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Research Article

Effect of supplementing *Saccharomyces cerevisiae* to milk on the growth performance, white blood cell profile, skeletal indices, and rectal temperature in neonatal Simmental calves

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ABSTRACT- This study was conducted using 30 newborn calves with an average weight of 42.55 ± 0.98 kg in a completely randomized design comprising five treatments and six replicates over a 42-day period. *Saccharomyces cerevisiae* (*S. cerevisiae*) yeast was mixed with the milk and administered to the calves in the morning. The experimental diets included: (1) a control diet without yeast, (2) 2.5 grams of live yeast, (3) 5 grams of live yeast, (4) 2.5 grams of inactive yeast, and (5) 5 grams of inactive yeast per calf per day. The calves were fed milk twice daily, in the morning and evening. Body weight, skeletal indices, and rectal temperature (RT) were recorded every 21 days, while blood samples were collected at the end of the study. Additionally, milk composition was analyzed. The results indicated that supplementation with 2.5 grams of inactive yeast resulted in the highest daily weight gain and the lowest feed conversion ratio ($P < 0.05$). Except for eosinophil counts, other white blood cell components remained unaffected. Yeast supplementation had no significant effect on RT or skeletal growth indices ($P < 0.05$). However, the addition of both live and inactive *S. cerevisiae* to the milk increased milk dry matter content, density, and conductivity. In conclusion, the supplementation of milk with live or inactive *S. cerevisiae* improved the growth performance of suckling calves.

INTRODUCTION

Feed additives play a crucial role in improving gut health in animals, thereby enhancing digestion rates and overall performance (Frizzo et al., 2011). Various microbial species have been approved as feed additives, demonstrating significant benefits in livestock production. Among these, yeasts are a prominent category of feed additives known for their potential to enhance gut health. While numerous yeast species exist, only three are commonly used in commercial applications: (1) *Saccharomyces*, (2) *Candida*, and (3) *Kluyveromyces*. One of the most widely utilized yeasts is *Saccharomyces cerevisiae* (*S. cerevisiae*), commonly known as baker's yeast, which is extensively used in bread making, nutritional supplementation, and alcohol production. This yeast is also a key industrial microorganism for the synthesis of single-cell proteins. Taxonomically, *S. cerevisiae* belongs to the Ascomycotina subgroup, Saccharomycetaceae family, and Saccharomyces genus. Due to rumen microbial dynamics, it enhances milk production in dairy cattle. Research on live *S. cerevisiae* as a feed additive in dairy cattle continues to be an area of scientific interest. Yeast is a rich source of essential nutrients, including vitamins, amino acids, peptides, minerals, organic acids, antioxidants, oligosaccharides, and beta-glucans (Zhang et al., 2024). The fermentation process in the rumen relies heavily on its microbial population, and yeast contributes vital vitamins, enzymes, and various cofactors. These stimulate microbial activity and growth rates (Dawson et al., 1990). Research has shown that yeast cultures improve feed utilization (Robinson and Erasis, 2009), feed conversion efficiency, growth rates (Lascano et al., 2009), and nutrient digestibility (Wohlt et al., 1991), while also being a cost-effective supplementation strategy (Hutjens, 1996). Ghazanfar et al. (2015) recommended incorporating *S. cerevisiae* into livestock diets to enhance growth performance and overall health in dairy calves. Additionally, yeast supplementation has been shown to positively influence hematological parameters, contributing to improved animal health (Agazzi et al., 2014). Further studies by Lascano et al. (2012) and Lesmeister et al. (2004) have demonstrated that yeast enhances hemicellulose hydrolysis and the digestibility of specific nutrients. Moreover, the inclusion of yeast culture has been linked to improved mineral absorption and overall metabolic health in animals (Dolezal et al., 2012). Thus, based on previous researches, this study hypothesizes that

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supplementation with live and inactive *S. cerevisiae* at two levels (2.5 and 5 g) may influence growth performance, white blood cell composition, and skeletal parameters in Simmental calves.

MATERIALS AND METHODS

This study was conducted on a 400-head cattle farm owned by Mr. Abedini in Karimabad village, Gorgan, Iran. Simmental calves were separated from their mothers immediately after birth and transferred to individual stalls following initial weighing. The stall floors were lined with straw to enhance calf comfort and health, with daily cleaning and replenishment of bedding. During the first two days, calves were fed colostrum at a rate of 10% of their body weight. From the third day onward, whole milk replaced colostrum, and weaning was completed by day 42. A total of 30 newborn calves, with a mean weight of 42.55 ± 0.98 kg, were assigned to a completely randomized design with five treatments and six replications over a 42-day period. Yeast was mixed with milk and administered to the calves in the morning. The calves received milk twice daily, in the morning and evening. The experimental diets were as follows: (1) control diet (without yeast), (2) diet supplemented with 2.5 grams of live *S. cerevisiae*, (3) diet supplemented with 5 grams of live *S. cerevisiae*, (4) diet supplemented with 2.5 grams of inactive *S. cerevisiae*, and (5) diet supplemented with 5 grams of inactive *S. cerevisiae*. Each morning at 8:00 AM, calves were provided with a starter feed mixed with alfalfa (80 with 20). Fresh water was available ad libitum. Details of the experimental diet composition are presented in Table 1. The live yeast supplement (Yupro) was obtained from Tekgen Biot Company, while the inactive yeast (Istos) was supplied by Soren Tech Tos Mashhad Company. Calves were weighed at the beginning of the study and subsequently every 21 days to assess body weight changes. Daily feed intake was recorded by weighing the feed provided in the morning and measuring the residual feed before the next feeding. Milk samples were collected for compositional analysis, including protein content, pH, fat, lactose, and total solids (Agazzi et al., 2014). These analyses were conducted at the Faculty of Animal Sciences, Gorgan University of Natural Resources and Agriculture (Gorgan, Iran). Skeletal growth parameters were measured on day 1 (birth) and every 21 days thereafter. The recorded parameters included body length, withers height, pelvic length, abdominal circumference, chest girth, wrist circumference, ankle circumference, pin bone interval, hip interval, the distance between the hip bones and pin bones, and pelvic width (Azizi Nasab et al., 2020).

Blood samples were collected from the jugular vein before the morning feeding on day 42 of the experiment. The samples were placed in 5 mL tubes for determination of eosinophils, monocytes, lymphocytes, and neutrophils. Hematological analysis was performed using an Autoanalyzer (Eko Milk Total Ultrasonic, Milk Analyzer Type, Milkana KAM98-2A, Bulgaria). rectal temperature (RT) was measured once a week, every 21 days, following the method described by Bokharaeian et al. (2023). A digital fever thermometer was inserted approximately 3 cm into the rectum and held in place for one minute until a stable reading was obtained. Statistical analysis was conducted using ANOVA with the mixed-effects model procedure of SAS, considering the period as a repeated measure. Data were analyzed using the General Linear Model (GLM) procedure of SAS (SAS Institute, 2001) based on the following statistical model: $Yij = \mu + Ti + \varepsilon ij$, where Yij represents the observed values, μ is the overall mean, Ti denotes the treatment effect, and εij is the residual error. Results were reported with significance considered at $P < 0.05$, while tendencies were noted for P values between 0.05 and 0.10.

Table 1. Ingredients and chemical composition of the experimental diet (% of diet Dry Matter)

Ingredient	Amount (%)
Alfalfa	20.0
Corn	22.4
Barley	24.0
Soybean meal	12.8
Rapeseed meal	10.4
Wheat bran	8.0
Minerals and Vitamins supplement*	0.96
Salt	0.64
Sodium Bicarbonate	0.8
Composition of nutrients	
Dry matter (%)	87.92
Crude protein (%)	18.28
Crude fat (%)	2.91
NDF (%)	27.37
ADF (%)	14.64
Ash (%)	5.00
Non-fiber carbohydrate (%)	46.44
Metabolizable energy (Mcal/kg)	3.04
Ca (%)	0.52
P (%)	0.36

* Premix of vitamins and minerals contained per kilogram of supplement: vitamin A: 800000 IU/kg; vitamin D3: 70000 IU/kg, vitamin E: 4000 IU/kg, vitamin C: 3000 mg, Magnesium 35000 mg. Manganese: 3000 mg; Zinc: 4000 mg; Copper: 1000 mg; Selenium 30 mg; Calcium: 120000 mg; I: 20 mg; Cobalt 10 mg; Phosphorus 30000 mg; Monensin: 1800 mg

RESULTS AND DISCUSSION

Growth performance: Table 2 presents the results for dry matter intake (DMI), initial weight, final body weight, average daily gain (ADG), and feed conversion ratio (FCR). Calves supplemented with 2.5 g of non-live yeast exhibited the highest average daily weight gain ($P < 0.05$) compared to other groups. The effectiveness of yeast supplementation is influenced by multiple factors, including the yeast strain, dosage, overall health and diet of the calves, and their productivity levels (Benedetti et al., 2024). Previous studies have linked the improved daily weight gain in *S. cerevisiae* supplemented calves to enhanced rumen development, which facilitates better nutrient absorption, particularly butyrate produced by *Butyrobacterium* spp. (Xiao et al., 2016). Similar findings were reported by Kumar and

Ramana (2008), who observed increased weight gain in calves fed yeast-supplemented diets. Although initial and final body weights were similar across groups, animals supplemented with both live and non-live yeast exhibited significantly better FCR ($P < 0.05$) and higher ADG ($P < 0.05$) compared to the control group. These results align with the findings of Kumar and Ramana (2008), who reported improved FCR in yeast-supplemented diets. However, research on yeast extract (YE) supplementation in dairy cattle has shown inconsistent results, suggesting a complex mechanism of action. Variability in findings may be attributed to the differences in yeast strain, dosage, dietary composition, lactation period, and experimental methodologies (Zhang et al., 2024). These factors collectively contribute to the diverse responses observed among individual animals.

Skeletal growth: The effects of live and non-live S. *cerevisiae* yeast supplementation on skeletal growth indices are shown in Table 5. These factors included body length, withers height, pelvic length, abdominal circumference, chest girth, wrist circumference, ankle circumference, pins interval, hips interval, the interval between the hip bones and the pin, and pelvic width. The values were assessed in calves at 0, 21, and 42 days, and the results are presented in Table 3. Our findings indicated that yeast supplementation had no significant effect on skeletal dimensions ($P < 0.05$). These results are consistent with those of Zantoni and Heinrichs (2003) and Lascano et al. (2009), who reported no changes in skeletal growth parameters in lactating calves fed yeast cultures. Similarly, our findings align with those of Khan et al. (2011) and Azizinasab et al. (2020), who also observed no significant impact of yeast supplementation on skeletal growth in young calves.

The chemical composition of milk following the addition of live and non-live *S. cerevisiae* is presented in Table 4. The results show that yeast supplementation significantly increased the levels of solids-not-fat (SNF), milk density, conductivity, and protein content. Adding 2.5 and 5 grams of yeast to the milk resulted in higher dry matter and protein levels, as expected. Additionally, viscosity and density are key indicators for investigating the flow characteristics of milk as well as explaining several sensory and quality properties of the product.

White blood cells: The blood parameter values, presented in Table 5, fall within the normal physiological range, indicating good overall health. Supplementation with yeast culture led to a significant increase in eosinophil levels ($P < 0.05$). These findings align with the research of Agazzi et al. (2014) and Heinrich et al. (2003), who reported that the addition of probiotics influences blood parameters. YE and its derivatives contain various immunomodulatory compounds that can directly and indirectly affect both pathogens and elements of the immune system. One key constituent, the polysaccharide β-glucan, acts as a biological response modifier. This compound enhances the innate immune response by stimulating macrophage and neutrophil activity, and it also strengthens adaptive immunity by boosting antibody production. Zhang et al. (2024) observed that YE supplementation improves antioxidative and immunological serum responses in mid-lactation dairy cows, suggesting its potential benefits for both rumen and blood metabolism during this critical period.

RT readings across all groups remained consistent, ranging from 38.33 to 39.00 °C (Table 6). Notably, the suckling calves displayed no signs of disease or metabolic abnormalities, as their body temperature remained stable and within normal limits, indicating no critical health concerns. These findings are in line with those of Benedetti et al. (2024), who observed similar RT patterns across different groups.

Table 2. Effects of adding live and non-live *Saccharomyces cerevisiae* to the milk on the growth performance of suckling calves

Parameter	Treatment					SEM	P-Value
	Control	2.5 gr of live yeast	5 gr of live yeast	2.5 gr of non-live yeast	5 gr of non-live yeast		
Initial weight (kg/day)	43.50	43.33	43.33	40.66	42.00	0.29	0.062
Final body weight (kg/day)	63.50	66.50	66.83	67.33	65.83	0.83	0.066
Dry matter intake (starter ration + milk) (g/day)	1654.25^{a}	1539.89^{ab}	1480.78^{b}	1457.15^{b}	1469.74^{ab}	19.17	0.012
Daily weight gain (kg/day)	0.47^{b}	0.55^{ab}	0.56^{ab}	0.63^{a}	0.56^{ab}	0.0009	0.002
Feed conversion ratio	3.63^{a}	2.78^{ab}	2.69^{bc}	2.32^{c}	2.62^{bc}	0.07	0.0002

$^{a-c}$ The difference of numbers in each row with dissimilar letters is significant ($P < 0.05$). SEM: Standard error of the means.

Table 3. Effects of adding live and non-live *Saccharomyces* yeast to milk on the skeletal growth of weanling calves

Parameter	Control	Treatment				SEM	P-Value
		2.5 gr of live yeast	5 gr of live yeast	2.5 gr of non-live yeast	5 gr of non-live yeast		
Body length (cm)							
Beginning	80.50	84.50	79.79	78.00	76.17	2.426	0. 3100
Day 21	82.33	84.67	82.67	86.67	81.50	2.706	0.6440
Day 42	86.50	87.83	85.17	87.83	83.00	2.873	0.7330
Withers (cm)							
Beginning	75.84	75.84	73.17	69.84	73.17	1.721	0.1148
Day 21	77.33	77.16	75.70	76.50	75.83	2.113	0.9639
Day 42	82.17	82.33	79.33	78.83	77.50	2.091	0.4064
Pelvic length (cm)							
Beginning	80.17	83.67	85.84	77.84	78.50	2.265	0.0865
Day 21	83.00	84.83	81.83	82.83	80.50	2.896	0.8725
Day 42	84.83	87.67	84.67	85.17	81.67	2.722	0.6568
Abdominal circumference (cm)							
Beginning	69.17	82.00	70.67	70.17	71.00	5.995	0.5476
Day 21	84.17	81.83	71.50	81.17	76.17	0.195	0.0649
Day 42	89.67	90.50	80.83	83.33	81.16	3.523	0.1710
Chest girth (cm)							
Beginning	76.84	83.13	73.17	70.17	68.76	3.199	0. 5100
Day 21	78.83	83.67	78.16	77.33	75.83	2.086	0.1722
Day 42	81.67	74.83	80.50	79.17	77.50	5.992	0.9362
Wrist circumference (cm)							
Beginning	12.17	12.84	13.67	11.84	11.84	0.557	0.1281
Day 21	12.83	12.67	13.33	13.33	12.83	0.668	0.8763
Day 42	13.50	13.67	14.17	13.17	13.50	0.557	0.8067
Ankle circumference (cm)							
Beginning	22.34	14.17	15.17	11.50	12.00	4.482	0.4556
Day 21	12.83	13.33	13.83	12.67	13.00	0.793	0.8483
Day 42	13.83	13.83	14.67	13.50	13.67	0.765	0.8441

Table 3. Continued

Parameter	Control	Treatment					
		2.5 gr of live yeast	5 gr of live yeast	2.5 gr of non-live yeast	5 gr of non-live yeast	SEM	P-Value
Pins interval (cm)							
Beginning	17.50	5.33	5.84	6.00	5.17	4.889	0.3381
Day 21	7.50	7.00	6.83	7.17	6.17	0.375	0.1892
Day 42	8.00	8.00	7.16	7.67	6.50	0.397	0.613
Hips interval (cm)							
Beginning	12.50^{ab}	13.00^{ab}	13.84^{a}	13.00^{a}	10.50^{b}	0.720	0.0370
Day 21	13.00	13.50	12.33	12.84	10.84	0.685	0.0962
Day 42	14.17	16.0	14.0	15.0	13.17	0.862	0.2169
The interval between two hip bones and the pin (cm)							
Beginning	16.00	18.84	17.66	15.67	15.67	4.646	0.0483
Day 21	16.50	18.17	17.17	15.84	16.00	0.757	0.2082
Day 42	18.57	20.0	19.67	19.84	18.00	0.619	0.1346
Pelvic width (cm)							
Beginning	21.84ab	23.00ab	24.33a	20.84b	20.83b	0.7477	0.0115
Day 21	22.33ab	22.33ab	25.00a	21.17b	21.17b	0.684	0.0033
Day 42	25.00	26.50	25.83	25.33	24.67	1.074	10.7711

$^{a-c}$ The difference of numbers in each row with dissimilar letters is significant ($P < 0.05$). SEM: Standard error of the means.

Table 4. Determining the chemical composition of milk after adding live and non-live *Saccharomyces cerevisiae*

Parameter	Control	Treatment					
		2.5 gr of live yeast	5 gr of live yeast	2.5 gr of non-live yeast	5 gr of non-live yeast	SEM	P-Value
SNF	8.35^{b}	9.17^{ab}	9.18^{ab}	9.25^{a}	9.20^{a}	0.187	0.0293
Protein	3.09^{b}	3.45^{ab}	3.45^{ab}	3.46^{a}	3.46^{a}	0.081	0.033
Fat	3.3	3.12	3.11	3.13	3.11	0.041	0.0702
Lactose	4.75	5.16	5.04	5.08	5.05	0.123	0.2599
pH	6.26	6.31	6.32	6.31	6.31	0.015	0.1880
Water	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Density	29.75^{b}	33.00^{ab}	33.00^{ab}	33.32^{a}	33.21^{a}	0.749	0.0295
Freezing point	55.57	42.28	60.40	60.95	60.55	8.185	0.4739
Conductivity	3.69^{b}	3.91^{a}	3.94^{a}	3.98^{a}	3.98a	0.033	0.0004

$^{a-c}$ The difference of numbers in each row with dissimilar letters is significant ($P < 0.05$). SEM: Standard error of the means.

Table 5. The effect of adding to milk of live and non-live *Saccharomyces cerevisiae* yeast on white blood cells in suckling calves

Parameter	Control	Treatment				SEM	P-Value
		2.5 gr of live yeast	5 gr of live yeast	2.5 gr of non-live yeast	5 g of non-live yeast		
Eosinophil	1.50a	0.34b	0.00b	0.00b	0.34b	0.202	0.0001
Monocyte	2.17	1.34	2.17	2.17	1.84	0.398	0.5663
Lymphocyte	69.84	69.50	68.00	68.17	56.00	4.334	0.1131
Neutrophil	29.84	29.34	34.00	32.67	39.50	4.434	0.5073
Neutrophil to Lymphocyte ratio	0.268	0.33	0.38	0.38	0.48	0.167	0.8868

[a-c] The difference of numbers in each row with dissimilar letters is significant ($P < 0.05$). SEM, standard error of the means.

Table 6. The effect of adding to milk of live and non-live *Saccharomyces cerevisiae* yeast on the average rectal temperature in suckling calves

Parameter	Control	Treatment				SEM	P-Value
		2.5 gr of live yeast	5 gr of live yeast	2.5 gr of non-live yeast	5 g of non-live yeast		
Beginning	38.67	38.83	39.00	39.00	38.99	0.162	0.5311
Day 21	38.33	38.75	38.67	38.99	38.17	0.254	0.1888
Day 42	38.41	38.08	38.00	38.25	38.17	2.187	0.4633

CONCLUSION

The findings of this study suggest that supplementing milk with live and inactive *S. cerevisiae* yeast at a dosage of 2.5-5 g per animal daily significantly improves several aspects of calf health. This supplementation enhances daily milk intake, feed conversion efficiency, and immune response, particularly by increasing eosinophil activity. Notably, these benefits were observed without any detectable negative side effects, making this approach a promising strategy to optimize calf nutrition and overall well-being during the suckling phase.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Taghi Ghoorchi and Kosar Ghezelsofli; Methodology: Kosar Ghezelsofli; Software: Abdolhakim Toghdory; Validation: Taghi Ghoorchi, Abdolhakim Toghdory, and Mostafa Hosseinabadi; Formal analysis: Abdolhakim Toghdory; Investigation: KosarGhezelsofli, Resources: Taghi Ghoorchi; Data curation: Taghi Ghoorchi; Writing—original draft preparation: Taghi Ghoorchi; Writing—review and editing: Taghi Ghoorchi; Visualization: Mostafa Hosseinabad; Supervision: Taghi Ghoorchi; Project administration: Taghi Ghoorchi; Funding acquisition: Taghi Ghoorchi.

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

ETHICAL STATEMENT

This study was performed in line with the principles of the Gorgan University of Agricultural Science and Natural Resources. Approval was granted by the Ethics Committee of University B (Date2024. No20.1115).

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