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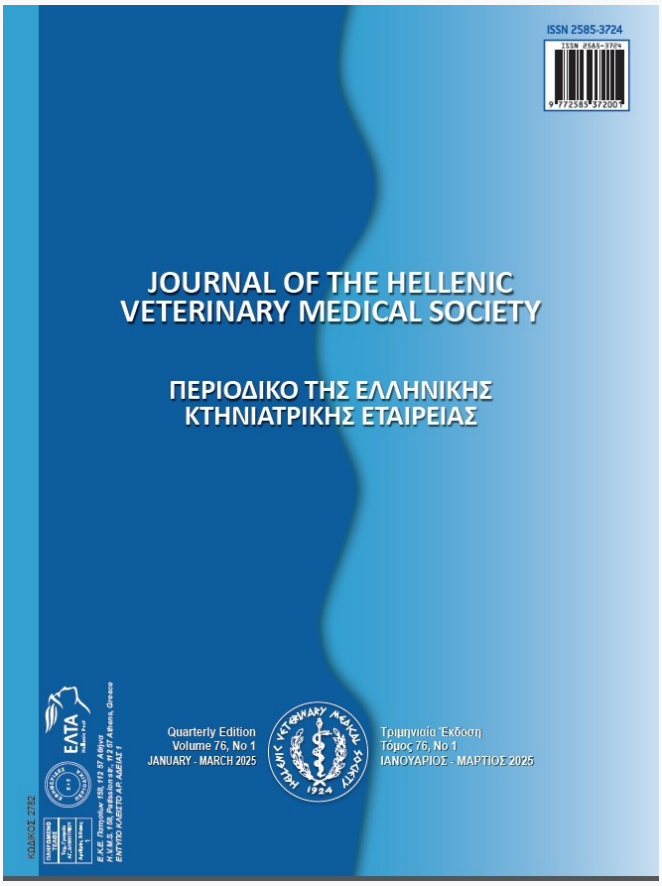

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Research article
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Using yeast-based probiotic as dietary supplement during transition period to improve performance of dairy cows

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ABSTRACT: A study was conducted to investigate the effect of live *Saccharomyces cerevisiae* yeast supplementation on dairy cow dry matter intake (DMI), milk production, milk composition and somatic cell count (SCC), rumen pH, and serum parameters (glucose, urea, alanine aminotransferase; ALT and aspartate aminotransferase; AST) during transition. Totally 24 multiparous dairy cows were randomly assigned to 3 groups (eight cows/group) fed the same diets supplemented without probiotics (Control group) or with probiotics at the rate of 15 or 30 g live yeast culture/cow daily for 5 weeks (transition period; from 2 weeks' pre-partum to 3 weeks' post-partum) in a completely randomised design. Dry matter intake (DMI) and milk yield were recorded daily and the milk composition, somatic cell count (SCC), rumen pH, and serum parameters were measured every week. A higher DMI and greater average daily milk yield were found in *Saccharomyces cerevisiae* treated groups as compared to the control groups ($P<0.05$). There was no significant difference in rumen pH between the control and 15g yeast-supplemented groups, but the higher dose of yeast increased ($P<0.05$) rumen pH. In probiotic-fed cows, milk fat was increased ($P<0.05$) and somatic cell count decreased ($P<0.05$), while milk protein and solid not fat (SNF) were unchanged. The concentration of serum glucose and urea was increased ($P<0.05$) in cows fed 15 or 30 g/d yeast compared with those of control at both pre- and post-partum periods. Overall, data showed the identical ($P>0.05$) effect of both yeast levels on milk yield and quality, which suggests the use of a lower level of 15 g/d in practice.

***Keywords*:** dairy cow; milk; probiotic; performance; yeast

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INTRODUCTION

The transition period (the last 3 weeks of gestation to the first 3 weeks of lactation) is an important part of the dairy cow's life cycle. In the transition period, the animal go through many challenges at calving and/or afterward. In order to achieve the lowest calving issues and to maintain good milk production throughout the remainder of the lactation period, it seems necessary to modify the diet of dairy cows with different feed additives (Sretenović et al., 2008; Cattaneo et al., 2022).

European Union have banned using animal feed additives containing antibiotic growth promoters such as ionophores due to increase in bacteria resistance, residual effects on milk and meat products, and harmful effects on human health (Calsamiglia et al., 2007, Elghandour et al., 2022). It is therefore crucial for nutritionists and production managers to find alternatives that will alleviate these side effects and problems associated with antibiotic withdrawal from diets and reduce diseases in animals. A recommended solution is to use some feed additives, such as the direct fed microbials; i.e., bacterial, fungal and yeast which are ingredients that can cause an animal to respond in non-nutritional ways such as altering rumen pH, increasing fiber digestion through stimulating cellulolytic bacteria, enhancing propionate production thus increasing growth, or altering metabolic processes (Soltan and Patra, 2021; Mahesh et al., 2021). In dairy cows, a feed additive containing yeast culture may be most beneficial if fed before parturition (Cattaneo et al., 2022). Since probiotics affect rumen microflora, they are considered possible antibiotic replacements (Auclair, 2001). Probiotics, defined as live microbial feed supplements that contain *Lactobacillus*, *Bacillus*, and/or *Saccharomyces*, thus enhance the host animal's gastrointestinal microbial balance (Mahesh et al., 2021). *Saccharomyces cerevisiae* as probiotic has been used for decades as preventative and therapeutic agent for diarrhea and other gastrointestinal disorders in ruminant and non-ruminant species and because of its ability to convert sugars (e.g. glucose, maltose) into ethanol and carbon dioxide (baking, brewing, distillery, liquid fuel industries), it is industrially important (Auclair, 2001). GRAS (Generally Recognized As Safe) status is granted to *S. cerevisiae* by the US Food and Drug Administration (Mahesh et al., 2021). Despite the numerous studies on the effect of *S. cerevisiae* in dairy cows, the results are still controversial; for example, some studies have found increase in milk production (Nasiri et al., 2019; Enculescu, 2021); others identified a trend (Cattaneo et al., 2022) or no significant effect (Halfen et al., 2020; Lim et al., 2021).

Additionally, it has been suggested that yeast can be effective under oxidative stress (Vieira et al., 2014; Cattaneo et al., 2022) by promoting antioxidant enzyme activities (Singh et al., 2014) or stabilizing intestinal microbiota and their metabolic activities (Pridmore et al. 2008), as it is very stressful for the animal during parturition; the period following parturition is marked by a great reduction of dry matter intake, so animals enter a negative energy balance which can result in many health problems when high amounts of milk are not accompanied by adequate intake of nutrients. Therefore, regarding limited research on dairy cows during the transition period, the purpose of this study was to determine how dietary supplementation with yeast *S. cerevisia* in viable form affects dairy cow's performance pre- and post-parturition.

MATERIALS AND METHODS

Animals, diets, and management

Twenty-four Holstein multiparous dairy cows (parity: 2.5 ± 0.6; BW: 641 ± 49 kg; daily milk yield in the last lactation: 34 ± 3.6 kg (mean ±SD)) were obtained from a commercial herd and housed in a free-stall barn that contained pens under similar standard environmental and management conditions for the whole experimental period from April to May 2022. The cows were randomly divided into 3 groups (eight cows/group) fed identical diets during the experiment consisting of concentrate mixture and roughage which were supplemented without (Control group) and with yeast culture (Treated group) at the rate of 15 or 30 g/cow live yeast daily for 5 weeks during the transition period. Live yeast culture (*S. cerevisiae*, SC47, 1×10^9 cfu/g, UNIQUE CELL, Tehran, Iran) as a dried powder was hand-mixed with about 100 g of concentrate and was top-dressed to cows daily before feeding. During the pre- and post-partum periods, the control and trial groups received a ration composed of forages and commercially available concentrate and were balanced in all nutrition parameters. The concentrate consisted of yellow corn, soybean meal, wheat bran, molasses, and a mineral-vitamin supplement. Feeds were offered to animals in all groups two weeks before the expected calving date, and until 3 weeks after parturition. Cows in all groups were fed based on milk yield and reproductive status accord-

ing to (NRC, 2001). The proportion of concentrate in the ration (on a dry matter basis) was higher than 40%. Forages were distributed ad libitum, and the concentrated quantity varied according to the milk yield. Free access to water was available and feeding was made ad-libitum for 2 times (in the morning and evening)/24-h.

Data collection

The dry matter intake (DMI)/day was estimated from the difference between daily feed delivery per group and residual feed which was collected and weighed every other day during pre- and post-partum periods. Milk was evaluated quantitatively per day and qualitatively per week. The quantitative record was the average milk yield/head/day. The animals were milked twice daily at 5:00 am and 3:00 pm throughout the experimental period. Morning and evening samples were mixed homogenized and stored at -20˚C in containers containing $K_2Cr_2O_7$ as a preservative. The qualitative record consisted of milk composition (fat, protein, and solids-not-fat; SNF) recognized by a milk scanner (Model 133 B), and the somatic cell count (SCC) recorded according to the microscopic method (EN ISO 13366-1:1997). Rumen pH was recorded weekly from the rumen fluid collected via stomach tube with a vacuum pump 5 h after morning feeding. Blood samples were collected before morning feeding for two-week periods pre- and 3 weeks post-partum via the jugular vein into an evacuated sterile tube. The blood was allowed to clot at room temperature (about 30 min after collection), centrifuged at 3000 RPM for 10 min, and then serum was aspirated. The serum was kept in plastic tubes at -20˚C until analysis. Samples were analyzed by enzymatic method using an autoanalyzer (Selectra E vital scientific, The Netherlands) to determine glucose, urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

Statistical analysis

The data were analyzed using the General Linear Model (GLM) procedures of Statistical Analysis Systems (SAS, 2001). Tukey's multiple range tests separated differences between dietary treatment means. A value of $P < 0.05$ was considered significant.

RESULTS

Table 1 presents the effects of dietary supplementation with probiotic (*S. cerevisiae*, g/cow/day) on dairy cows' DMI and rumen pH during the transition period. A higher DMI was observed during pre- and post-partum periods in *S. cerevisiae* treated groups compared to the control group (P<0.05). The cows fed 30 g *S. cerevisiae* had higher ruminal pH compared to those fed 0 or 15 g probiotic (P<0.05). However, ruminal pH remained unchanged between control and 15 g *S. cerevisiae* fed groups (P>0.05).

The effect of dietary supplementation with yeast *S. cerevisiae* on milk yield, composition and SCC during the transition period is shown in Table 2. A comparison of *S. cerevisiae* treated groups with control groups found greater average daily milk yield (P<0.05) during the first, second, and third weeks of lactation. Milk fat was increased (P<0.05) and SCC was decreased (P<0.05) in yeast-fed cows than those that did not administer this additive, while milk protein and SNF were unchanged.

A comparison of the effects of dietary supplementation with *S. cerevisiae* during transition period on dairy cows' serum parameters is presented in Table 3. The inclusion of yeast in the diet elevated (P<0.05) the serum concentration of glucose and urea in cows more compared to those of the control group, while ALT, AST, and ALP were the same among the three groups.

DISCUSSION

Table 1. Effect of dietary supplementation of *S. cerevisiae* (g/cow/day) on dairy cows DMI and rumen pH during transition period.

Item	Control	15 g probiotic	30 g probiotic	SEM	P-value
During pre-partum period					
DMI (kg/d)	8.83^{b}	10.05^{a}	9.96^{a}	0.275	0.008
Rumen pH	5.95^{b}	6.15^{b}	6.70^{a}	0.119	0.0006
During post-partum period					
DMI (kg/d)	10.70^{b}	11.61^{a}	12.01^{a}	0.224	0.002
Rumen pH	5.78^{b}	6.09^{ab}	6.60^{a}	0.155	0.004

^{a,b}Means within the same row with uncommon superscript differ significantly (P<0.05).

Table 2. Effect of dietary supplementation of *S. cerevisiae*, (g/cow/day) on milk yield, composition and SCC during transition period.

Item	Control	15 g probiotic	30 g probiotic	SEM	P-value
1st week of lactation					
milk yield (kg/d)	19.71[b]	21.71[a]	21.50[a]	0.315	0.0003
Fat (%)	4.06[b]	4.55[a]	4.61[a]	0.121	0.007
Protein (%)	3.73	3.41	3.40	0.168	0.31
Solids not fat (%)	12.27	12.10	12.19	0.384	0.95
Somatic cell(×10³/mL)	158.4	150.1	149.6	3.56	0.17
2nd week of lactation					
milk yield (kg/d)	24.21[b]	25.84[a]	26.00[a]	0.437	0.015
Fat (%)	3.64[b]	4.00[a]	4.16[a]	0.084	0.0009
Protein (%)	3.54	3.52	3.75	0.080	0.099
Solids not fat (%)	10.34	10.23	10.55	0.169	0.39
Somatic cell (×10³/mL)	170.9[a]	157.8[ab]	155.4[b]	3.69	0.015
3rd week of lactation					
milk yield (kg/d)	28.07[b]	32.97[a]	32.95[a]	0.182	<.0001
Fat (%)	3.15[b]	3.83[a]	3.79[a]	0.111	0.0004
Protein (%)	3.36	3.95	3.92	0.220	0.12
Solids not fat (%)	11.18	11.52	11.15	0.400	0.77
Somatic cell (×10³/mL)	174.3[a]	163.5[b]	162.8[b]	2.76	0.012

[a,b]Means within the same row with uncommon superscript differ significantly (P<0.05).

Table 3. Effect of dietary supplementation of yeast *S. cerevisiae*, (g/cow/day) on dairy cows serum parameters during transition period.

Item	Control	15 g probiotic	30 g probiotic	SEM	P-value
During pre-partum period					
Glucose (mg/dL)	81.90[b]	84.69[a]	84.14[a]	0.601	0.009
Urea nitrogen (mg/dL)	20.69[b]	24.52[a]	25.61[a]	0.577	<0.001
ALT (U/L)	14.63	14.45	14.87	0.510	0.84
AST (U/L)	34.49	33.92	34.42	1.716	0.97
ALP (U/L)	20.74	20.41	20.86	0.211	0.32
During post-partum period					
Glucose (mg/dL)	72.56[b]	76.85[a]	76.08[a]	0.863	0.005
Urea nitrogen (mg/dL)	15.52[b]	17.02[a]	18.01[a]	0.368	0.0004
ALT (U/L)	14.57	14.30	14.58	0.485	0.90
AST (U/L)	35.35	35.04	35.26	1.121	0.98
ALP (U/L)	8.36	8.50	8.57	0.342	0.91

[a,b]Means within the same row with uncommon superscript differ significantly (P<0.05).

Dry matter intake and rumen pH

Consistent with our results herein, increase in DMI after calving following administration of live *S. cerevisiae* was observed by Bach et al. (2018) and Cattaneo et al. (2022), however, they did not found any significant effect on DMI during prepartum period. Cattaneo et al. (2022) proposed that the delayed effect of yeast probiotic on DMI (during prepartum) might be due to the adaptation period needed for observing the effects. On the other hand, Nasiri et al. (2019) reported that prepartum DM intake was greater in yeast-fed cows; however, this difference disappeared after parturition. A multiple analysis of feeding live yeast (*S. cerevisiae)* to dairy cows showed no effect on DMI postpartum (de Ondarza et al., 2010). Although, the mechanism of how yeast can increase feed intake is still not well understood, it has been suggested that improvement in fiber digestion as a result of *S. cerevisiae* supplementation increases passage rate and then DMI (Dann et al., 2000). Another possible reason may be due to the fact that live *S. cerevisiae* has a scavenging effect on oxygen that benefits the anaerobic microorganisms involved in ruminal digestion process (Newbold et al., 1996).

The higher rumen pH values in cows fed live yeast

compared to the control group herein could be attributed to the promoting properties of yeast on cellulolytic and lactate-utilizing bacteria, thus decreasing the risk of subacute ruminal acidosis after calving (Perdomo et al., 2020). Accordingly, increasing lactate utilizers in the rumen help maintain rumen pH above 6.0 and improve fiber digestion (Sretenović et al., 2008). In addition to stabilizing rumen pH, probiotics provide nutrients, inhibit the growth of bacteria, and destroy lactate by competing with bacteria (Ayad et al., 2013). Shi et al. (2019) concluded that despite of similar rumen pH in cows supplemented with or without *S. cerevisiae* fermentation product, addition of yeast during the calving transition may reduce the range of rumen pH after calving and the duration of subacute ruminal acidosis by the end of the calving transition.

Milk yield and composition

In the present study, increase in DMI was accompanied by increase in milk production, as Moallem et al. (2009) reported that live yeast supplementation will enhance milk yield only when DMI is increased. Similar results on DMI and milk production were observed when cows received 3.3 g/d live yeast (Bach et al., 2018). However, Yalcin et al. (2011) found higher milk production in cows fed live yeast culture than control group without any changes in DMI, possibly due to higher organic matter digestibility which then provided greater fermentable energy for milk production. Bruno et al. (2009) suggested that yeast supplementation could increase milk yield since it enhanced DM digestibility and thus increased microbial protein yields and glucogenic substrates. Contrast to our results, Olagaray et al. (2019) and Sauls-Hiesterman et al. (2021) reported that milk production was not affected by *S. cerevisiae* fermentation product during the first 6 and 7 weeks postpartum, respectively, however, they observed that milk fat percentage was increased by *S. cerevisiae* supplementation which is in agreement with our results herein. Higher milk fat content in early lactation may indicate higher fat mobilization, however, lack of effect on body weight and body condition score (BCS) (data not shown) makes that unlikely. In our study, increased in milk fat content in *S. cerevisiae* fed cows compared to the control can be partly explained by higher rumen pH as Bauman and Lock (2006) stated that elevated rumen pH can prevent shifting in rumen biohydrogenation (lower trans 10 C18:1), thus reducing the milk fat depression. Moreover, fiber-degrading bacteria that primarily produce acetate; the main precursor for de novo fatty acid synthesis, have greater proliferation at higher rumen pH (Olagaray et al., 2021). It has been reported that live yeast supplementation improved *Fibrobacter* and *Ruminococus* (fiber-degrading bacteria) and stimulated *Megasphera* and *Selenomonas* (lactate-utilizing bacteria) (Bitencourt et al., 2011), and also increased relative abundance of *Bacteroidales* (Bach et al., 2019). Overall, a multilevel meta-analysis performed on 99 trials from 49 peer-reviewed studies in lactating dairy cows showed that supplementation with yeast probiotic (*S. cerevisiae*) was associated with increased milk yield (+ 0.69 kg/d) and milk fat content (+ 0.06%) (Abdelli et al., 2022).

In agreement with our findings, somatic cells were decreased in cows fed *S. cerevisiae* fermentation product during the first 4 weeks postpartum (Zaworski et al., 2014). Similarly, Yuan et al. (2015) reported that yeast supplementation decreased somatic cells in early lactation cows. Sretenović et al. (2008) also found that the trial group supplemented with yeast (a commercial preparation of live yeast cultures combined with probiotics and enzymes) had lower somatic cells than the control group, suggesting better cow udder health. Contrary to our results, Nasiri et al. (2019) did not observe any difference between cows fed with or without live yeast in case of somatic cells. The decreasing response of somatic cells to administration of *S. cerevisiae* may be due to improvement in immunity, since Jensen et al. (2008) found cytotoxic effect of natural killer cells and activation of B lymphocytes in the presence of *S. cerevisiae* in cell culture.

Blood serum metabolites

Blood metabolites are a very good indicator of animal condition. Blood glucose and urea nitrogen (urea N) levels are used to evaluate the nutritional status of ruminants (Hammond et al., 1994). The concentrations of glucose and serum urea N were within the normal range of 40-100 and 10-25 mg/dL, respectively (Merck Veterinary Manual, 2009). Alhough, most of the studies using yeast probiotic did not observe any significant effects on glucose and/or urea concentrations (Al Ibrahim et al., 2010; Olgaray et al., 2019; Nasiri et al., 2019; Sauls-Hiesterman et al., 2021; Cattaneo et al., 2022), higher contents in our study can be ascribed by higher DMI and/or higher DM or protein digestibilities leading to greater production of propionate (one of the main precursors for hepatic glucose synthesis) or ammonia, or both (Miller-Webster et al., 2002; Zaworski et al., 2014)

which suggest that *S. cerevisiae* may accelerate adaptation to the pre- or post-partal diets and their nutrient utilization. Zaworski et al. (2014) reported that *S. cerevisiae* fermentation product supplementation did not affect serum concentrations of glucose and urea N during transition period, nevertheless, during the first 48 h after calving, the cows fed with *S. cerevisiae* fermentation product had higher serum glucose and urea N concentrations. Blood urea levels are closely correlated with how efficiently dietary protein is utilized and it has been stated that plasma urea N levels are a reliable indicator of protein degradation in the rumen and protein intake post-rumen (Hristov et al., 2010). Opposed to our findings, Halfen et al. (2020) observed that yeast culture supplementation resulted in lower blood urea in mid-lactation dairy cows, indicating more efficient conversion of ruminal ammonia into microbial protein and decrease ammonia concentration in the rumen. In our study, increase in plasma urea as a result of addition of *S. cerevisiae* suggests the increased rate of protein catabolism (Ahmadzadeh et al., 2018). However, more evaluations of ruminal, metabolic, and milk parameters related to nitrogen recycling are needed to be investigated. AST, ALT and ALP are used to diagnose biliary and liver damage (Silanikove and Toimkin, 1992). Our data on blood concentrations of hepatic enzymes were within the normal intervals (Merck Veterinary Manual, 2009). In agreement with the present results, Sretenović et al. (2008) and Mostafa et al. (2014) also reported the unaffected activity of AST, ALT and ALP by commercial yeast culture (*S. cerevisiae*) in dairy cows' serum.

Overall, the inconsistency between studies can be due to the feeding strategy, feed composition, dry matter intake, types and dosages of yeast used, physiological stage, stress, health status, and age of the animals (Mikulec et al., 2010; Nocek et al., 2011).

CONCLUSION

A study concluded that adding yeast *S. cerevisiae* to the diet of dairy cows during the transition period resulted in an increase in milk yield and milk fat content. A similar effect was found for both yeast levels on dairy cow performance, which suggests that lower levels (15g) should be used in practice.

CONFLICT OF INTERST

Conflicts of interest are declared by the authors.

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