

## Impact of live yeast and selenium supplementation on blood metabolites and rumen pH of young bulls after long-transport to the fattening unit



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### ARTICLE INFO

#### Article history:

Received 16 February 2024

Revised 6 November 2024

Accepted 8 November 2024

Available online 17 November 2024

#### Keywords:

Adaptation

Antioxidants

Cattle

Stress

Welfare

### ABSTRACT

Long-distance transport and the receiving phase at the fattening unit are sources of stress for young beef cattle. This randomised controlled study involved 80 Charolais young bulls that underwent 12 h of transport from France to Italy and aimed at testing whether the animals have some benefits from the supplementation of live yeast and selenium through slow-release boluses and diet. The bulls were randomly allocated into two supplementation groups of 40 animals each, named **Yeast** and **Control** groups. Bulls of the Yeast group received a supplementation of *Saccharomyces cerevisiae* and selenium-enriched yeast ( $1.5 \times 10^{10}$  CFU/bull per day of live yeast and 1.5 mg/bull per day of selenium) by two slow-release ruminal boluses 1 day before leaving France, and a live yeast supplemented diet once in Italy ( $8 \times 10^9$  CFU/bull per day of live yeast). Yeast and control bulls underwent the same manipulations. Individual BW and complete blood metabolic profile were assessed at the arrival to the Italian fattening unit (**day 0**), after 7 days (**day 7**), and at the end of the receiving phase (**day 30**). The rumen environment was continuously monitored through reticulum-rumen sensors that measured several parameters in a subsample of 60 bulls, equally distributed between Yeast and Control groups. *Saccharomyces cerevisiae* and selenium supplementation did not affect growth performance and metabolic profile. However, the supplementation stabilised the rumen environment by limiting the daily pH amplitude and SD and the inter-animal variability. The Yeast group increased the time spent ruminating (+39 min/day) at day 30 compared to Control group. More stable ruminal conditions are important to support beef cattle health during the receiving period at the fattening unit, when animals face the delicate transition to high-energy diets.

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### Implications

Young beef cattle face health and welfare challenges during the receiving period after long transport. This study investigated whether dietary live yeast and selenium could support rumen health by alleviating the impacts of transport oxidative stress and adaptation to the new diet. Results showed that yeast supplementation can improve rumination time and help stabilise rumen conditions, promoting resilience in beef cattle during the receiving period. For the livestock industry, yeast and selenium supplementation may represent a preventive strategy to protect rumen func-

tion, enhancing animal welfare and reducing potential health issues at the start of the fattening cycle.

### Introduction

The use of direct-fed microbes such as fungi and yeast has increased exponentially in the last decades to improve beef cattle performances (Peng et al., 2020). In the meta-analysis of Batista et al. (2022), yeasts improved animals' performances such as final BW and ADG. Among the different yeasts available, the most used in cattle is *Saccharomyces cerevisiae* or its cell wall (Thrune et al., 2009; Sartori et al., 2017; Peng et al., 2020). The yeast cell wall and live *Saccharomyces cerevisiae* improve the activity of lactic acid-utilising microorganisms, reducing the lactic acid accumulation

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in the rumen and thus increasing pH and total rumen microflora (Marden et al., 2008; Chaucheyras-Durand and Durand, 2010). Both live yeast and yeast cell wall can effectively reduce the damage of subacute ruminal acidosis (SARA; Vyas et al., 2014), promoting a more favourable rumen environment to the growth of cellulose-consumers microorganisms (McAllister et al., 2011). Finck et al. (2014) reported that, if supplemented to beef cattle during the receiving period at the fattening unit, *Saccharomyces cerevisiae* has positive effects on DM intake and ADG. The polysaccharides of the yeast cell wall have direct and indirect positive effects on the immune system of animals, helping to mitigate stress and reduce the incidence of diseases (Broadway et al., 2015). Stress events depress the immune system, promoting viral replication and secondary bacterial infections in the respiratory tract (Wilson et al., 2017). For instance, the transition from pasture based to intensive indoor system, along with changes in temperature and humidity, act as stressors for cattle, increasing the likelihood of respiratory diseases (Louie et al., 2018). Yeast serves as a potential organic substrate for selenium, a trace element with antioxidant properties in cattle (Mehdi and Dufresne, 2016). Selenium can be supplied to livestock either as inorganic mineral salt or as organic yeast enriched with selenomethionine (Korhola et al., 1986). Aktas et al. (2011) demonstrated that vitamin E + selenium and vitamin A + D + E reduce lipid peroxidation and prevent oxidative stress due to long-term transport. Transport can induce oxidative stress in cattle by increasing malondialdehyde levels, a marker of free radical damage (Chirase et al., 2004; Wernicki et al., 2006; Burke, 2007) which has been linked to a higher risk of bovine respiratory disease in transported calves (Chirase et al., 2004). Furthermore, Salles et al. (2014) recorded significant effects of selenium supplementation on the immune system such as the increase of macrophage phagocytic activity in 30-day-old calves with high serum selenium levels, and other studies (Rowntree et al., 2004; Guyot et al., 2007) observed that selenium supplementation in cows before calving boosts immunoglobulin concentrations in calves. To date, selenium metabolism and the availability of this element through enriched yeast administration should be more deeply investigated to understand if organic selenium has the potential to prevent oxidative stress.

The young stocks born in the French cow-calf pasture-based farms are usually commingled in dedicated collection centres before being truck loaded and transferred to the Italian fattening units (Herve et al., 2020). Animals are stratified according to their BW in the same collection centre, inevitably promoting the commingling of animals from different areas and farms (Santinello et al., 2022a; Santinello et al., 2022b). After the transport to the Italian fattening unit, beef cattle face a receiving period to the new housing and management system. Over the first 20–30 days from arrival, the diet gradually shifted from a forage-based to a high-energy-based diet with concentrates representing more than 60% of the total matter at the end of the transition period (Cozzi, 2007). Commingling, transport, and change in climate, housing, and feeding are stressful factors that predispose the incoming animals to disease outbreaks (Taylor et al., 2010; Santinello et al., 2020, Santinello et al., 2024). According to Rumor et al. (2015), the receiving phase is the most critical for the mortality of the French beef cattle imported in Italy (around 900 000 beef cattle/year). Furthermore, Earley et al. (2017) observed that transport induces changes in the physiological profile of young cattle. Blood parameters like cortisol and creatine kinase have been used as stress indicators in transported beef cattle, as they may increase dramatically after transport (Damte et al., 2018). The present study dealt with the receiving phase (i.e., from the day of arrival to the 1st month of the fattening phase) of French Charolais young bulls at the Italian fattening unit after long-distance transport. The aim was to evaluate the effects of the live yeast *Saccharomyces*

*cerevisiae* and a selenium-enriched yeast supplementation on beef cattle growth performances, plasma traits, and rumen environment measured by reticulum-rumen devices.

## Material and methods

### Animals and experimental design

All experimental procedures were approved by the Animal Care and Use Committee of the University of Padova (Ethical Approval Code: n. 400074 5/2021). This randomised controlled study was carried out in a commercial beef cattle fattening unit located in Porto Viro (Rovigo, Veneto, Italy) using 80 Charolais young bulls imported from France in October 2021. Forty bulls were selected from each of two neighbouring collection centres in the Auvergne region (France), where animals were weighed and vet-checked for their health status and separated to create homogeneous batches stratified for BW. The veterinary check involved a thorough examination to ensure that the selected animals were free from cough and any symptoms of bovine respiratory diseases, diarrhoea, and locomotor issues. Only animals that passed this health screening were included in the study. Each batch was then split into two balanced groups: one received the slow-release boluses supplementation (Yeast group, n = 20 bulls) and the other was used as Control group (n = 20 bulls). The Yeast and Control animals underwent the same handling during the bolus administration and transport from France to Italy to obtain homogeneous conditions. The two groups were randomly created using the “RAND” function in Microsoft Excel spreadsheet, assigning animals to the two groups using their ear tag ID to identify them and maintaining homogeneous groups in terms of BW and age. Then, animals of the two groups were marked with four different colours to identify the intended allocation pen once at the Italian fattening unit (Fig. 1). Bulls of the Yeast group had an average BW of  $445 \pm 11.8$  kg and  $442 \pm 16.3$  kg, and an average age at arrival of  $289 \pm 20.1$  days and  $282 \pm 27.8$  days in the first and second collection centre, respectively. The animals of the Control group had an average BW of  $444 \pm 12.0$  kg and  $444 \pm 15.5$  kg, and an average age at arrival of  $284 \pm 22.9$  days and  $284 \pm 26.0$  days in the first and second collection centre, respectively.

The transport of the bulls to the Italian fattening unit was carried out by the same type of truck providing an average space allowance of 2 m<sup>2</sup>/animal, in compliance with the current European regulations for cattle transport (European Commission, 2018). Each truck driver provided straw for bedding and checked the functioning of the truck drinkers before starting the uploading of the animals. The travel lasted 12 h covering 950 km from 2100 to 0900 h of the day after. Once at destination, the two batches of bulls were housed according to the colours into eight pens of 10 mates each. The first truck transported the animals housed in pens 5, 6, 7, and 8, and the second truck those in pens 1, 2, 3, and 4. Pens 1, 2, 7, and 8 hosted the bulls of the Control group (n = 40) and pens 3, 4, 5, and 6 the bulls of the Yeast group (n = 40; Fig. 1). Each pen had a deep litter straw bedding with a space allowance of 7.5 m<sup>2</sup>/bull and a manger space of 0.5 m/bull. All pens were equipped with two waterers to allow free access to drinking water.

### Experimental supplementation and diet

Upon departure, animals were fed hay and water *ad libitum*. The day before departure, a trained veterinarian provided two slow-release boluses to the young bulls belonging to the Yeast group using a bolus gun. The boluses, weighing 70 g each, contained live *Saccharomyces cerevisiae* CNCM I-1077 (Levucell SC® 10 TITAN, Lallemand SAS, Blagnac, France) and a selenium-enriched yeast

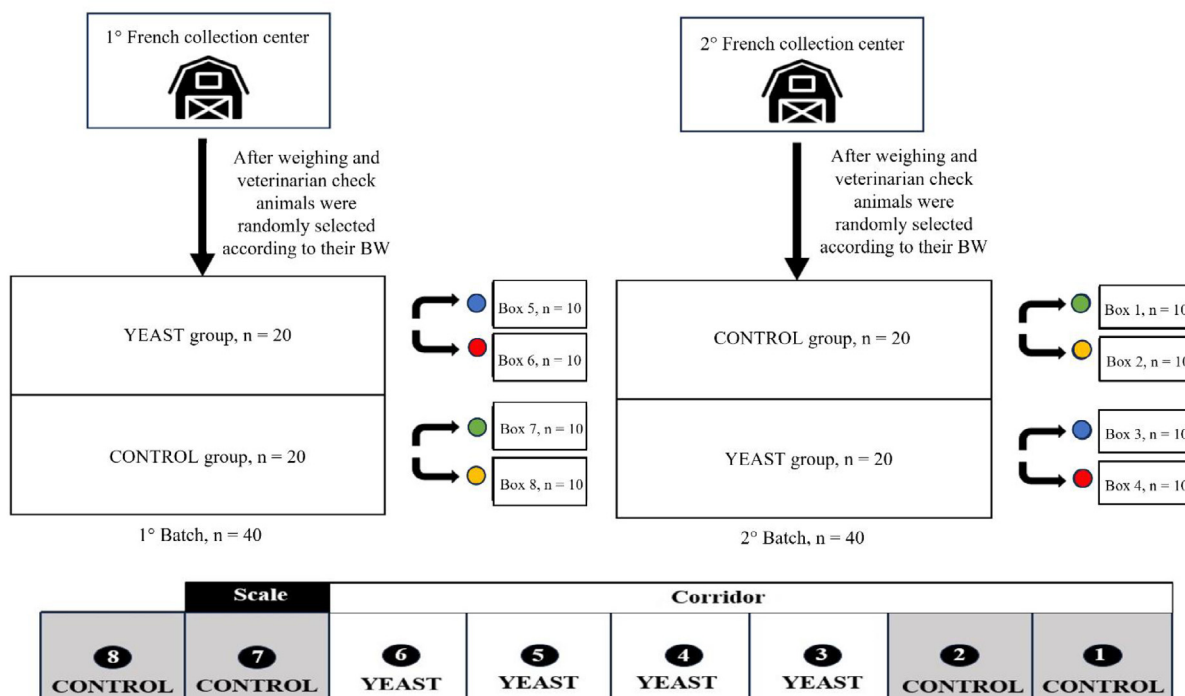


Fig. 1. Experimental design and Charolais young cattle allocation.

(ALKOSEL<sup>®</sup>, Lallemand SAS, Blagnac, France) produced from *Saccharomyces cerevisiae* NCYC R397. The two boluses were designed for releasing a total of 1.5 g/day of live yeast (i.e.,  $1.5 \times 10^{10}$  CFU/day) and 1.5 mg/day of selenium (i.e., 0.95 ppm selenomethionine for a target DMI of 8 kg/day) over 1 week of bolus administration.

After arrival to Italy, young bulls were fed hay and increasing amount of a diet during the 1<sup>st</sup> week that was replaced by a higher-energy diet in the following weeks. Details on diet compo-

Table 1

Nutrient and chemical analysis of the diets administered to Charolais young cattle in the 1<sup>st</sup> week of arrival to the Italian fattening unit (Diet 1) and in the following weeks until day 30 (Diet 2).

Item	Diet 1	Diet 2
Ingredients, % DM basis		
Corn silage	24.5	32.6
Meadow hay	18.5	—
Wet sugar beet pulps	12.6	11.7
Corn meal	11.5	23.7
Wheat bran	11.4	9.40
Dehydrated alfalfa	10.2	9.40
Soybean meal	9.30	11.4
Mineral premix <sup>1</sup>	2.00	1.80
Nutrient analysis		
DM, %	55.4	52.0
Ash, % DM	5.70	5.20
CP, % DM	14.0	15.2
EE, % DM	2.30	2.60
aNDF, % DM <sup>2</sup>	39.2	30.6
ADF, % DM	20.7	18.4
Starch, % DM	20.0	30.6
uNDF, % DM <sup>3</sup>	9.40	8.10

<sup>1</sup> The mineral premix contained: 19.1% calcium; 0.33% phosphorus; 0.17% agnesium; 0.06% sodium.

<sup>2</sup> Amylase treated NDF.

<sup>3</sup> Undigested NDF.

sition and analysis are reported in Table 1. Both diets were provided *ad libitum* as a total mixed ration once a day at 1000 h, and it was estimated that during the 1<sup>st</sup> week, each animal ate around 8 kg/day of DM. The diet of the Yeast group differed only for the inclusion of yeast supplementation (Levucell SC<sup>®</sup> 10 TITAN) to achieve a theoretical daily dose of 100 g/bull of yeast premix (i.e.,  $8 \times 10^9$  CFU/bull per day of live yeast). This dosage was chosen based on the positive outcomes in terms of ADG and feed efficiency reported in previous studies on beef cattle during challenging periods, such as the receiving phase (Smith et al., 2020; Parra et al., 2021). Samples of the diets were collected weekly and kept frozen at  $-20$  °C until chemical analyses. Samples were dried in a forced-air oven at 103 °C overnight for DM determination, ground in a Retch mill type SK (Bauknecht, Stuttgart, Germany) to pass a 1 mm screen, and analysed for several parameters. The ash content was determined by combustion at 550 °C for 4 h. Nitrogen was determined following the Dumas method (AOAC, 2023) by the combustion digestion of the sample at 900 °C in excess of oxygen by Dumatherm<sup>®</sup> (Gerhardt GmbH & Co, Königswinter, Germany) as described by Mihaljev et al. (2015), and feedstuff CP content was then calculated as nitrogen  $\times$  6.25. Ether extract content was determined following the indication of European Commission Regulation No. 152/2009 (European Commission, 2009). Fibre fractions were analysed and expressed as NDF assayed with a heat-stable amylase and expressed exclusive of residual ash, and ADF was expressed exclusive of residual ash, according to Mertens et al. (2002). The starch content was determined by enzymatic method (McCleary et al., 2019). The undigestible NDF was determined after *in vitro* fermentation for 240 h (Raffrenato et al., 2018).

Animals' performance and blood sampling collection

All bulls were individually weighted on three dates after transport: at the arrival to the fattening unit (day 0), after 7 days (day

7), and at the end of the receiving period after 30 days (**day 30**). At day 0, all animals were unloaded from the trucks and weighed. In compliance with the current European regulations on cattle transport (European Commission, 2018), bulls had free access to water during transport. On days 7 and 30, the bulls were deprived of feed overnight but had access to water. Individual blood samples were collected by a veterinarian at each of the weighing sessions after the veterinary examination of each animal. Animal handling and moving were performed by trained personnel to reduce stressful conditions and the procedures were conducted only after the veterinarian check of animal health. An electronic livestock scale ( $\pm 1$  kg) was used to individually weigh the bulls. Blood samples were collected by the veterinarian of the fattening unit in Italy. All samples were taken from the jugular vein of each bull using 9 mL Vacuette® LH – lithium heparin blood collection tubes (Greiner Bio-One GmbH, Kremsmünster, Austria) and placed into a refrigerated box filled with dry ice until the end of the sampling session. Blood tubes were centrifuged at  $1\,500 \times g$  for 15 min at  $4^\circ\text{C}$  within 2 h after the collection, and plasma was aliquoted and stored into 2 mL cryovials (S.I.A.L. Cryovials, Rome, Italy) at  $-80^\circ\text{C}$  until the analysis. Blood analyses were carried out at the laboratory of the Istituto Zooprofilattico Sperimentale delle Venezie (Legnaro, Italy) and blood parameters included the protein profile (albumin, creatinine, globulins, total proteins, total blood urea), the energy profile (cholesterol, glucose, triglycerides), the hepatomuscle profile (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatine kinase, lactate dehydrogenase, gamma-glutamyl transferase, total and direct bilirubin), and the mineral profile (calcium, chlorine, iron, potassium, magnesium, sodium, phosphorous, selenium). The levels of cortisol and non-esterified fatty acids were assessed as well. All plasma components were analysed by biochemical analyser (COBAS C501, Roche Diagnostics GmbH, Mannheim, Germany) using commercial diagnostic kits from Roche Diagnostics. The non-esterified fatty acid determination was performed with enzymatic colorimetric methods (Randox Laboratories Ltd., Ardmore, United Kingdom) using COBAS C501 instrumentation.

#### Monitoring of rumen environment

Two different reticulum-rumen devices were used to monitor changes in the rumen environment during the receiving period of the Charolais young bulls at the Italian fattening unit. Devices were rumen fluid resistant (materials tested by the German Agricultural Society, DLG). A total of 60 bulls were equipped with the reticulum-rumen sensors that were inserted at the two French collection centres after blood sampling. Thirty-four bulls (17 Yeast and 17 Control) received the first reticulum-rumen device (**RR-T**, SmaXtec animal care GmbH Belgiergasse 3, Graz, Austria, TX-1442A) to monitor rumen temperature, rumination time, and activity of the animal. Other 26 bulls (13 Yeast and 13 Control) received the second device (**RR-pH**, SX2, SmaXtec animal care GmbH Belgiergasse 3, Graz, Austria, SX2, TX-2142P) that was equipped with a pH electrode to measure reticulum-rumen pH (accuracy:  $\pm 0.1$  pH unit) for 90 days. The animals that received the RR-T bolus had an average BW of  $444 \pm 14.4$  kg and  $445 \pm 13.4$  kg, and an average age at arrival of  $280 \pm 22.5$  days and  $288 \pm 24.5$  days for Yeast group ( $N = 17$ ) and Control group ( $N = 17$ ), respectively. The animals that received the RR-pH bolus had an average BW of  $445 \pm 10.4$  kg and  $445 \pm 10.1$  kg, and an average age at arrival of  $290 \pm 23.0$  days and  $281 \pm 23.4$  days for Yeast group ( $N = 13$ ) and Control group ( $N = 13$ ), respectively. Trained veterinarians used a specific gun provided by the producers to administer the boluses, following the manufacturer's instructions. In the Yeast group, 13 animals received both the slow-release yeast bolus and the RR-pH device, while 17 animals received both the slow-

release yeast bolus and the RR-T device. These animals were part of the treated group. In the control group, 13 animals received only the RR-pH device, and 17 animals received only the RR-T device. Thus, both the treated and control animals underwent the same handling and management during bolus administration, transport, and on-farm movements in France and Italy. The RR-T and RR-pH devices were  $105 \times 35$  mm and  $132 \times 35$  mm (length  $\times$  diameter), respectively. Both devices recorded information every 10 min for a total of 144 measures/day. The internal memory of the devices allowed to store the recorded data of temperature and pH for 50 days. The RR-pH was calibrated using a buffer solution (pH = 7) before the insertion into the rumen environment. The validity of the devices was tested in the study of Klevenhusen et al. (2014), who used rumen-cannulated dairy cows as reference for pH. Both devices transferred their information to a base station placed in the receiving barn of the fattening unit, through a wireless system. Because the devices need 1 or 2 days to be stabilised inside the reticulum, the data from the insertion at the French collection centres to the first day after animals' arrival to the Italian fattening unit were not interpretable. Since bulls generally stayed 1 or 2 days in the collection centres, there was no option to consider using RR bolus data at this stage in the current study.

#### Statistical analysis

BW and blood parameters were analysed using the SAS software 9.4 (SAS Institute Inc., Cary, NC). Individual ADG was calculated for the two subsequent interim periods (day 0 to day 7, and day 7 to day 30) and for the whole receiving phase (day 0 to day 30). The ADG of bulls was analysed using a linear model that considered the fixed effects of yeast supplementation group (Yeast, Control), pen nested within yeast supplementation group, and age at arrival as covariate. To assess the trend of BW and blood parameters during the receiving period (day 0 to day 30), the following linear mixed repeated model was used:

$$y_{ijklmn} = \mu + \text{supplementation}_i + \text{pen}_j(\text{supplementation}_i) + \text{time}_k + \text{age}_l + (\text{supplementation} \times \text{time})_{ik} + \text{bull}_m + e_{ijklmn}$$

where  $y_{ijklmn}$  is the dependent variable;  $\mu$  is the overall intercept of the model;  $\text{supplementation}_i$  is the fixed effect of the  $i$ th supplementation group ( $i = \text{Yeast, Control}$ );  $\text{pen}_j(\text{supplementation}_i)$  is the fixed effect of the  $j$ th pen ( $j = 1-8$ ) nested within the  $i$ th supplementation group;  $\text{time}_k$  is the fixed effect of the  $k$ th day of measurement ( $k = 0, 7, 30$ );  $\text{age}_l$  is the fixed effect of age of bull at arrival modelled as covariate;  $(\text{supplementation} \times \text{time})_{ik}$  is the fixed interaction effect between supplementation group and day of measurement;  $\text{bull}_m$  is the random effect of the  $m$ th bull ( $m = 1-80$ )  $\sim N(0, \sigma_{\text{bull}}^2)$ , where  $\sigma_{\text{bull}}^2$  is the bull variance; and  $e_{ijklmn}$  is the random residual  $\sim N(0, \sigma_e^2)$ , where  $\sigma_e^2$  is the error variance.

Before applying the model to blood parameters, values outside the mean  $\pm 3$  SD were discarded as outliers and creatine kinase was log-transformed to reach normal distribution. Total and direct bilirubin were returned by the laboratory as binary variables and were considered physiological when  $< 2.5$   $\mu\text{mol/L}$  and  $< 1.5$   $\mu\text{mol/L}$ , respectively. Therefore, two categories for total and direct bilirubin were created and chi-squared test was performed to assess any differences across time (days 0, 7, 30) or between supplementation group (Yeast, Control).

Data recorded by the sensors for the reticulum-rumen parameters were analysed using the R software v. 4.1.2. Individual data collected every 10 min during the 4 days preceding the weighing and sampling time points on day 7 and day 30 were extracted and summarised daily. Data on day 0 were not available due to the sensors' technological limitations described above. Then, daily

indicators of mean pH, pH amplitude (difference between the maximum and minimum pH), pH SD, mean activity, rumination (min/day), and mean temperature (°C) were calculated. In addition, daily relative pH indicators (NpH) were calculated according to Villot et al. (2018); briefly, signal processing was applied to raw RR-pH values to calculate the NpH by filtering and normalising data to remove inter-individual variability, sensor drift, and sensor noise. Both the sensitivity and specificity of NpH by Villot et al. (2018) ranged from 0.82 to 0.88 to diagnose SARA. Accordingly, an animal was considered positive to SARA on a daily base if NpH decreased by more than 0.3 units for more than 50 min, and daily amplitude of NpH varied by more than 0.8 units and/or its SD was above 0.2 units. The percentage of animals under SARA was calculated for each period and group (day 7 and day 30 for Yeast and Control groups). Additionally, a statistical method based on the 'change-point' package of R software (Killick et al., 2022) was implemented to detect the significant number of daily pH drops in the RR-pH kinetics curve. A daily pH pattern for each animal was calculated by averaging each 10-min measurement of the 4 days for each period (day 7 and day 30). The daily reticulo-rumen indicators at day 7 and day 30 were analysed separately with the following model:

$$y_{ijklmn} = \mu + \text{supplementation}_i + \text{age}_j + \text{day}_k + \text{truck}_l + \text{bull}_m(\text{pen}_n) + e_{ijklmn}$$

where  $y_{ijklmn}$  is the response variable;  $\mu$  is the overall intercept of the model;  $\text{supplementation}_i$  is the fixed effect of the  $i$ th supplementation group ( $i = \text{Yeast, Control}$ );  $\text{age}_j$  is the fixed effect of age of bull at arrival to the fattening unit modelled as covariate;  $\text{day}_k$  is the fixed effect of the  $k$ th day ( $k = 1-4$ );  $\text{truck}_l$  is the fixed effect of the  $l$ th truck ( $l = 1, 2$ );  $\text{bull}_m(\text{pen}_n)$  is the random effect of the  $m$ th bull ( $m = 1-80$ ) nested within the  $n$ th pen ( $n = 1-8 \sim N(0, \sigma_{\text{bull}(\text{pen})}^2)$ ), where  $\sigma_{\text{bull}(\text{pen})}^2$  is the bull within pen variance; and  $e_{ijklmn}$  is the random residual  $\sim N(0, \sigma_e^2)$ , where  $\sigma_e^2$  is the error variance. Due to a lack of statistical significance, the fixed interaction effect between supplementation and time (day) was removed from the model used to process the daily reticulo-rumen indicators. Binary outcomes created for SARA positive/negative days were evaluated using the same effects in a generalised linear mixed model with a binomial distribution. A multiple comparison of least squares means for the supplementation group effect was performed using Bonferroni's test. The inter-animal variability within group was evaluated through the Levene test using the 10 min averaged measures by day calculated on both periods (day 7 and day 30). For all the analyses, statistical significance was set at  $P < 0.05$  and a trend of significance was set at  $0.05 \leq P < 0.10$ .

Despite the randomised controlled design of our study, there are possible limitations to consider. While we ensured treatments were applied without "spillover" effects and measurements of BW, blood metabolites, reticulo-rumen pH, and reticulo-rumen temperature were objective, interactions between animals within the same pen may have occurred. This could have affected the assumption of complete independence of measurements. Although the animals were selected to be similar in BW and age to minimise variability and manger space allowed each animal of the same pen to eat at the same time, inherent biological differences, such as feeding behaviour and thus hierarchy, could have impacted the outcomes. The choice of the individual animal as the experimental unit was based on the precise nature of the treatments and objective measurements. Given our environmentally controlled conditions (i.e., same transport method, animal handling in France and Italy, animal management in Italy, manger space, pen dimension), this approach was suitable for statistical inference at individual level. However, these limitations should be considered when interpreting the results and assessing their broader applicability.

## Results

### Growth performances and plasma traits

Control and Yeast bulls started their fattening cycle with similar BW (day 0; BW = 417 and 416 kg, respectively; SEM = 2.70;  $P > 0.05$ ) and no differences due to the yeast supplementation were recorded neither at day 7 (BW = 433 kg for both groups; SEM = 2.79 kg;  $P > 0.05$ ) nor at day 30 (BW = 469 and 468 kg, respectively; SEM = 3.32 kg;  $P > 0.05$ ) as depicted in Fig. 2. There was no significant effect of yeast on any of the calculated ADG ( $P > 0.05$ ; Table 2). Least squares means of plasma traits for sampling time and yeast supplementation effects are presented in Table 3. Except for magnesium, all the plasma traits significantly varied over sampling time ( $P < 0.05$ ). Most of them decreased over time, except for globulins, calcium and iron which had an opposite trend. Plasma cortisol decreased from day 0 to day 30 reaching the nadir at day 7. Yeast supplementation led to overall higher values of creatine kinase, potassium, and selenium and lower values of alkaline phosphatase ( $P < 0.05$ ; Table 3). Interaction between sampling time (day) and supplementation did not affect any blood traits ( $P > 0.05$ ), with the only exception of selenium that had the highest value on day 7 for the Yeast group ( $P < 0.05$ ; Fig. 3).

### Effects of live yeast supplementation on reticulum-rumen traits

Least squares means of reticulum-rumen traits measured at day 7 and day 30 through the RR-T and RR-pH sensors are reported in Table 4. Compared to the Control group, Yeast group had significantly lower daily pH amplitude, SD, and percentage of animals positive to SARA at day 7, with a higher number of pH drops ( $P < 0.05$ ). On day 7, Yeast group tended to ruminate more compared with Control group. Yeast supplementation did not affect reticulum-rumen sensor traits at day 30, except for rumination time. Bulls of the Yeast group ruminated on average 39 min/day more than Control group ( $P < 0.05$ ; Table 4). Fig. 4 depicts the daily reticulum-rumen pH pattern measured at day 7 and day 30. The daily pattern of raw pH showed a positive trend from the early morning to early afternoon and a negative trend from the late afternoon that continued overnight. The Yeast group showed a significantly lower variability in daily rumen pH compared to the Control group, either at day 7 ( $P < 0.05$ ) or at day 30 ( $P < 0.05$ ). This is depicted in Fig. 5, which shows the inter-animal variability of daily rumen pH at day 7 and day 30.

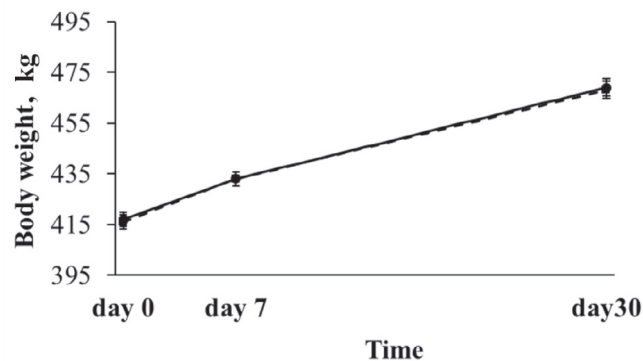


Fig. 2. Least squares means and SE of BW of Charolais young cattle over time (day 0 = arrival to Italy; day 7 = 7 days after arrival to Italy; day 30 = 30 days after arrival to Italy) according to the supplementation group (Yeast = dashed line,  $n = 40$  bulls; Control = solid line,  $n = 40$  bulls) ( $P > 0.05$ ).

**Table 2**

Average daily gain from arrival (day 0) to day 30 of the receiving period at the fattening unit of Charolais young cattle for supplementation group effects.

Trait	Supplementation		SEM	P-value
	Control group	Yeast group		
Bulls (n)	40	40		
Average daily gain (kg/day)				
from day 0 to day 7	2.30	2.35	0.21	0.87
from day 7 to day 30	1.69	1.66	0.08	0.83
from day 0 to day 30	1.84	1.83	0.07	0.91

**Table 3**

Least squares means and SEM of plasma traits of 80 Charolais young cattle for time (day 0, 7 and 30) and supplementation group effects.

Trait	Time <sup>1</sup>			SEM	P-value	Supplementation			SEM	P-value
	day 0	day 7	day 30			Control	Yeast			
Bulls (n)	80	80	80			40	40			
Protein profile										
Albumin (g/l)	34.5 <sup>a</sup>	31.5 <sup>b</sup>	31.0 <sup>b</sup>	0.26	<0.001	32.2	32.4	0.26	0.62	
Creatinine (µmol/l)	145 <sup>b</sup>	157 <sup>a</sup>	115 <sup>c</sup>	2.64	<0.001	139	139	2.88	0.93	
Globulins (g/l)	41.8 <sup>b</sup>	43.6 <sup>a</sup>	42.5 <sup>ab</sup>	0.59	<0.001	42.6	42.7	0.61	0.95	
Total protein (g/l)	76.2 <sup>a</sup>	75.2 <sup>b</sup>	73.8 <sup>c</sup>	0.50	<0.001	75.0	75.1	0.54	0.90	
Urea (mmol/l)	3.71 <sup>a</sup>	3.24 <sup>b</sup>	2.89 <sup>c</sup>	0.10	<0.001	3.34	3.22	0.09	0.33	
Energy profile										
Cholesterol (mmol/l)	2.40 <sup>a</sup>	2.09 <sup>b</sup>	1.57 <sup>c</sup>	0.05	<0.001	1.97	2.08	0.06	0.18	
Glucose (mmol/l)	5.24 <sup>a</sup>	4.91 <sup>b</sup>	5.01 <sup>b</sup>	0.06	<0.001	5.01	5.10	0.06	0.24	
Triglycerides (mmol/l)	0.23 <sup>a</sup>	0.18 <sup>b</sup>	0.17 <sup>b</sup>	0.01	<0.001	0.19	0.19	0.004	0.44	
Non-esterified fatty acids (meq/l)	0.98 <sup>a</sup>	0.70 <sup>b</sup>	0.28 <sup>c</sup>	0.03	<0.001	0.66	0.65	0.03	0.85	
Hepato-muscle profile										
Alkaline phosphatase (U/l)	133 <sup>a</sup>	78.9 <sup>b</sup>	74.8 <sup>b</sup>	3.74	<0.001	102 <sup>a</sup>	89.9 <sup>b</sup>	3.94	0.04	
Alanine aminotransferase (U/l)	28.1 <sup>a</sup>	22.9 <sup>b</sup>	21.9 <sup>b</sup>	0.50	<0.001	24.3	24.3	0.52	0.96	
Aspartate aminotransferase (U/l)	112 <sup>a</sup>	84.6 <sup>b</sup>	70.0 <sup>c</sup>	2.23	<0.001	90.4	87.6	2.07	0.34	
Creatine kinase (U/l) <sup>2</sup>	399 <sup>a</sup>	220 <sup>b</sup>	138 <sup>c</sup>	1.07	<0.001	205 <sup>b</sup>	258 <sup>a</sup>	1.06	0.01	
Lactate dehydrogenase (U/l)	1 399 <sup>a</sup>	1 142 <sup>b</sup>	1 030 <sup>c</sup>	16.85	<0.001	1 213	1 167	18.2	0.08	
Gamma glutamyl transferase (U/l)	15.3 <sup>a</sup>	12.4 <sup>b</sup>	11.6 <sup>b</sup>	0.45	<0.001	13.1	13.0	0.45	0.87	
Total bilirubin <sup>3</sup>										
>2.5 µmol/l	49	55	18	–	<0.001	60	63	–	0.73	
<2.5 µmol/l	31	25	62	–		100	97	–		
Direct bilirubin <sup>3</sup>										
>1.5 µmol/l	36	25	3	–	<0.001	32	33	–	0.89	
<1.5 µmol/l	44	55	77	–		128	127	–		
Mineral profile										
Calcium (mmol/l)	2.43 <sup>ab</sup>	2.40 <sup>b</sup>	2.44 <sup>a</sup>	0.01	0.03	2.42	2.43	0.01	0.85	
Chlorine (mmol/l)	100 <sup>a</sup>	97.1 <sup>b</sup>	96.4 <sup>c</sup>	0.21	<0.001	97.8	98.0	0.20	0.40	
Iron (µg/dl)	60.8 <sup>c</sup>	111 <sup>a</sup>	98.1 <sup>b</sup>	3.10	<0.001	89.2	90.5	2.70	0.72	
Potassium (mmol/l)	4.68 <sup>b</sup>	5.04 <sup>a</sup>	4.72 <sup>b</sup>	0.04	<0.001	4.76 <sup>b</sup>	4.87 <sup>a</sup>	0.04	0.04	
Magnesium (mmol/l)	0.83	0.83	0.82	0.01	0.76	0.84	0.82	0.01	0.25	
Sodium (mmol/l)	140 <sup>a</sup>	140 <sup>a</sup>	138 <sup>b</sup>	0.21	<0.001	139	139	0.19	0.71	
Phosphorus (mmol/l)	2.31 <sup>a</sup>	2.33 <sup>a</sup>	2.14 <sup>b</sup>	0.03	<0.001	2.25	2.27	0.03	0.60	
Selenium (µg/l)	63.4 <sup>b</sup>	89.5 <sup>a</sup>	85.3 <sup>a</sup>	2.12	<0.001	70.1 <sup>b</sup>	88.7 <sup>a</sup>	2.29	<0.001	
Stress profile										
Cortisol (nmol/l)	60.4 <sup>a</sup>	48.3 <sup>b</sup>	56.5 <sup>ab</sup>	3.61	<0.001	51.3	58.9	3.55	0.14	

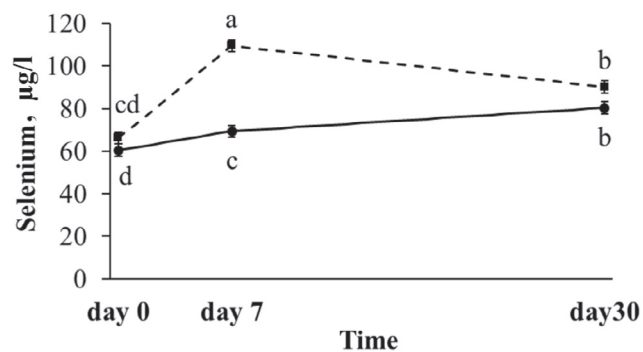
<sup>1</sup> day 0 = upon arrival to Italy; day 7 = after 1 week of arrival to Italy; day 30 = after 4 weeks of arrival to Italy.<sup>2</sup> Back-transformed values.<sup>3</sup> Total and direct bilirubin are reported as percentage of samples that were above or below the thresholds and were analysed using Chi-squared test.a,b,c Means with different superscripts within the same blood parameter and effect differ significantly ( $P < 0.05$ ).

## Discussion

### Growth performances and plasma traits

Several studies have tested the effects of yeast or yeast-based product supplementation on ADG of beef cattle under different conditions (Broadway et al., 2015; Sartori et al., 2017; Pancini et al., 2020). However, their results were inconsistent and difficult to compare due to the differences in cattle age, diet composition, yeast strains, and dosage. These different responses suggest that the yeast supplementation interacts with many variables. Control

and Yeast bulls had similar BW at the arrival to the fattening unit. During the 1<sup>st</sup> week, they were fed a moderate energy content in total mixed ration and then they were switched to a higher energy diet for the remaining receiving phase. Body weight increased similarly in both Control and Yeast groups, with ADG that was numerically higher between day 0 and day 7 compared to that from day 7 and day 30. This was likely due to a compensation for the weight loss due to the transport stress. Body weight shrinkage is one of the most common effects of long transport in cattle due to factors like distance and duration of the travel, driving quality, animal handling procedures, feed and water restrictions, and climate con-



**Fig. 3.** Least squares means and SE of selenium concentration in plasma of Charolais young cattle for the interaction effect between supplementation group (Yeast = dashed line, n = 40 bulls; Control = solid line, n = 40 bulls) and sampling time (day 0 = arrival to Italy; day 7 = 7 days after arrival to Italy; day 30 = 30 days after arrival to Italy;  $P < 0.05$ ). <sup>a,b,c,d</sup> Different superscripts indicate a significant difference ( $P < 0.05$ ).

ditions (González et al., 2012; Van Engen and Coetzee, 2018). Yeast supplementation did not affect growth performance, confirming the results from previous studies (Cozzi et al., 2011; Sartori et al., 2017; Pancini et al., 2020). Sgoifo Rossi et al. (2017) did not observe differences in the final BW of bulls that received yeast and selenium supplementation, but they observed an overall improved ADG in the supplemented group compared to the control group. This difference, however, was marked between 19 and 54 days after the arrival to the fattening unit; it is possible, therefore, that the observation period of the present study (30 days) was not long enough to detect differences in growth performances.

**Table 4**

Effects of yeast supplementation on reticulo-rumen traits of Charolais young cattle at day 7 and day 30. Bulls equipped with bolus for pH measurement were 13 per group (Yeast and Control), and bulls equipped with bolus for assessment of rumen activity, temperature, and rumination time were 17 per group.

Trait	Supplementation		SEM	P-value
	Control	Yeast		
<b>Day 7<sup>1</sup></b>				
N of bulls with pH bolus	13	13	–	–
Daily pH mean	6.94	6.97	0.06	0.69
Daily pH amplitude	0.68 <sup>a</sup>	0.58 <sup>b</sup>	0.03	0.04
Daily pH SD	0.17 <sup>a</sup>	0.13 <sup>b</sup>	0.01	0.01
Time of NpH <sup>2</sup> below –0.3, min/d	55.0	19.0	2.12	0.20
pH drops, number/d	3.85 <sup>b</sup>	4.54 <sup>a</sup>	0.29	0.03
Daily SARA-positive, % of animals <sup>3</sup>	36.0 <sup>a</sup>	12.8 <sup>b</sup>	6.00	0.01
N bulls with temperature bolus				
Activity, reference unit	6.52	6.56	0.24	0.91
Rumination, min/d	396	428	13.1	0.09
Temperature, °C	39.9	39.8	0.05	0.82
<b>Day 30<sup>4</sup></b>				
N of bulls with pH bolus	13	13	–	–
Daily pH mean	7.01	6.95	0.11	0.56
Daily pH amplitude	0.75	0.76	0.08	0.88
Daily pH SD	0.17	0.17	0.02	0.92
Time of NpH <sup>2</sup> below –0.3, min/d	15.3	29.0	0.40	0.42
pH drop, number/d	4.11	4.10	0.29	0.99
Daily SARA-positive, % of animals <sup>3</sup>	16.1	17.8	7.00	0.85
N of bulls with temperature bolus				
Activity, reference unit	4.43	4.77	0.27	0.36
Rumination, min/d	347 <sup>b</sup>	386 <sup>a</sup>	12.6	0.03
Temperature, °C	39.9	39.9	0.03	0.76

<sup>1</sup> 4 days of measures before day 7 sampling time point.

<sup>2</sup> Daily relative pH indicators.

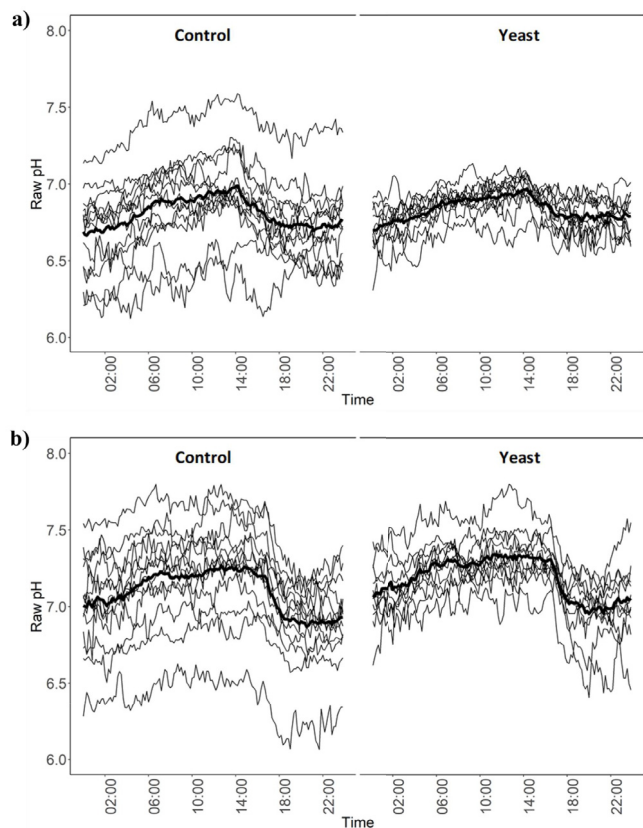
<sup>3</sup> An animal was considered positive to subacute ruminal acidosis (SARA) on a daily basis if NpH decreased by more than 0.3 units for more than 50 min, and daily NpH amplitude varied by more than 0.8 units and/or its SD was above 0.2 units.

<sup>4</sup> 4 days of measures before day 30 sampling time point.

<sup>a,b</sup> Means with different superscripts within the same rumen parameter line differ significantly ( $P < 0.05$ ).

Almost all the plasma traits varied significantly over sampling time. The temporal pattern of protein, energy, hepato-muscle, mineral, and stress profile markers denoted a framework of stress induced by the commingling and transport that led to some degree of energy deficit, muscular damage, dehydration, and hepatic stress, even if almost all the traits remained within the physiological range. Santinello et al. (2024) evaluated the same animals during transport from France to Italy and observed that signs of stress and recovery efforts began even before the departure. All the parameters tended to stabilise during the receiving phase denoting a progressive recovery of physiological homeostasis. A reliable example in this regard is the pattern observed for creatine kinase (marker of muscular damage), gamma-glutamyl transferase (marker of hepatic health), and non-esterified fatty acids (markers of energy deficit) that peaked at day 0 to progressively decrease at the following two sampling dates. Values of plasma traits recorded at day 30 might represent a reference for Charolais young bulls under intensive fattening, as the animals at the end of the receiving phase should have adapted to the new housing and feeding conditions.

Yeast supplementation scarcely affected the metabolic profile. Selenium is a fundamental component of the glutathione peroxidase selenoproteins, which are the main responsible for the protection of cells from oxidative stress, and it is also involved in the metabolism of the thyroid hormones (Mehdi and Dufrasne, 2016). Usually, French young bulls are raised on pasture and selenium is among the most deficient trace elements in grazing cattle not receiving a specific mineral supplementation, despite its positive effects on health, including improved immune competence (Arthington and Ranches, 2021). The few overall variations in the metabolic traits that were observed for the Yeast group were difficult to explain based on yeast and selenium metabolism and phys-



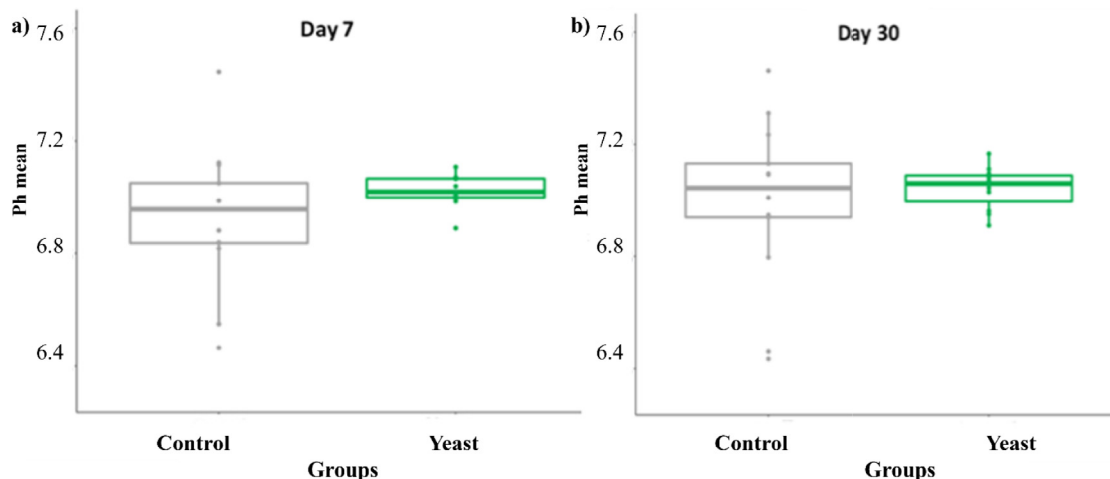
**Fig. 4.** Charolais young cattle daily reticulo-rumen pH pattern of Yeast and Control groups (13 bulls each) at day 7 (a) and day 30 (b)\*. \* Individual data collected every 10 min during the 4 days preceding the weighing and sampling time points on day 7 and day 30 were extracted and summarised daily.

ological function. The overall lower level of alkaline phosphatase recorded in Yeast compared with Control bulls was in contrast with the findings of Arthur (1988a) and Jia et al. (2019), who observed higher levels of the enzyme in calves and steers, respectively, with higher availability of selenium. Creatine kinase also showed an opposite trend compared to results from the literature, where higher levels of selenium were linked to lower levels of this

enzyme, due to the protective role of the selenoproteins on the muscular cells (Arthur et al., 1988b). Marcato et al. (2018) suggested that creatine kinase can increase due to the restoration of homeostasis when cattle are physiologically stressed. The difference in potassium concentration between the two supplementation groups has not a clear explanation. No other minerals showed significant variations (except for selenium) and there is no evidence in the literature that relates live yeast-selenium and potassium. Conversely, considerably higher levels of selenium in the Yeast group were expected, due to its supplementation. Consistently, selenium was affected by the interaction between the supplementation group and sampling time. Selenium supplementation by boluses should have promoted its marked increase in the plasma recorded on day 7. Then, plasma concentration progressively decreased, reaching again the levels of the Control group at day 30. No other plasma traits were affected by the supplementation × sampling time interaction, so yeast supplementation did not affect the metabolic profile under challenging conditions (day 0 and day 7) and after the receiving period (day 30). Cozzi et al. (2011) showed that higher selenium availability led to greater antioxidant activity in beef cattle, with increased serum total antioxidative capacity, but during a longer supplementation. However, the primary focus of the present study was on the transfer of organic selenium through yeast into the bloodstream, rather than on the reduction of oxidative activity in the animals' metabolism following selenium supplementation. Therefore, no specific marker of oxidative stress was investigated. As a result, the findings of this study did not allow for direct conclusions about the role of selenium to reduce the oxidative stress of the animals.

*Live yeast supplementation and rumen environment*

The values of pH (average of 6.95) are considered high for the category of young beef cattle especially between day 7 and day 30, with higher values for day 30. This could be partially explained by the observed difference (−0.2 pH units) between pH measured in the free rumen liquid compared to reticulum location (Neubauer et al., 2018). Therefore, the normalised RR-pH indicators (Villot et al., 2018) were integrated to investigate the yeast supplementation on rumen characteristics. At the receiving phase, a significantly lower number of bulls were recorded as positive to SARA in the Yeast group compared to the Control group which could be explained by previous findings indicating that bulls supplemented with yeast had higher level of butyrate and lower level



**Fig. 5.** Overall evolution of the inter-cattle variability of daily reticulo-rumen pH mean for Yeast (YEAST, green line; n = 13) and Control (CONT, grey line; n = 13) bulls over the 4 days before sampling at day 7 ( $P < 0.05$ ; a) and day 30 ( $P < 0.05$ ; b).



of lactate in the rumen fluid (Magrin et al., 2018). Moreover, on the same day during the initial feeding phase, the Yeast group had a significantly greater frequency of daily pH drops than the Control group, while maintaining a similar mean pH. A drop of ruminal pH in cows is associated with the ingestion of fermentable carbohydrates and the accumulation of high amounts of short-chain fatty acids, due to their slow absorption by the rumen wall. A low number of pH drops with an abrupt decrease measured daily have been reported in dairy cows subjected to an induced SARA challenge compared to the same animals fed a forage-rich diet (Villot et al., 2023). Considering that both groups commenced the fattening cycle with equivalent BW and feeding regimens, this variation in pH fluctuation may be linked to differences in feeding behaviour. This observation aligns with previous findings of De Vries and Chevaux (2014), who reported improvements in meal pattern, including more frequent meals that tended to be smaller and shorter for cows supplemented with the same yeast strain. Rumination time improved in the Yeast group (+39 min/day compared with Control group at day 30). This result agreed with previous findings on meal pattern and is encouraging when considering that bulls at the end of the receiving phase shift to high-energy diets. In this study, the time of total mixed ration delivery was at 1000–1100 h, and this explains why the daily reticulum-rumen pH patterns had a decrease from early morning to early afternoon and a negative trend from the late afternoon to around 1800 h, both at day 7 and day 30. Moreover, the inter-individual variability of the rumen conditions can also produce different animals' responses to fattening phase. The young bulls of this study had a specific and lower inter-animal variability for daily pH fluctuation within the supplementation group, likely due to a specific meal patterning, hierarchy, and microbial characteristics of the rumen homogenised by the addition of the live yeast. It was evident how, at both times, yeast supplementation stabilised the rumen pH of the Yeast group, thus significantly reducing the inter-animal variability. This could represent an important aid in bulls' diet and feeding management. In their drifting analysis, Crossland et al. (2019) reported that moving the animals through the facilities can cause a significant disturbance in pH values for several days. The 80 Charolais young bulls used in this study were commingled from different farms of origin and long transported to a completely new environment made of different housing, management, and feeding. All that has shown to be a relevant source of stress which in turn could have influenced rumen pH values and pattern. The supplementation of yeast can stabilise the pH variance (Crossland et al., 2019) and this might explain why a significant reduction of daily pH amplitude and SD was observed at day 7. The complexity around cattle handling from pasture to collection centres made it difficult to anticipate the RR bolus insertion before arrival to the collection centres. Indeed, the requirement for a couple of days settling the RR sensors data acquisition prevented us from valorising the valuable data during transport since animals did not stay longer than 1–2 days before transport to Italy. The opportunity to apply the RR sensors and the nutritional bolus in a future study before the commingling procedure would represent a great improvement for both RR data collection and supplementation efficacy.

The receiving phase of beef cattle at the final fattening unit is challenging for health and welfare, as animals have to recover from the stress of the transfer from their farms of origin, and they are exposed to a completely new environment, housing solutions, and feeding programmes. In this scenario, cattle may have some benefit from the dietary supplementation with yeast and selenium. The outcomes of this study revealed that yeast supplementation did not improve the growth performance of imported Charolaise young bulls nor affected their metabolic profile, but it helped to stabilise the conditions of their rumen environment by limiting

the daily pH amplitude and SD. As a response to these better ruminal conditions, the animals boosted their daily time spent ruminating measured at the end of the receiving phase, when they were fed a high-energy diet. Therefore, yeast supplementation seems to be a positive support to the rumen environment in cattle exposed to stressful conditions. This could be an interesting finding also when thinking about the high-energy diets generally fed to beef cattle during the remaining fattening period.

### Ethics approval

All the experimental procedures were approved by the Animal Care and Use Committee of the University of Padova (Ethical Approval Code: n. 400074 5/2021).

### Data and model availability statement

None of the data were deposited in an official repository. However, data and models are available upon request.

### Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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### Declaration of interest

University of Padova authors have no conflicts of interest. Clothilde Villot, Eric Chevaux, and Bruno Martin are employed by Lallemand SAS.

### Acknowledgement(s)

The authors would like to thank the fattening unit Azienda MEA (Porto Viro, Rovigo, Italy) where the trial was conducted.

## Financial support statement

This study was supported by Lallemand SAS (Blagnac, France), research grant number: DE\_M\_ALTRECOMM22\_01.

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