Italian Journal of Animal Science

ISSN: 1828-051X (Online) Journal homepage: www.tandfonline.com/journals/tjas20

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To cite this article: Silvia Sandrini, Vera Perricone, Alessia Pea, Clara Cenati, Giovanni Savoini, Gianluca Baldi & Alessandro Agazzi (2025) Live yeast supplementation improves apparent nutrient digestibility of high-fibre diet in mature quarter horses, Italian Journal of Animal Science, 24:1, 1381-1389, DOI: 10.1080/1828051X.2025.2515539

To link to this article: https://doi.org/10.1080/1828051X.2025.2515539

Published online: 18 Jun 2025.

ITALIAN JOURNAL OF ANIMAL SCIENCE
2025, VOL. 24, NO. 1, 1381–1389
https://doi.org/10.1080/1828051X.2025.2515539

RESEARCH ARTICLE

Live yeast supplementation improves apparent nutrient digestibility of high-fibre diet in mature quarter horses

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ABSTRACT

This study aimed to evaluate the effects of live yeast (*Saccharomyces cerevisiae*, BioCell® S12, DBVPG 48 SF, Mazzoleni spa, Bergamo, Italy) in mature horses fed a high forage:concentrate ratio diet on apparent nutrients digestibility. Eight American Quarter Horse (475.5 kg; 8.5 years) were supplemented with *S. cerevisiae* (3 g/horse/d; LY) or not (CTR) in a two-periods crossover design of 28 days each, with a 7-days adaptation period, and a confinement in the last 5 days. Body weight (BW) was measured at 0, 15, 23 and 28 days for each period, and dry matter intake (DMI) determined during confinement. Individual faecal samples were collected for five consecutive days at the end of each period (23-28d). Data were analysed using a MIXED procedure for repeated measurements of SAS. No effects of *S. cerevisiae* supplementation were observed on BW and DMI during the whole trial. Yeast improved apparent nutrient digestibility of dry matter (58.22% vs. 53.95%; ±1.38; $p < 0.05$), organic matter (59.86% vs. 55.35%, ±1.38; $p = 0.04$), crude protein (59.71% vs. 55.09%, ±1.44; $p = 0.04$), neutral detergent fibre (52.53 vs. 46.65%, ±1.78; $p = 0.04$), acid detergent fibre (47.96% vs. 41.02%, ±2.03; $p = 0.03$), and cellulose (59.43% vs. 53.56%, ±1.75; $p = 0.04$), with a positive trend on hemicellulose (57.61% vs. 52.86%, ±1.59; $p = 0.06$). No significant effects were observed on apparent digestibility of crude fat, non-structural carbohydrates and digestible energy. In the present study, the administration of *S. cerevisiae* to mature Quarter horses on high forage:concentrate diet significantly improved the apparent nutrient digestibility, with especial remark to fibrous fractions of the diet.

HIGHLIGHTS

- *S. cerevisiae* improves nutrient digestibility in mature horse
- Yeast increases fibre utilisation of high-forage diet
- Yeast supplementation does not change energy digestibility

ARTICLE HISTORY
Received 10 February 2025
Revised 29 May 2025
Accepted 30 May 2025

KEYWORDS
Horse; live yeast; apparent digestibility; high fibre diet

Introduction

Horses have a peculiar digestive system organisation which classifies them between monogastric and ruminant species (Ericsson et al. 2016; Harris et al. 2017). The horse gastrointestinal tract (GIT) facilitates the fermentation of dietary fibre through the degradation and utilisation of cellulose and hemicellulose. This process is primarily carried out by specialised microbial populations in the hindgut (De Fombelle et al. 2003; Dougal et al. 2013), ultimately resulting in the production of volatile fatty acids as an energy source (Jouany et al. 2009).

However, the balance of gut microbial populations can be altered by several factors, such as diet composition and other nutritional factors, as seen in the administration of poor-quality forages or high-starch diets (Harris et al. 2017; Murray et al. 2017).

Various nutritional approaches are proposed to maintain an optimal balance of the microbial community in horses and enhance the digestive processes, including the administration of probiotics and prebiotics (Garber et al. 2020; Ganda et al. 2023). In this regard, yeast inclusion in the diet could lead to positive effects on horses' GIT, improving the digestion of different nutrients (Jouany

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et al. 2008, 2009; Agazzi et al. 2011; Salem et al. 2016; Murray et al. 2017).

Among the different yeast strains available on the market, *Saccharomyces cerevisiae* is the most employed in horse nutrition (de Moura et al. 2016; Perricone et al. 2022). *In vivo* and *in vitro* studies on the efficacy *S. cerevisiae* strains on nutrients digestibility are anyway still relatively limited, with a primary focus on fibre fractions and protein digestibility. These studies show variable results due to different factors, such as the amount of yeast provided or the forage to concentrate ratio of the diet (Perricone et al. 2022). The literature suggests that the positive effects of *S. cerevisiae* supplementation on fibre fractions may be linked to its support of cellulose-degrading bacteria in the hindgut, which is usually associated with improved nutrient digestion, especially regarding the available fibre fractions (Medina et al. 2002; Lattimer et al. 2007; Coverdale 2016). The hypotheses regarding the effectiveness of *S. cerevisiae* on fibre digestibility may be based on its ability to maintain an optimal pH in the hindgut, thereby increasing the number of cellulolytic bacterial (Jouany et al. 2008; Elghandour et al. 2014, 2016). Additionally, a second proposed mechanism of action could involve *S. cerevisiae*'s oxygen scavenging ability, which supports the activity of anaerobic fibrolytic bacteria in the hindgut (Shepherd et al. 2012; Julliand and Grimm 2016).

Despite the positive results obtained from *S. cerevisiae* dietary supplementation on fibre digestibility in horses, a knowledge gap still exists regarding its effectiveness with respect to some other nutrients. Specifically, few, if any, studies have addressed the digestibility of other energy-related sources such as fat (Agazzi et al. 2011; Mackenthun et al. 2013; Palagi et al. 2017) or non-structural carbohydrates (NSC), which contribute to the horse's dietary energy availability. This lack of knowledge may stem to the general consensus that yeasts manly yield to positive effects in the hindgut, as the site of fibre digestion, while fat and NSC are mainly digested in the small intestine. Although different aspects still require further elucidations, including those explored using omics techniques (Perricone et al. 2022), it has been reported that *S. cerevisiae* may help limit undesirable changes in the intestinal ecosystem (Medina et al. 2002; Morgan et al. 2007; Jouany et al. 2008, 2009; Agazzi et al. 2011), potentially improving the gut health and indirectly affecting nutrient digestibility in the proximal intestine.

Thus, the aim of the present study was to enhance the understanding of live yeast supplementation (BioCell® S12, *S. cerevisiae* DBVPG 48 SF, Mazzoleni spa, Bergamo, Italy) on nutrients apparent digestibility in mature Quarter horses, with a particular focus on dietary energy sources.

Material and methods

Ethical approval

Animals in the study were used according to European Union Directive 2010/63/EU covering the protection of animals used for experimental or other purposes and according to the recommendation of Commission 2007/526/CE covering the accommodation and care of animals used for experimental and other scientific purposes. During the study, appropriate animal health and welfare inspections were carried out. Care, handling, and sampling of animals defined in the present study were approved by the University of Milan Animal Care and Use Committee (OPBA n° 159_2019).

Experimental design and animals housing

Eight Quarter horses (5 males and 3 females; age: 8.0 ± 1.83 years; body weight: 475.31 ± 31.29 kg) from "Badi Farm" (Sumirago, Varese, Italy) were enrolled in a two-periods crossover design with four subjects in each experimental group consisting, respectively, in 3 males and 1 female and 2 males and 2 females. At the beginning of the study, horses were blocked by age, body weight (BW) and sex and were randomly distributed to one of the two experimental groups. The crossover design consisted of two experimental periods (P1 and P2, respectively) of 28 days each with the daily supplementation of live yeast to treated animals. Before starting P1, an adaptation period of 7 days to the common basal diet was performed for all the horses involved in the trial. A wash-out period of 15 days was adopted between P1 and P2 with the administration of the common basal diet to all the subjects. The horses were individually housed in 3×3 m boxes, with ad libitum access to fresh water and continuously maintained on dust-free wood shavings, to avoid any nutrients ingestion other than the experimental diets. During the first 23 days of each period, the horses had a daily exercise of 1–2 h with the rider into a yard with sand, to avoid any other feed intake except the experimental diets.

In the last five days of each experimental period horses were confined 24 h/day in individual boxes and daily samples of fresh faeces were collected in the morning from the upper portion after spontaneous defaecation, avoiding any contamination with the litter. Vaccination and deworming practices were consistent with farm protocol; horses were vaccinated annually for tetanus, Western equine encephalitis, Eastern equine encephalitis, West Nile, influenza, equine herpes virus (EHV-1 and EHV-4), strangles and rabies.

Diets and dietary treatments

The chemical composition of forages and concentrates fed during the trial are reported in Table 1. The basal diet was formulated to meet or exceed the nutrient requirement for horses having moderate work intensity as suggested by the National Research Council (NRC 2007) and consisted of a forage:concentrate ratio equal to 87:13 DM basis, fed at a rate of 2.4% BW (Table 2). Horses were fed twice day (7:30 AM and 5:30 PM) two equal amounts of the basal diet. Total daily amount of mix hay (ryegrass, timothy and clover) for each horse was weighed and divided into two separate meals and stored in nets. The total daily amount of pelleted commercial mixture, fibrous mixture cubes and cracked corn was previously mixed, then divided in two plastic daily bags of equal amounts for each horse and administered twice day as for hay. The dietary treatments consisted in the administration of the basal diet with (LY) or without (CTR) 3 g/horse/d of *S. cerevisiae* (DBVPG 48 SF with $12*10^9$ cfu/g, BioCell S12®, Mazzoleni spa, Bergamo, Italy) individually top-dressed over the morning concentrate meal. The yeast dosage was assigned considering our previous research (Agazzi et al. 2009) and following the suggested recommend dosage from the producer.

Recorded parameters and samples collection

Individual BW was determined using an electronic scale (2 m × 1 m, capacity 1500 kg; sensitivity ±0.5 kg) on day 0, 15, 23 and 28 of each P1 and P2. Individual consumption of hay and concentrates were recorded daily during the 5–days confinement period, accounting for the refusals in the following morning after daily administration. During each collection period (day 23 to day 28), confined horses were monitored in the morning every 30 min for spontaneous defaecation and 200 grams of fresh faeces were collected from the upper part to avoid any contamination with the litter. Collected individual faecal samples were stored in single plastic bags at 5 °C, subsequently transferred within 3 h to −20 °C, pending analysis. Hay samples (500 g) were daily collected for each horse during confinement and subsequently stored as for faecal

Table 2. Ingredients and calculated chemical composition of high-fibre basal diet provided to mature quarter horses fed 3 g/d *S. cerevisiae.*

Ingredients[a]	Kg, as fed
Mix Hay	10.00
Pelleted commercial mixture	0.84
Fibrous mixture cubes	0.76
Cacked corn	0.75
Total amount provided	12.35
Chemical composition	%DM
DM	93.97
CP	10.27
CF	1.91
NSC	21.69
NDF	60.08
ADF	32.42
ADL	4.45
Hemicellulose	27.65
Cellulose	27.97
Ash	6.05
DE, kcal/kg	2234.92

[a]DM = dry matter; CP = crude protein; CF = crude fat; NSC = non-structural carbohydrates; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; DE = digestible energy.

Table 1. Chemical analysis of hay, pelleted mixture, fibrous cubes and cracked corn administered to mature quarter horses fed 3 g/d *S. cerevisiae.*

Nutrients (%DM)[c]	Mix Hay	Pelleted commercial mixture[a]	Fibrous mixture cubes[b]	Corn, cracked
DM	95.26	88.82	88.80	87.77
OM	93.91	92.03	92.10	98.63
CP	9.95	14.92	11.10	8.84
CF	1.66	3.58	1.86	3.63
NDF	65.40	48.22	47.33	9.60
ADF	35.94	20.83	23.95	3.33
ADL	4.68	5.28	3.77	0.91
Hemicellulose	29.45	27.39	23.38	6.27
Cellulose	31.26	15.55	20.18	2.42
Ash	6.09	7.97	7.90	1.37
AIA[c]	1.75	1.01	1.62	0.08
NSC	16.90	25.31	31.81	76.56
DE, kcal/kg	2092.32	2425.31	2497.84	3813.23

[a]Based on wheat bran, alfalfa, barley, corn, oat, soybean meal, carobs and soybean oil.
[b]Based on couch grass hay, ryegrass, barley, oat, vetch and sainfoin.
[c]DM = dry matter; OM = organic matter; CP = crude protein; CF = crude fat; NSC = non-structural carbohydrates; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; AIA = acid insoluble ash; DE = digestible energy.

samples. Assuming a total transit time of 36 h in the case of high-fibre diets (Agazzi et al. 2011), hay sampling started one and half day before the beginning of the daily faecal collection to maintain the adequate correspondence between ingested diets and collected faeces. Pelleted commercial mixture, fibrous mixture cubes, and cracked corn were sampled (500 g each) at the beginning of P1 and P2 and stored as for faecal and hay samples pending analyses.

Preparation of the samples for chemical analyses

Hay, concentrates, and faecal samples were analysed according to the protocols of the Association of Analytical Communities Official Methods of Analysis (AOAC 2005) to determine dry matter (DM) (method 930.15), crude protein (CP) (method 2001.11), neutral detergent fibre (NDF) (method 2002.04), acid detergent fibre (ADF) (method 973.18), acid detergent lignin (ADL) (AOAC 973.18), and ash (method 942.05). Crude fat (CF) content was determined by DM 21/12/1998 GU n° 31 08/02/1999, Suppl. 13 protocol, and Non-structural carbohydrates (NSC) content was calculated as 100–CP-CF-NDF-ash. Acid-Insoluble Ash (AIA) content was measured following Reg. CE n. 152/2009 and used as an internal marker to determine the apparent digestibility of the experimental diets as reported by Van Keulen and Young (1977). Organic matter (OM) content was calculated as follows: DM – ash. Hemicellulose content was determined as NDF-ADF, and cellulose content as ADF-ADL. Digestible Energy (DE) content of the diet was estimated as proposed by Pagan et al. (1998):

$$\text{DE (Mcal/kg DM)} = 2188 + 12.18\text{CP\%} - 9.37\text{ADF\%} - 3.83\text{hemicellulose \%} + 47.18\text{EE\%} + 20.35\text{NSC\%} - 26.3\text{ash\%}.$$

Digestibility values were calculated according to the method of Schaasftra et al. (2019).

Statistical analysis

When designing the experiment, G-Power software (Faul et al. 2009) was used to compute the actual power, setting an α-error probability of 0.05. The obtained actual power and effect size were 0.88 and 2, respectively. Body weight, feed intake and nutrients apparent digestibility were analysed with SAS On Demand For Academics (https://www.sas.com/it_it/software/on-demand-for-academics.html) using mixed procedure for repeated measurements. The applied model accounted for the effects of treatment, time, period, sequence of treatment, and the interactions between treatment × time, treatment × period and treatment × time × period, adjusted by Tukey–Kramer test. Diet, hay and concentrate intakes were expressed as percentage of BW. No missing data was present for all the calculated digestibility coefficients. Before submitting the digestibility coefficients to statistical analysis, data were checked for normal distribution by Kolmogorov–Smirnov, Cramer–von Mises and Anderson–Darling tests. Outlier data were detected by the Median Absolute Deviation Method (Leys et al. 2013) using ±2.0 MAD values (eliminating likely outliers). No outliers were detected for NDF, ADF, ADL, hemicellulose, cellulose, ash, and digestible energy, while one value was deleted for dry matter and organic matter, 2 values for crude protein and crude fat, and 3 values were eliminated for non-structural carbohydrates. In each case, the horse represented the unit for statistical analysis and significance was set for $p \leq 0.05$.

Results and discussion

Dry matter intake and body weight

In the present trial the administration of *S. cerevisiae* to mature horses did not lead to increased DMI in the five days of confinement when considering the whole diet ($p = 0.96$) or exclusively the provided forage ($p = 0.96$) or concentrates ($p = 0.83$) (Table 3). Similarly, no significant differences were detected among the experimental groups considering the days of sampling during confinement, P1 and P2, and the interaction between treatment × day and treatment × period ($p > 0.05$). Our findings are in accordance with Morgan et al. (2007) that did not observe any influence of yeast supplementation in mature horses when fed diets with both high- and low-quality forage, either on forage or concentrate intake. Similarly, in our previous trial (Agazzi et al. 2011) we found that DMI was not changed when horses on high-fibre diets received 4.6×10^{10} cfu/d of live yeast *S. cerevisiae*. However, different results were previously evidenced by Jouany et al. (2008), who reported higher values of DMI when horses were fed diets with a high amount of fibre in the diet and were supplemented with 4.5×10^{9} cfu/d of *S. cerevisiae*.

In the present study, the BW of experimental groups was similar at the beginning of the trial ($p > 0.05$) and no significant effects were observed throughout the experiment between LY and CTR horses ($p = 0.94$) (Table 4). Moreover, no effect of sampling day ($p = 0.83$), period ($p = 0.22$) or the interaction between treatment × day ($p = 0.99$), treatment × period ($p = 0.49$) or treatment × day × period

Table 3. Daily DMI (% of BW) and feeding behaviour of mature quarter horses fed (LY) or not (CTR) 3 g/d *S. cerevisiae* during 5–days of confinement.

		Days of confinement							*p*-values				
		1	2	3	4	5	Means[a]	SEM	Treat	Day	Period	Treatment*day	Treatment*period
Forage	CTR	1.94	1.95	1.95	1.95	1.95	1.95	0.051	0.961	1.000	0.183	1.000	0.412
	LY	1.95	1.95	1.95	1.95	1.95	1.95						
Concentrates	CTR	0.43	0.42	0.43	0.42	0.43	0.42	0.011	0.825	0.920	0.184	0.992	0.450
	LY	0.43	0.43	0.43	0.42	0.43	0.43						
Ration	CTR	2.37	2.36	2.37	2.37	2.38	2.37	0.062	0.935	0.999	0.182	1.000	0.418
	LY	2.38	2.37	2.37	2.37	2.38	2.37						

Abbreviations: CTR:control group fed the basal diet; LY: treated group fed the basal diet plus 3 g/d *S. cerevisiae S. cerevisiae* (BioCell® S12, DBVPG 48 SF, Mazzoleni spa, Bergamo, Italy).
[a]Data are presented as the mean values of individual DM intake for P1 and P2.

Table 4. Body weight of mature quarter horses on high-fibre diet fed (LY) or not (CTR) 3 g/d *S. cerevisiae.*

		Days of confinement					
Period		0	15	23	28	SEM	*p*-value
P1	CTR	467.00	476.00	476.88	473.37	17.962	0.935
	LY	469.13	480.00	483.75	482.75		
P2	CTR	485.12	487.25	495.50	497.00		
	LY	480.00	481.25	487.75	487.50		
P1 and P2							
	CTR	476.06	481.63	486.19	485.19	12.703	
	LY	474.56	480.63	485.75	485.12		

Abbreviations: CTR: control group fed the basal diet; LY: treated group fed the basal diet plus 3 g/d *S. cerevisiae S. cerevisiae* (BioCell® S12, DBVPG 48 SF, Mazzoleni spa, Bergamo, Italy).

($p = 0.99$) were found. Beside the lack of significant differences between LY and CTR horses during the whole trial period, both groups gained approximately 2% of initial BW. However, the observed increase in BW in LY and CTR is lower than the approximately 10% previously evidenced by Jouany et al. (2008); the relatively low BW increment we observed in our study could be related to the physical activity allowed to our horses during the first 23 days of each period, whereas horses used by Jouany et al. (2008) had less possibility to move because fistulated. Also, BW changes in yeast-supplemented horses were previously evaluated by some other authors in the past years (Morgan et al. 2007; Agazzi et al. 2011; Moura et al. 2011), but without outlining any significant differences with respect to not-supplemented subjects. It is generally recognised that an increase in BW is not the primary expected effect of live yeast administration in horses. Rather, the enhancement of the intestinal microbial population and the modulation of the gut environment are expected to improve nutrient digestibility (Elghandour et al. 2020).

Apparent digestibility of nutrients

In our trial, a positive effect of the administration of *S. cerevisiae* to mature horse fed high forage:concentrate diet were evidenced on apparent digestibility of nutrients (Table 5). Dry matter and OM apparent digestibility was improved in LY horses ($p \le 0.05$) according with previous studies (Miraglia et al. 1999; Jouany et al. 2008; Agazzi et al. 2009; 2011; Garber et al. 2020). We hypothesise that the significant improvement in DM and OM in present trial could be the consequence of the increased apparent digestibility observed for the fibre fractions with respect to the high forage:concentrate ratio in the administered diet.

Results from our study also evidenced a significant increase in apparent digestibility of dietary CP in yeast-supplemented horses ($p = 0.04$). It is historically reported that CP digestibility in horses is decreased as the percentage of forage NDF increases (Vander Noot and Gilbreath 1970; LaCasha et al. 1999); then, high-quality forages show higher CP digestibility than low-quality forages (Morgan et al. 2007). Anyway, an improvement of CP digestibility was observed when adding yeasts to low-quality forage diets (Switzer et al. 2003; Morgan et al. 2007) as in our case, where the NDF content of mix hay was 65.40% on DM basis.

The administration of *S. cerevisiae* in the present trial positively effected the apparent digestibility of NDF, ADF, and cellulose ($p = 0.04$; $p = 0.03$, and $p = 0.04$, respectively), with a trend for enhanced hemicellulose digestibility ($p = 0.06$) (Table 5). The obtained results for the fibrous fractions in the present trial align with those reported in earlier studies (Glade 1991; Medina et al. 2002) and have been subsequently confirmed by more recent publications. These studies found that *S. cerevisiae* supplementation, when using high forage:concentrate diets, led to an improvement in the apparent digestibility of fibre (Agazzi et al. 2009, 2011; Garber et al. 2020). The mechanisms of microbial breakdown of the plant material show large similarities between horses and ruminants (Elghandour et al. 2020), suggesting that *S. cerevisiae* may have comparable beneficial effects on the digestive microbial populations and the balance of the digestive microbial ecosystem in both species (Jouany et al. 2009). Specifically, the positive effects of *S. cerevisiae*

Table 5. Apparent digestibility coefficients (%) of nutrients in mature quarter horses on high-fibre diet fed (LY) or not (CTR) 3 g/d *S. cerevisiae.*

	Treatment								
Item	CTR	LY	SEM	Treatment	Day	Period	TxD	TxP	TxDxP
DM	53.95	58.22	1.38	0.051	<0.01	<0.01	0.435	0.194	<0.001
OM	55.35	59.86	1.38	0.041	<0.01	<0.01	0.322	0.238	<0.001
CP	55.09	59.71	1.44	0.043	<0.01	<0.01	0.502	0.242	<0.001
CF	22.84	28.38	3.01	0.217	<0.01	<0.01	0.234	0.233	<0.001
NSC	80.67	81.97	1.80	0.617	0.095	0.854	0.489	0.826	0.069
NDF	46.65	52.53	1.78	0.038	<0.001	<0.001	0.414	0.155	<0.001
ADF	41.02	47.96	2.03	0.033	<0.001	<0.001	0.488	0.0925	<0.001
Hemicellulose	52.86	57.61	1.59	0.056	<0.001	<0.001	0.377	0.349	<0.001
Cellulose	53.56	59.43	1.75	0.035	<0.001	<0.001	0.403	0.091	<0.001
DE	63.26	66.08	1.15	0.108	<0.001	<0.001	0.267	0.298	<0.001

Abbreviations: CTR: control group fed the basal diet; LY: treated group fed the basal diet plus 3 g/d *S. cerevisiae* (BioCell® S12, DBVPG 48 SF, Mazzoleni spa, Bergamo, Italy).

observed in horses in the present trial can be attributed to its ability to survive the transit through the initial part of the horse's digestive tract, reaching the caecum and colon, where it exerts its positive effects on fibre fractions (Medina et al. 2002; Jouany et al. 2008).

However, *Saccharomyces cerevisiae* does not appear to colonise the caecum and the colon. Instead, its positive effects may be related to the viable yeast cells present in the large intestine ecosystem (Nagaraja et al. 1997; Wunderlich et al. 2023), and their higher activity (Jouany et al. 2008). Additionally, *S. cerevisiae* may help preserve the pH in the hindgut, while also reducing the concentrations of lactic acid ammonia, and altering the (acetate + butyrate)/propionate ratio by increasing the molar percentage of acetate when high-fibre diets are fed to horses (Medina et al. 2002; Wunderlich et al. 2023).

These mechanisms of action suggest that *S. cerevisiae* enhances the fibrolytic activity of the resident bacteria in the hindgut, without increasing the total number of cellulolytic bacteria. However, results over the years are in some cases contrasting, with some studies indicating that *S. cerevisiae* did not positively impact the digestion of any fibre fraction (Glade and Biesik 1986; Hall et al. 1990; Mackenthun et al. 2013). Beside the yeast dosage and the forage:concentrate ratio of the diet, the observed variability of results could also be related to the quality of the hay administered or to the abrupt introduction of a new hay type, even when its composition is similar. Such chance can disrupt the hindgut microbial ecosystem, negatively impacting digestibility (Grimm et al. 2016). These results agree with those reported by Di Francia et al. (2008) and Zicarelli et al. (2024) in buffaloes and, according to Ghazanfar et al. (2017), should be attributed to the improved digestive ecosystem also by the production of further enzymes.

In the present trial, we did not observe significant effects of *S. cerevisiae* supplementation on CF apparent digestibility. These results align with our previous studies (Agazzi et al. 2009; 2011) where horses on a 70:30 forage:concentrate diet with 1.60% fat content were supplemented with *S. cerevisiae* at a rate of 2 g/d and 4.6×10^{10} CFU/d, respectively. Similarly, later studies by Mackentun et al. (20132011) and Gobesso et al. (2018) did not find a significant improvement in CF digestibility when horses were fed 1–3 g/d of *S. cerevisiae*, regardless of whether on maintenance or exercise regimens.

Non-structural carbohydrates can constitute a significant portion of the dietary energy provided, even in diets with a high forage:concentrate ratio. For example, common cool-season hay can contain between 10% and 20% NSC, and higher levels can be found in several commercial feeds (Loos et al. 2024). In our study NSC content in the diet was 21.69% on DM basis, which is acceptable for healthy horses. No significant improvement of the apparent digestibility was evidenced, although there was a trend ($p = 0.069$) for the interaction between treatment, day, and period.

To the best of our knowledge, the present study is the first to examine the overall apparent digestibility of NSC in horses fed yeast, whereas other studies have primarily focused on starch digestibility alone, often with no significant results (Taran et al. 2016; Gobesso et al. 2018). Although a limitation of the present trial is the lack of collected data related to the gut environment, we speculate that the absence of a significant improvement in NSC apparent digestibility may be due to the inability of the yeast dosage used to induce significant changes in the small intestine microbiota and environment. Unfortunately, few studies have investigated the effect of *S. cerevisiae* on the horse foregut microbiota; only Julliand et al. (2018) reported reduced concentrations of amylolytic bacteria and lactate-utilizing bacteria in the stomach of horses fed a high-starch diet. Further research, particularly using -omics techniques, would be valuable in the future to deepen our understanding of the yeast's mechanisms of action throughout the entire gastro-intestinal tract (Perricone et al. 2022).

Beside the possible effects of yeast administration on NSC apparent digestibility, it should be noted that yeast could also contribute to beneficial effects on insulin resistance in horses. This was demonstrated by Loos et al. (2024), who tested the protective effects of a blend of omega-3 fatty acid sources, glutamine, vitamin E, and active brewer's yeast in a NSC challenge in horses affected by insulin dysregulation.

The increased apparent digestibility of the fibrous fractions in the present trial was expected to increase the total amount of DE available to the horse, although a similar DMI between LY and CTR was observed. However, we did not detect a significant improvement of DE apparent digestibility ($p = 0.11$), which was consistent with the lack in significant changes in BW between the experimental groups. Our findings align with previous studies, which reported no differences in gross energy digestibility and BW when adding yeast culture or live yeasts to high- or low-quality forages diets in mature horses (Morgan et al. 2007), or in high-fibre or high-starch diets in ponies (Garber et al. 2020). However, it is worth noting that de Rezende et al. (2012) observed an improvement in the apparent digestibility of DE in *S. cerevisiae* supplemented horses in training.

Conclusions

The present trial demonstrated that the administration of *S. cerevisiae* (Biocell S12®, Mazzoleni spa, Bergamo, Italy) to mature horses on high-fibre diets significantly improved the apparent digestibility of most nutrients, particularly the fibre fractions. In this view, the administration of the yeast could be particularly beneficial in feeding programs where concentrates inclusion is limited or where maintaining digestive efficiency is a priority.

Specifically, by enhancing the breakdown of fibrous components, yeast supplementation may help stabilise hindgut conditions, support gut health, and optimise the use of forage-based diets. These effects can contribute to better maintenance of body condition, reduced digestive stress, and more efficient feeding strategies, making live yeast a useful addition in both leisure and performance horse management.

However, in our study no significant effects were observed on energy-related nutrients such as NSC and CF. As others previously published manuscripts, our work has some limitations in fully understanding the yeast's mechanism of action in the gastrointestinal tract of horses. The effects of *S. cerevisiae* on microbiota composition across the entire gastrointestinal tract of horses remain poorly investigated, with most available information focusing solely on the hindgut environment, typically using faeces as a proxy of the distal colon. A comprehensive understanding of how yeast affects the microbiota throughout the whole gastrointestinal tract could be a critical point for the future research. Achieving this goal could be facilitated by applying nutrigenomic, metabolomic and proteomic approaches, that could provide valuable insights into microbiota kinetics, molecular mechanisms and newly generated metabolites or active molecules that could reflect the yeast's effects on the overall health of the animal.

Acknowledgements

Authors acknowledge Mazzoleni spa (Bergamo, Italy) for providing the tested compound and all the support. The authors acknowledge support from the University of Milan through the APC initiative.

Authors contribution

Silvia Sandrini: Investigation, Data curation and analysis, Writing- Original draft & editing, Writing-Review and editing; Vera Perricone: Data curation and analysis, Writing- Original draft & editing, Writing-Review and editing; Alessia Pea: Writing-Review and editing; Clara Cenati: Writing-Review and editing; Giovanni Savoini: Conceptualisation, Writing-Review and editing; Gianluca Baldi: resources; Alessandro Agazzi: Resources, Conceptualisation, Methodology, Supevision, Investigation, Writing-Review and editing. All authors have read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data supporting the findings of the present study are available on request from the corresponding authors.

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