de Manabí, Ecuador, durante los meses de septiembre a diciembre de 2015, con una duración de 120 días. Se emplearon doce animales en un diseño de sobre cambio o Cross over. Se evaluaron cuatro tratamientos: (1) pastoreo + concentrado, (2) pastoreo + concentrado + 100 ml/L de hidrolizado, (3) pastoreo + concentrado + 150 mL/L de hidrolizado y (4) pastoreo + concentrado + 200 ml/L de hidrolizado). Los animales pastaron *Megathyrus maximus* en potreros de 0,40 ha. Se determinó: materia seca, proteína, fibra en detergente neutro y ácido, fósforo, calcio, celulosa, hemicelulosa y contenido celular del pasto. La producción de leche se evaluó cada 15 días, para determinar la calidad de la leche se midieron los contenidos de proteína, grasa, sólidos totales y acidez. **Resultados:** La producción de leche mostró un incremento superior a 1 L al emplear hidrolizado en la ración. El porcentaje de proteína y grasa de la leche mayor se obtuvo en T3 con 4,34 y 4,87 %, con diferencias respecto al resto de los tratamientos. Lo contrario sucedió para los sólidos totales donde T2 mostró el mayor porcentaje con 13,70 %. Las variables densidad y acidez no reflejaron diferencias entre los tratamientos. **Conclusiones:** La suplementación con el hidrolizado

enzimático de cerveza tuvo un efecto positivo sobre producción y calidad de la leche, fundamentalmente cuando se emplearon 150 y 200 ml/L en la ración de los animales.

**Palabras clave:** Suplementación, calidad de la leche, rendimiento productivo, grasa de la leche, hidrolizado enzimático.

## Introduction

Finding low-cost feed additives that increase animal production performance has become both a priority and a challenge (Sosa-Cossio et al., 2018). A wide variety of supplements are used to improve production efficiency and ruminal physiology in cattle (Bustamante et al., 2021). Among these substances are antibiotic growth promoters (AGPs), which influence metabolic processes and improve feed utilization efficiency (Barrera et al., 2022).

The use of AGPs has been restricted in many regions of the world due to the emergence of residual effects in food and problems of microbial resistance associated with diseases in humans and animals (Cruz-Aviña et al., 2023). Thus, the emergence of less expensive alternatives—such as brewer’s yeast—has created the possibility of replacing traditional protein meals (*Saccharomyces cerevisiae*) (Marrero et al., 2010). Thanks to its high protein content (40–45%) and the presence of other beneficial compounds—such as vitamins, minerals, oligosaccharides, organic acids, amino acids, peptides, antioxidants, and  $\beta$ -glucans—brewer’s yeast can be used as a dietary supplement, as these components support the growth of ruminal microorganisms (Choque, 2020).

The brewing industry generates by-products that can be used either dry or wet in ruminant diets. This would prevent environmental pollution and would also constitute a source of non-degradable protein (NDP) in the rumen (Quispe-Paxipati et al., 2019). Various studies have shown that these by-products promote cattle development—both in milk production and fattening—when degraded by rumen microorganisms (Ávila & Calderón, 2020). This is because they improve the rumen environment by reducing the amount of oxygen, promoting anaerobiosis, and stimulating the growth of cellulolytic bacteria, thus improving the animal’s productive response (Ávila & Calderón, 2020).

Beer industry derivatives are also used in Ecuador to achieve higher animal yields, since the microorganisms found in yeasts have a high content of proteins, carbohydrates, lipids, vitamins, and minerals (Sghirla-Herrería & González-Marcillo, 2020). Yeasts are considered an important source of products with probiotic activity, either as live strains or as cell wall derivatives (Choque, 2020). These substances have demonstrated immunostimulatory activity in farm animals and have also improved digestive physiological processes, which contributes to better production results (Choque, 2020).

The objective of this study was to evaluate the effect of supplementing Brown Swiss cows with an enzymatic brewer's yeast hydrolysate on milk production and quality.

## Materials and methods

Location and duration of the experiment. This research was conducted at the facilities of the Manabí Polytechnic Agricultural School, located in El Limón, Bolívar canton, Calceta parish, Manabí province, Ecuador. The study took place between the coordinates of 0049' 27.9" south latitude, 80010'47.2" west longitude, and at an altitude of 15 meters above sea level (m.a.s.l.) from September to December 2015. The climate characteristics are reflected in Table 1.

**Table 1**

*Climatic Characteristics of the Study Site: El Limón, Bolívar, Calceta, Manabí, Ecuador (September–December 2015)*

|                              |                                    |
|------------------------------|------------------------------------|
| Average annual temperature   | 25.6 °C                            |
| Average annual precipitation | 838,7 mm                           |
| Average relative humidity    | 78 %                               |
| Heliophany                   | 1158 hours of sunshine °C per year |
| Evaporation                  | 1365,2 cm                          |

*Note.* Adapted from Manabí Water Resources Regulatory Corporation (CRM). (2023). *Carrizal-Chone project using GPS (El Limón Geographical Landmark)*. Manabí, Ecuador.

Handling the experiment. The experiment lasted 120 days (September to December 2015), during which the effects of brewer's yeast hydrolysate were evaluated in crossbred Brown Swiss cattle. Twelve cows were selected, each one with an average of 90 ( $\pm$  10) days of lactation, 5.43 L/cow production, and 450 kg ( $\pm$  3 kg) weight. The cows were dewormed, and their initial weights were recorded at the start of the experiment. The study was carried out in two phases: a 15-day adaptation period to allow the animals to adjust to the feed, followed by a 120-day experimental phase (Castro-Rincón et al., 2020).

The enzymatic hydrolysate used was obtained from brewer's yeast (HELC, Hidrolizado Enzimático de Levadura Cervejera); it was produced in a 10 L capacity bioreactor, for which 9 L of yeast cream at 20% dry matter, 200 g of NaCl, and 300 g of molasses were added; the pH was adjusted (5-6) with 37% NaOH; 1 L of enzymatic crude was supplied; subsequently, it was stirred at 60 rpm at 50°C for 10 h; then the temperature was raised to 71°C 15 minutes before finishing the process; and then it was packaged in clean bottles in an oven at 180°C and hermetically sealed, according to the methodology established by Pérez et al. (2006), to then be evaluated in lactating cows.

The animals grazed together in 0.40 ha paddocks planted with savoy grass (*Megathyrus maximus*), without fertilization or irrigation. With continuous 60-day rotations, the average pasture availability was 30 kg of DM/LU/rotation. Each animal received a daily ration of concentrate equivalent to one kilogram, which was mixed with the enzymatic hydrolysate of brewer's yeast and offered in the shelter, with water available ad libitum in all cases (Desnoyers et al., 2009).

Variables under study. The bromatological composition of the grass was determined: DM, CP, ash, OM, P, and Ca according to AOAC (2016); and NDF, ADF, ADL, cellulose (Cel), hemicellulose (Hcel), and cell contents (CC) according to Goering & Van Soest (1970). Milk production was assessed every 15 days during the milking routine (6:00 a.m. and 2:00 p.m.). At each milk weighing, 20 samples were collected to determine quality. These samples included protein, fat, total solids, and acidity, using the methodology of Kirk and Egan (1996).

Experimental design and treatments. The results were analyzed according to a crossover design, following the methodology described by Castro-Rincón et al. (2020). The treatments consisted of access to grazing plus concentrate, along with supplementation using enzymatic hydrolysate of brewer's yeast at doses of 100, 150, and 200 mL. Four treatments were established, each with three replications, as shown in Table 2.

**Table 2**

*Experimental Treatments in Dairy Brown Swiss Cows Supplemented with Brewer's Yeast Hydrolysate in El Limón, Bolívar, Calceta, Manabí, Ecuador (September–December 2015)*

| Treatments                                      | Code | Rep. | Experimental units | Total number of experimental units |
|-------------------------------------------------|------|------|--------------------|------------------------------------|
| Witness Grazing + Concentrate                   | T0   | 3    | 1                  | 3                                  |
| Grazing + Concentrate + 100mL of HELC, per cow. | T1   | 3    | 1                  | 3                                  |
| Grazing + Concentrate + 150mL of HELC, per cow  | T2   | 3    | 1                  | 3                                  |
| Grazing + Concentrate + 200mL of HELC, per cow  | T3   | 3    | 1                  | 3                                  |
| Total                                           |      |      |                    | 12                                 |

Note. HELC = Enzymatic hydrolysate of brewer's yeast (*enzymatically hydrolyzed brewer's yeast*).

Statistical analysis. The Shapiro-Wilk test (Massey, 1995) was used to assess the normality of the data distribution, and Bartlett's test (1937) was applied to verify homogeneity of variances. An analysis of variance was performed, and means were compared using Tukey's test at a significance level of  $p < 0.05$ . Additionally, the analyses were conducted using SPSS version 22 for Windows (International Business Machines Corporation [IBM], 2021).

## Results

The analysis of the chemical composition of *Megathyrsus maximus* highlighted the specific characteristics of this species within the study region. Crude protein values of 10.19%, NDF of 52.48%, and cellular content of 47.52% were observed, to mention the most important elements when including a feed in the diet of ruminants, especially when the hydrolysate acts on fibrous feeds (Table 3).

**Table 3**

*Chemical composition of grass Megathyrsus maximus in El Limón, Bolívar, Calceta, Manabí, Ecuador, September-December 2015*

| Indicators       | (%)   | DE±   |
|------------------|-------|-------|
| DM               | 30,34 | 0,95  |
| CP               | 10,19 | 0,23  |
| Ca               | 0,54  | 0,023 |
| NDF              | 52,48 | 1,569 |
| ADF              | 27,79 | 1,067 |
| ADL              | 3,68  | 0,464 |
| Cellulose        | 24,16 | 0,665 |
| Hemicellulose    | 24,64 | 0,796 |
| Cellular content | 47,52 | 1,567 |

Note. DM = Dry matter; CP = Crude protein; Ca = Calcium; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin.

The milk production results showed differences in the three hydrolysate levels when compared to the control group. It is noteworthy that milk production increased by 1.42, 1.09, 0.91, 1.02, and 1.11 kg per day in each experimental treatment compared to the control group (Table 4).

**Table 4**

*Milk production of Brown Swiss cows supplemented with beer hydrolysate in El Limón, Bolívar, Calceta, Manabí, Ecuador, September-December 2015*

| Milk production per month/cow in kg | Treatments (hydrolysate levels in mL/L) |                   |                   |                   |      |       |
|-------------------------------------|-----------------------------------------|-------------------|-------------------|-------------------|------|-------|
|                                     | 0                                       | 100               | 150               | 200               | EE±  | P     |
| First                               | 5,34 <sup>b</sup>                       | 6,49 <sup>a</sup> | 6,65 <sup>a</sup> | 6,79 <sup>a</sup> | 0,36 | 0,031 |
| Second                              | 5,23 <sup>b</sup>                       | 6,87 <sup>a</sup> | 6,81 <sup>a</sup> | 6,32 <sup>a</sup> | 0,67 | 0,012 |
| Third                               | 5,77 <sup>b</sup>                       | 6,78 <sup>a</sup> | 6,44 <sup>a</sup> | 6,68 <sup>a</sup> | 0,39 | 0,021 |
| Room                                | 5,38 <sup>b</sup>                       | 6,93 <sup>a</sup> | 6,99 <sup>a</sup> | 6,40 <sup>a</sup> | 0,75 | 0,042 |
| Average                             | 5,43 <sup>b</sup>                       | 6,76 <sup>a</sup> | 6,72 <sup>a</sup> | 6,54 <sup>a</sup> | 0,48 | 0,031 |

Note. a, b Different letters within the same column indicate significant differences according to Tukey's test ( $p < .05$ ).

Milk quality showed a higher protein content when the 200 ml/l hydrolysate treatment was used, with increases of 0.9 and 1.54 percentage points, with differences compared to the control (Table 5). Total solids were highest in the 150 mL/L treatment, reaching 13.70%. No significant differences were observed among treatments for density and acidity. Notably, the control group and the 150 mL/L treatment showed no differences across any of the measured indicators.

**Table 5**

*Milk Quality of Brown Swiss Cows Supplemented with Brewer's Yeast Hydrolysate in El Limón, Bolívar, Ecuador (September–December 2015)*

| Indicators (%) | Treatments (hydrolysate levels in ml/l) |                    |                    |                    |       |       |
|----------------|-----------------------------------------|--------------------|--------------------|--------------------|-------|-------|
|                | 0                                       | 100                | 150                | 200                | EE±   | P     |
| Protein        | 3,44 <sup>b</sup>                       | 3,13 <sup>c</sup>  | 3,44 <sup>b</sup>  | 4,34 <sup>a</sup>  | 0,07  | 0,001 |
| Fat            | 3,33 <sup>c</sup>                       | 3,20 <sup>d</sup>  | 3,57 <sup>b</sup>  | 4,87 <sup>a</sup>  | 0,06  | 0,002 |
| Density        | 1,03 <sup>a</sup>                       | 1,03 <sup>a</sup>  | 1,02 <sup>a</sup>  | 1,03 <sup>a</sup>  | 0,01  | 0,234 |
| Total solids   | 12,68 <sup>b</sup>                      | 12,81 <sup>b</sup> | 13,70 <sup>a</sup> | 10,65 <sup>c</sup> | 0,19  | 0,002 |
| Acidity        | 0,18 <sup>a</sup>                       | 0,18 <sup>a</sup>  | 0,18 <sup>a</sup>  | 0,18 <sup>a</sup>  | 0,001 | 0,201 |

Note. a, b Different letters within the same column indicate significant differences according to Tukey's test ( $p < .05$ ).

## Discussion

The protein and fibrous component levels (Table 3) obtained in the present study were 10.19, 52.48, 27.79, and 3.68%. These results are consistent with those reported by Méndez-Martínez et al. (2018; 2019), who found protein values close to 11% under very similar temperature, humidity, and precipitation conditions in El Empalme, Ecuador. However, the NDF and ADF values recorded in this study were elevated approximately 60% and 30%, respectively which could be explained by the specific soil and climatic conditions of the region.

Plant species exist, reproduce, and persist in certain soil and climate contexts, which can be considered as tolerance to these conditions (Herrera et al. 2018). This is evident in the present study, where rainfall distribution has a significant influence on the productivity and quality of plant varieties of *Megathyrus maximus*. Hence, factors such as soil characteristics, fertilization, water availability, planting season, and management practices, among others, play a crucial role in determining the nutritional quality of plant species within production systems. Pastures are a clear example of this.

Excess water causes anoxia in roots, affecting their aerobic respiration and mineral and water absorption. If this is prolonged in non-tolerant species, carbon assimilation and translocation decrease, producing metabolic changes that activate anaerobic respiration, which implies lower energy efficiency and bio-productivity in plants. Meanwhile, water stress due to water deficit decreases cell wall concentration in forage leaves and stems, although their structural components (cellulose, hemicellulose, and lignin) and polyphenols vary. The latter is attributable to the plant's need to maintain high levels of carbohydrates in soluble forms during osmotic adjustments (Álvarez, 2019; Méndez-Martínez et al., 2020).

The positive effects of hydrolysate supplementation were observed, with levels of 100, 150, and 200 ml/l exceeding the control in milk production (Table 4). In our region, studies have reported that including 10 g of *Saccharomyces cerevisiae* in the diet of Holstein cows resulted in an increase in milk production of 1.57 kg per cow per day. Similarly, in southeastern Brazil during the summer, an increase of 1.4 kg per cow per day was observed (Mirzad et al. 2019). Thus, other authors have stated that the inclusion

of yeast in the cattle ration can improve the average production value by 3.6%, which is equivalent to 1 kg of milk per cow per day (Ávila & Calderón, 2020; Marrero et al., 2020).

In studies on the effect of enzymatic hydrolysate of *Saccharomyces cerevisiae* on ruminal degradation kinetics, Valenciaga et al. (2019) found increases in dry matter degradability and improved forage utilization. This study found that, when applying treatments of 150 and 200 ml/l, milk production and quality increased. This result is related to the effects of hydrolysate as an activator of yeast strains on the populations of total viable and cellulolytic bacteria used as a supplement for ruminants. (Casas, 2018; Sghirla-Herrería & González-Marcillo, 2020; Zhu et al., 2017), promote anaerobiosis and stimulate the growth of cellulolytic bacteria, which reduces the concentration of ammonium and increases the synthesis of microbial protein (Rodríguez et al., 2020).

Research on dairy cow diets has demonstrated that supplementing with yeast at levels of 10, 100, and 150 grams resulted in oxygen reductions between 46% and 89% (Marrero et al., 2020; Narváez et al., 2021; Sghirla-Herrería & González-Marcillo, 2020). This generates an increase in rumen microorganisms of close to 30%, which leads to better feed utilization, increasing energy and microbial protein production by 10 to 20%. The activating effect is due to the growth elements that yeasts provide for ruminal microorganisms, such as B vitamins, short-chain fatty acids (SCFAs) and branched-chain fatty acids, provitamins, and micronutrients (Ogunade et al., 2019).

Milk quality increased as hydrolysate levels increased by 0.9, 1.54, and 1.02% for protein, fat, and total solids (Table 5). This result is consistent with findings reported by Narváez et al. (2021), who observed milk protein values of 3.50% when 150 g of *Saccharomyces cerevisiae* was included in dairy cow rations. It should be noted that in this case the fat percentages (4.18%) were higher than those found in this study, which could have been due to the diet; intrinsic characteristics; physiological and body state; category and productive purpose of the animals; grazing hours; type and quality of the grass; consumption of legumes; and other management conditions, facilities, and comfort.

By including 10 g/L of yeasts (strain L25/7/13 of *S. cerevisiae*) in dairy cows, García et al. (2020) reported improvements in milk quality, mainly affecting milk protein with values of 3.2%, lower than those shown in this study when applying 150 and 200 ml/L of yeast hydrolysate (3.44 and 4.34%, respectively). In this sense, Desnoyers et al. (2009)

stated that the increase in milk production is related to a decrease in fat content. However, the results of this work reflected that supplementation with *Saccharomyces cerevisiae* caused a rise in milk production alongside an improvement in fat percentage; however, total solids did not increase and, in fact, decreased in the last treatment.

The addition of *Saccharomyces cerevisiae* to the cattle ration was shown to enhance ruminal activity, including the digestibility and degradability of forages (Kumprechtová et al., 2019), as well as changes in rumen fermentation patterns (pH modification, number, and type of microorganisms). On the other hand, López et al. (2021) reported in *in vitro* studies that the inclusion of an enzymatic hydrolysate of yeast (*S. cerevisiae*) in a ration for cattle had an activating effect on the population of cellulolytic bacteria in the rumen, showing the possibility that the adequate supply of this yeast increases ruminal degradability, fundamentally when the fiber content is high (Shi et al., 2019).

The use of yeast (*S. cerevisiae*), among which enzymatic hydrolysate appears in the ruminant feed, reflects great importance for livestock since its use allows improving the efficiency of energy use in ruminants (López et al. 2021), which can mitigate the emission of greenhouse gases such as methane, positively favoring the recovery of the environment. Consequently, many studies demonstrate the importance of using brewer's yeast hydrolysate, which highlights the activating effect on the populations of total viable and cellulolytic bacteria in the rumen, as well as the increase in these, which undoubtedly acts on fibrous foods to make them more efficient (Marrero et al., 2020; Sosa et al., 2020). This strongly underscores the relevance of this study.

## Conclusions

Supplementation with enzymatic hydrolysate from beer showed a positive effect on milk production and quality, particularly when 150 and 200 ml/L were used in the animals' rations. These concentrations resulted in the highest proportions of protein, fat, and milk density, with values of 4.34%, 4.87%, and 1.031%, respectively. Therefore, it is considered a performance-enhancing product in the dairy industry.

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**Authors' contributions.** Jacinto Alex Roca-Cedeño, as the thesis advisor, provided the research guide in accordance with the research guidelines and reviewed and consolidated the results. Shesnarda Elizabeth Basurto Rodríguez and Mayté Abigail Guamán Moposa developed the systematic search for research related to the subject matter under study, prepared the treatments, and carried out the verification of the results under the supervision of the advisor. They jointly developed the research process.

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