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The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Effect of fluidised dried yeast (*Saccharomyces cerevisiae)* supplementation on milk composition, somatic cell count and milk yield at different lactation stages in Polish Holstein-Friesian cows

A. Kalińska, M. Gołębiewski*, K. Wnęk-Auguścik, J. Slósarz, M. Kunowska-Slósarz, M. Balcerak, T. Przysucha, B. Kuczyńska, K. Puppel and M. Kot

Warsaw University of Life Sciences, Institute of Animal Science, Animal Breeding Division, Ciszewskiego Str. 8 02-786 Warsaw, Poland

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* Corresponding author:
e-mail: marcin_golebiewski@sggw.edu.pl

ABSTRACT. The nutrition of modern dairy cows is a crucial factor affecting milk yield, its parameters and production economics. Nutritional supplementation is often necessary to ensure that cows receive all the required proteins in their diet. The aim of the study was to analyse the impact of fluidised dried yeast (*Saccharomyces cerevisiae*) on daily milk yield, milk yield for all lactation stages, and udder condition. Milk samples were collected before the experiment, during a 4-week period of yeast supplementation, and 4 weeks after the experiment to analyse changes in milk composition and yield. The results demonstrated a significant positive effect ($P < 0.01$) on daily milk yield at all stages of lactation, as well as improved udder condition, as evidenced by lower somatic cell counts in milk. Therefore, the results have confirmed that fluidised dried yeast can be a valuable component of the diet for high-yielding dairy cows. However, further analyses are necessary to determine the long-term influence of yeast supplementation on milk production and the overall health of cows.

Introduction

The rumen, a specialised fermentation chamber in the digestive system of ruminants, contains a diverse population of anaerobic microorganisms, including bacteria and protozoa. These microorganisms are responsible for breakdown and degradation of various dietary components present in the diet of dairy cows. The rumen ecosystem plays a vital role in the efficient digestion and utilisation of feed by ruminants. Feeding high-concentrate diets, which are rich in easily fermentable carbohydrates, can lead to the development of subclinical ruminal acidosis (Sauvant et al., 1999).

In modern dairy farming, high-yielding dairy cows require precise nutrition management. A balanced diet is essential to achieve adequate milk production levels. While soybean meal is commonly used as a protein source, additional supplements are often required to ensure the proper protein level in the diet of dairy cows (Suriyapha et al., 2022). Therefore, scientists are exploring new and innovative solutions to improve dairy cattle nutrition. Previous studies have shown that yeasts (*Saccharomyces cerevisiae*) can be a valuable nutritional supplement for dairy cows because they increase feed intake and milk production, and improve milk quality (Alshaikh et al., 2002; Lehloenya et al., 2008; Yalcin et al., 2011; Zhao and Qin, 2011). Most research on dietary yeast has focused on supplementation with live yeasts (Cakiroglu et al., 2010; Yalcin et al., 2011; Bayat et al., 2015).

Additionally, studies have explored the combination of yeast supplementation with fat, which has shown positive effects on milk yield, milk fat

content, dry matter intake, and body condition score (Ajithakumar et al., 2017). However, the research on the use of different forms of yeast in dairy cattle nutrition is relatively limited. Alternative forms such as fluidised dried yeasts can be an interesting option for breeders and milk producers. This form of yeast offers potential advantages in terms of convenience and storage time.

Yeast can be effective in alleviating pH decline and reducing the risk of rumen acidosis in commercial cattle fed diets based on maize silage. When used at higher levels, yeast supplementation can provide further benefits by promoting increased fibre degradation in low-quality maize silages, as it enhances rumen fermentation processes (Guedes et al., 2008). Available literature suggests that yeast supplementation can positively affect rumen pH (Desnoyers et al., 2009). However, it is important to note that the effectiveness may vary depending on the specific strain of *S. cerevisiae* used as active dried yeast, as different strains have shown variations in their ability to modify the rumen fermentation pattern (Chung et al., 2011). *In vitro* experiments have also confirmed that the appropriate dosage of yeasts can improve feed fermentation (Jiao et al., 2018). Other authors have suggested that yeast culture supplementation significantly increases milk yield and tends to increase the fat, protein and lactose contents in milk (Yalcin et al., 2011). Administration of *S. cerevisiae* cultures was also shown to improve milk yield and milk components in heat-stressed cows (Schingoethe et al., 2004; Bruno et al., 2009).

The purpose of the study was to determine the effect of supplementation with *S. cerevisiae* yeast cultures on: 1) milk yield during different stages of lactation, 2) milk yield and milk composition, and 3) somatic cell count (SCC) in Polish Holstein-Friesian (PHF) cows.

Material and methods

Supplementation with yeasts applied in the present study does not require any approval of the research ethics committee, as yeasts are commonly used as a supplement in animal nutrition. Moreover, the manuscript does not include clinical trials.

Determination of the quantitative and qualitative composition of yeast (number of cells, type and species of fluidised dried yeast)

The research material consisted of 3 samples (D1, D2, D3) of fluidised dried yeast (*S. cerevisiae)* (AgroYeast, Krośniewice, Poland). Each sample weighed 200 g and was randomly selected from batches sent for the experiment (AgroYeast, Krośniewice, Poland). The number of yeast cells was determined using standard microbiological analysis. Fluidised dried yeast samples (10 g) were mixed with 90 ml of peptone water (Merck, Darmstadt, Germany) and subsequently homogenised in a stomacher machine (IUL Instruments, Barcelona, Spain). Decimal dilutions were prepared from the resulting mixtures. From each dilution, 100 µl was plated on a Petri dish with yeast extract glucose chloramphenicol agar (YGC). The inoculated plates were incubated for 5 days at a temperature of 25 °C. The analysis was performed three times, and the results were expressed as the logarithm of colony-forming units (CFU) per g.

Yeast quality (genetic and species homogeneity) was determined using methods described by Martorell et al. (2005) and Torriani et al. (2004). Yeast DNA was isolated using the ExtractMe DNA yeast kit (DNA Gdańsk, Gdańsk, Poland), according to the manufacturer's instructions. Isolates were labelled according to the yeast sample (D1, D2, D3) and assigned a corresponding letter of the alphabet (A-J). Genetic identification was conducted using PCR with specific primers (Merck, Darmstadt, Germany) with the following sequences:

1. YC1f CTT ATG CTT GGA ACC TCA AGA CA
2. YC2r AGA AGC AAC AAC AGC AAC AAC CCA A

PCR conditions were as follows: initial denaturation at 94 °C for 2 min; 40 cycles of denaturation at 94 °C for 1 min, annealing at 59 °C for 1 min, extension at 72 °C for 1 min, and final elongation 72 °C for 10 min. PCR products were separated electrophoretically using a 1% agarose gel (ThermoFischer Scientific, Waltham, USA). The expected PCR product derived from genomic DNA of *S. cerevisiae* had a size of 1710 bp. The appropriate DNA marker was used in electrophoresis.

Determination of thermostability of fluidised dried yeasts

Samples of the tested fluidised dried yeasts (1 g) were transferred into sterile Eppendorf tubes. The tubes were then incubated in a thermoblock (Bio-Rad, Hercules, USA) at a temperature of 80 °C for 30 min. Inoculations on YGC were made at 10-min intervals. This process was repeated as described previously in order to calculate the number of yeast cells. A 10^{-1} dilution was prepared by mixing 1 g of yeast with 9 ml of peptone water.

Animals and housing system

The experiment was conducted on a farm located in the north-eastern Poland. A herd of 360 PHF cows, both lactating and dry, was kept in a free-stall barn with shallow litter bedding. For the experiment, 320 lactating cows were divided into individual groups based on their milk yield and nutritional requirements. The farm participated in a milk recording system of the Polish Federation of Cattle Breeders and Dairy Farmers. Milk samples were collected using the AT4 recording method (www.pfhb.pl).

Nutrition management and feed quality

Lactating cows were divided into 3 groups according to their lactation stage. Dry cows were divided into two groups (1 – cows 5–6 weeks after drying; 2 – cows 2–3 weeks before delivery). Cows were fed *ad libitum* using the total mix ration (TMR) feeding system balanced according to the nutritional requirements of the cows. Additionally, the cows had continuous access to fresh water.

The feeds used in the TMR system were analysed to calculate their quality and composition. Representative samples (1 kg) of each feed and three samples of the complete TMR feed were placed in string bags and stored in a portable fridge until they were transported to the laboratory. These samples were subjected to a standard procedure for estimating the physicochemical composition of the feed. The samples were tested using an Infraxact apparatus (Labexchange, Burladingen, Germany) according to PB-19-02 2014.04.03 guidelines. The obtained results are presented in Table 1.

Table 1. Chemical composition of feed components and total mixed ration (TMR) samples used in the nutrition of cows

Sample	Ingredients, % DM							
	ADF	Protein	NDF	Ash	Starch	DM	Fat	Fibre
TMR1	13.78	6.87	21.38	3.92	6.87	44.65	1.09	9.81
TMR2	12.91	7.00	19.18	3.84	7.57	42.95	1.10	8.92
TMR3	13.57	7.59	21.98	4.11	6.40	46.57	0.97	10.08
Maize silage	8.54	3.94	13.91	1.78	12.03	37.85	1.34	7.35
Haylage	17.10	7.04	24.68	3.42	-	41.46	1.53	13.14
Grain middlings	-	12.59	-	1.41	54.28	1.14	11.90	2.90

TMR1 – total mixed ration 1, TMR2 – total mixed ration 2, TMR3 – total mixed ration 3; ADF – acid detergent fibre, NDF – neutral detergent fibre, DM – dry matter

Experimental design

The experiment was conducted in two stages. In the first stage, which lasted for two weeks, the cows' diet was supplemented with fluidised dried yeast *S. cerevisiae* at a rate of 10 g/day/cow (5 g twice a day) to prepare the rumen bacteria for yeast supplementation. In the second stage, which lasted for 30 days, the diet was supplemented with fluidised dried yeast at a rate of 15 g/day/cow (7.5 g twice a day). The amount of yeast supplementation was based on the available references and the author's previous studies. To incorporate the yeast into the cows' diet, it was mixed with concentrated feed and added to the other TMR components in a mixer-wagon. Particle size distribution of the TMR offered and TMR refused was measured once a week using a Penn State Particle Separator (PennState Extension, Pennsylvania, USA), as described by Kononoff et al. (2003). The average DMI kg/day intake in individual groups was as follows: 1 – 16.4 kg/day, 2 – 17.1 kg/day, 3 – 17.6 kg/day.

Milk sample collection

Cows were milked twice a day in the milking parlour, where milk production was controlled using milk meters. Milk samples (n = 945) were taken during the first stage of the experiment, in the fourth week after starting the second part of the experiment, and four weeks after the end of the study. Representative samples consisted of milk from morning and afternoon milking were taken from each lactating cow (250 ml). A preservative (Microtabs, Bentley, Warsaw, Poland) was added to each sample for milk composition analysis The analysis, including protein, fat, lactose, urea, dry matter, and ash content was carried out using MilkoScan FT 120 (Foos Electric, Warsaw, Poland), and SCC was calculated using Somacount 150 (Bentley, Warsaw, Poland). Milk performance records and milk analysis results were utilised to assess the influence of yeast supplementation on milk production during different lactation stages. Furthermore, the ratio of fat to protein and the contents of protein, fat, lactose, and urea were examined to estimate the metabolic status of the cows.

Economic production calculations

The economic effect of the supplement was calculated by analysing the cost of the yeast supplement and its effect on production. Milk costs were calculated using an algorithm based on the average rates of 0.08 PLN for fat and 0.16 PLN for protein for the experimental herd. The additional income associated with changes in milk production and milk quality was calculated based on the milk performance records.

Statistical analysis

Statistical analysis was conducted using only complete and verified results from the experiment, which consisted of 945 records. Data were calculated using IBM SPSS Statistics 23.0.0.3 software.

The effect of yeast supplementation on milk production was assessed according to the following model:

$$Y = \mu + A_i + B_j + C_k + D_l + (A_i \times B_j) + (A_i \times C_k) + e_{ijkl,}$$

where: μ – average, A_i – sample collection (1 – before supplementation, 2 – during supplementation, 3 – four weeks after the end of supplementation), B_j – lactation stage (1 – ≤ 40 days of lactation; 2 – 41–90 days of lactation, 3 – 91– 150 days of lactation, 4 – 151-200 days of lactation, 5 – 201– 300 days of lactation, 6 – ≥ 301 days of lactation), C_k – lactation month (1–12 lactation month), D_l – lactation number (1–4 or more), $A_i \times B_j$ – interaction between sample collection and lactation stage, $A_i \times C_k$ – interaction between sample collection and lactation month, e_{ijkl} – random error.

Results

Quantitative and qualitative yeast composition (number of cells, type and species of fluidised dried yeast)

Data from Table 2 and Figure 1 show that the yeast cell count in each sample was ≥ 9 log CFU/g and varied by 5.5%. The average CFU log in all samples was similar and ranged from 9.60 to 9.71 with low standard deviations for all samples.

Table 2. Number of yeast cells in the analysed yeast samples

Sample	Repetition 1, log CFU/g	Repetition 2, log CFU/g	Repetition 3, log CFU/g	Average, log CFU/g
D1	10.20	9.23	9.69	9.71 ± 0.49
D2	10.11	9.63	9.56	9.77 ± 0.30
D3	9.85	9.34	9.60	9.60 ± 0.25

D1 – yeast sample 1 , D2 – yeast sample 2, D3 – yeast sample 3. Data are presented as the logarithm of colony-forming units (CFU) per g. No statistical differences were observed

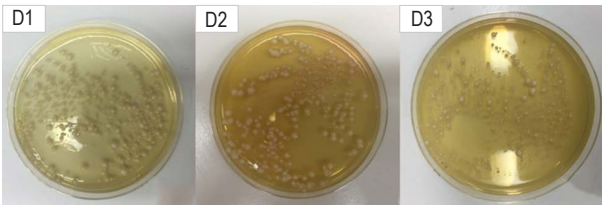

Figure 1. Fluidised dried yeast colonies on yeast extract glucose chloramphenicol agar (YGC)

D1 – yeast sample 1, D2 – yeast sample 2, D3 – yeast sample 3

Figure 1 presents the photographs of Petri dishes containing YGC agar showing yeast colonies after five days of incubation at 25 °C.

Samples were prepared from batches of yeasts labelled D1, D2, D3 for genetic analysis. Isolates for the PCR reactions were assigned letters A–J, respectively, depending on the sample (Table 3).

Table 3. Classification of yeast cell isolates

Yeast sample	Symbols of yeast isolates
D1	D1A, D1B, D1C, D1D, D1E, D1F, D1G, D1H, D1I, D1J
D2	D2A, D2B, D2C, D2D, D2E, D2F, D2G, D2H, D2I, D2J
D3	D3A, D3B, D3C, D3D, D3E, D3F, D3G, D3H, D3I, D3J

D1 – yeast sample 1 , D2 – yeast sample 2, D3 – yeast sample 3

DNA of the isolates was used in the PCR reaction. D1A-D1J – isolates from yeast sample D1, D2A-J – isolates from yeast sample D2, D3A-J – isolates from yeast sample D3.

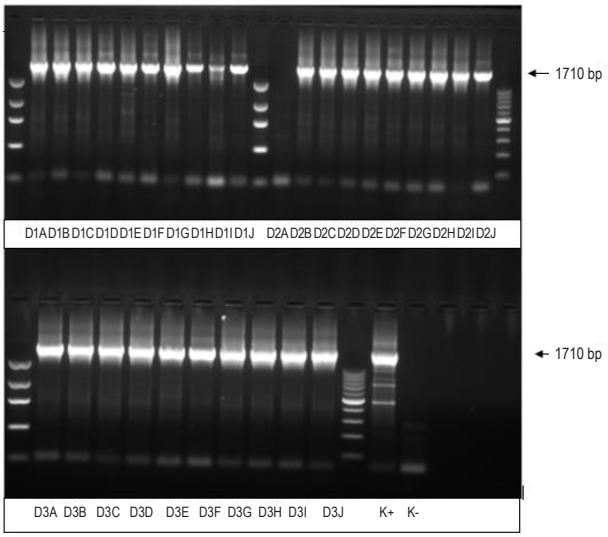

Figure 2. Electrophoretic separation of PCR products using a 1% agarose gel

K+ positive control, K– negative control

The data from Figure 2 shows that an appropriate band of 1710 bp was amplified for all isolates, as indicated by DNA marker.

The obtained data suggest that yeast samples D1 and D3 were 100% identical with the species *S. cerevisiae*. However, sample D2 showed 90% similarity (Table 4).

Table 4. Compatibility of isolates with *Saccharomyces cerevisiae*

Yeast sample	Compatibility with *Saccharomyces cerevisiae*, %
D1	100
D2	90
D3	100

D1 – yeast sample 1, D2 – yeast sample 2, D3 – yeast sample 3

Thermostability of fluidised dried yeast

The results of thermostability determination of the examined fluidised dried yeasts are presented in Table 5. The count of yeast cells in samples D1 and D3 decreased after 30 min of incubation at 80 °C. However, it was surprising to note that the thermostability results varied depending on the sample number, even though all the samples were taken from the same batch.

Table 5. Yeast cell count during the thermostability test (incubation at 80 °C for 30 min)

Yeast sample	'0' time, log CFU/g	After 10 min of incubation, log CFU/g	After 20 min of incubation, log CFU/g	After 30 min of incubation, log CFU/g
D1	9.20	9.06	8.41	3.04
D2	10.61	10.57	10.38	10.26
D3	9.89	9.88	9.03	6.11

D1 – yeast sample 1, D2 – yeast sample 2, D3 – yeast sample 3; CFU – colony-forming unit. No statistical differences were observed

Evaluation of the effect of yeast supplementation in PHF cows' diet on milk yield, milk quality, animal health and production economics

The milk yield and milk composition values recorded for the whole herd throughout the experiment are presented in Table 6. The average daily milk yield was approximately 23 kg and the milk contained 3.46% protein, 4.67% fat and 2.69% casein. However, the urea content was high (≥ 347 mg/ml) and SCC reached almost 526.650/ml of milk. The dry matter content was 13.68%, i.e. slightly higher than the typical level in Holstein-Friesian breed of cattle milk, while lactose and casein levels were 4.69 and 2.69, respectively. The fat-to-protein ratio was 1.37, which could indicate that some animals in the herd had subclinical or clinical ketosis.

Table 6. Analysis of milk yield and milk composition for the whole herd throughout the experiment

	N	Minimum value	Maximum value	Average	Standard error	Standard deviation
Milk, kg/day	945	6.00	50.00	23.13	0.26	7.35
Fat, %	945	1.23	9.70	4.67	0.04	1.07
Protein, %	945	2.20	6.25	3.46	0.02	0.48
Casein, %	945	1.66	4.90	2.69	0.01	0.39
Lactose, %	945	2.67	5.21	4.69	0.01	0.29
Dry matter, %	945	5.55	23.28	13.68	0.05	1.32
Urea, mg/l	945	3.16	593.00	347.28	2.72	75.07
SCC, x 10^3/ml	945	8.00	8308.00	526.65	39.56	1085.68
fat-to-protein ratio	945	0.54	3.02	1.37	0.01	0.35

N – number of samples; SCC – somatic cell count. No statistical differences were observed

Table 7 presents the effect of dietary supplementation of fluidised dried yeast *S. cerevisiae* on milk yield and milk composition. Milk samples were classified as: 1 – before supplementation, 2 – during supplementation, 3 – 4 weeks after the end of supplementation.

Table 7. Statistical analysis of milk yield and milk composition before (1), during (2) and after dietary supplementation (3) with fluidised dried yeast

Item		N	Average	Standard error	Significance
Milk, kg/day	1	315	22.87	0.394	$P = 0.00$
	2	315	24.75	0.501	
	3	315	21.64	0.453	
	all	315	23.13	0.265	
Fat, %	1	315	4.70	0.059	$P = 0.03$
	2	315	4.50	0.064	
	3	315	4.83	0.078	
	all	315	4.67	0.039	
Protein, %	1	315	3.47	0.029	$P = 0.00$
	2	315	3.38	0.030	
	3	315	3.55	0.031	
	all	315	3.46	0.017	
Casein, %	1	315	2.70	0.023	$P = 0.00$
	2	315	2.61	0.025	
	3	315	2.76	0.025	
	all	315	2.69	0.014	
Lactose, %	1	315	4.71	0.020	$P = 0.207$
	2	315	4.69	0.017	
	3	315	4.66	0.018	
	all	315	4.69	0.011	
Dry matter, %	1	315	13.76	0.080	$P = 0.02$
	2	315	13.46	0.078	
	3	315	13.84	0.089	
	all	315	13.68	0.048	
Urea, mg/l	1	315	297.20	3.718	$P = 0.00$
	2	315	374.75	3.616	
	3	315	372.23	5.104	
	all	315	347.28	2.721	
SCC, x 10^3/ml	1	315	511.90	70.839	$P = 0.784$
	2	315	504.80	64.993	
	3	315	567.92	69.645	
	all	315	526.65	39.564	
fat-to-protein ratio	1	315	1.38	0.021	$P = 0.758$
	2	315	1.36	0.021	
	3	315	1.37	0.024	
	all	315	1.37	0.013	

N – number of samples, SCC – somatic cell count; $P < 0.05$ indicates that the observed differences are statistically significant

The addition of fluidised dried yeast to the diet of cows was found to have a positive effect ($P < 0.01$) on milk performance (Table 7), as daily milk production increased by 1.88 kg compared to the results before the experiment. Moreover, the results clearly showed that there was a decrease in milk production after the

cessation of yeast supplementation ($P < 0.01$). The cows produced 3 kg less milk per day compared to the supplementation period, and milk yield was even lower compared to the values before the experiment. The fat content and protein content in the milk increased at the end of the experiment. No changes were observed for lactose and casein concentrations. It should be noted that these results were statistically significant, except for lactose, SCC and the fat-to-protein ratio.

Interestingly, there was a decrease in SCC during the yeast supplementation period compared to the results before the experiment and after the end of supplementation.

Figure 3 illustrates daily milk yield categorised by lactation stage (before, during, and after dietary supplementation with fluidised dried yeast). The data was analysed using the statistical model presented in the article. Cows during all lactation stages experienced a decrease in milk production at the end of the supplementation period comparing to their performance before the study (Figure 4). The curves for the pre-supplementation and post-supplementation groups in Figure 4 are steep, indicating significant changes in milk production during these periods.

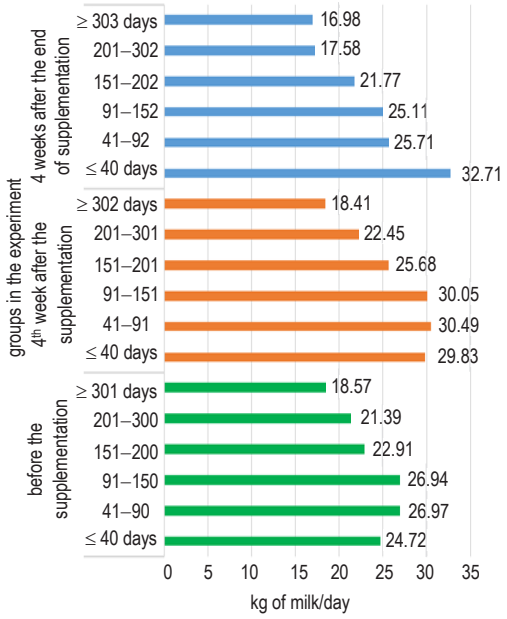

Figure 3. Daily milk yield in different lactation stages before, during, and after dietary supplementation with fluidised dried yeast, kg of milk/day

A relationship was found between milk yield and milk fat content (Figure 5). Milk from cows with the highest milk yield during yeast supplementation contained less fat (4.50%) than the other groups, i.e. before the experiment and after the end of the trial (Table 7).

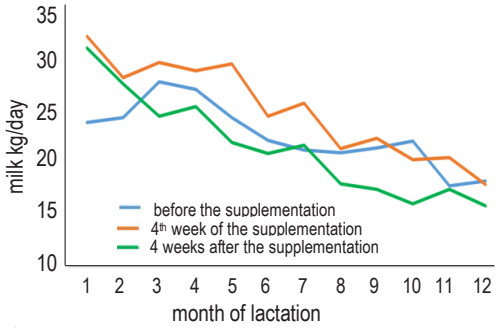

Figure 4. Lactation curves before, during, and after dietary supplementation with fluidised dried yeast supplementation

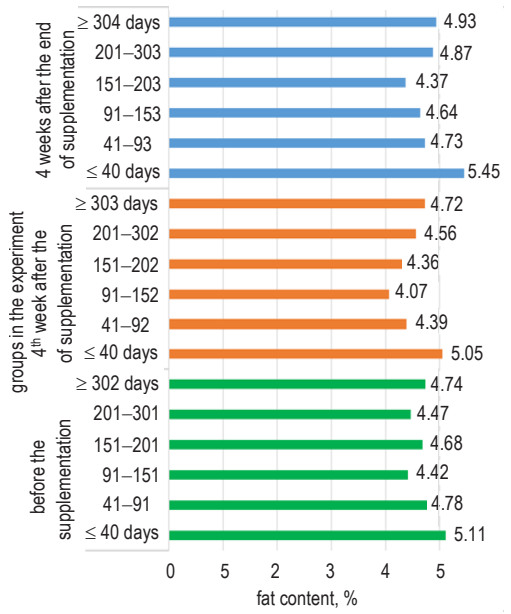

Figure 5. Fat content in milk from different lactation stages before, during, and after dietary supplementation with fluidised dried yeast

Changes in the fat content during different months of lactation are shown in Figure 6. Cows had higher milk yields during the experiment, resulting in a decrease in milk fat content (Table 7).

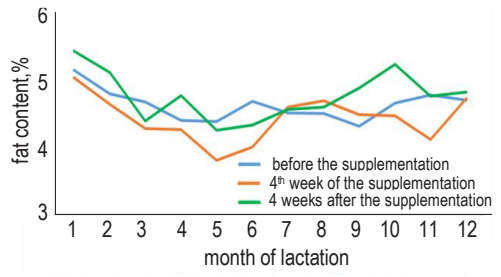

Figure 6. Changes in the fat content in the milk from cows used in the experiment

The protein and casein contents in the milk from cows used in the experiment were also analysed (Table 7), and changes in these parameters were similar to fat content alterations (Figures 7 and 8).

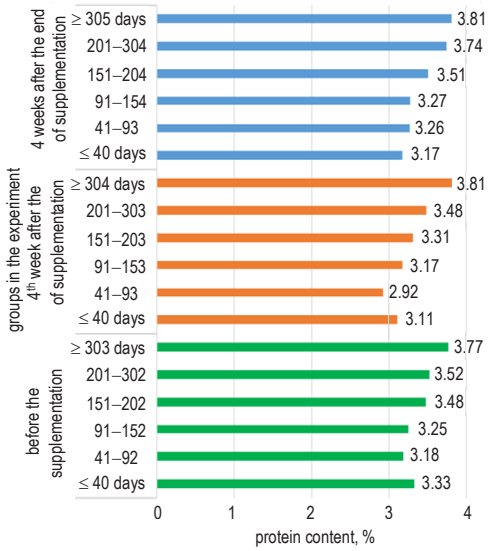

Figure 7. Milk protein content in different lactation stages before, during, and after dietary supplementation with fluidised dried yeast

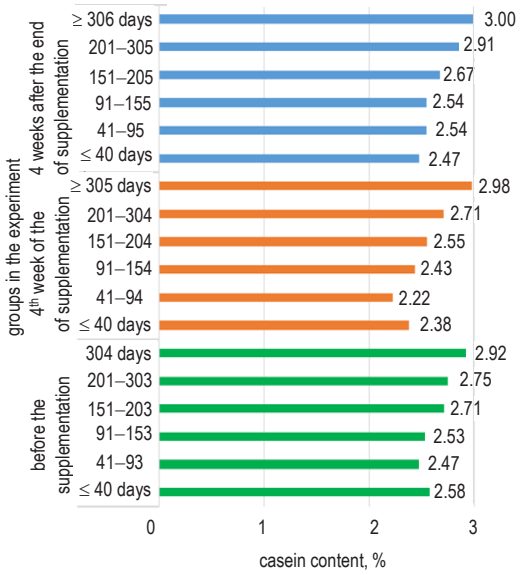

Figure 8. Casein content in milk in different lactation stages before, during, and after dietary supplementation with fluidised dried yeast

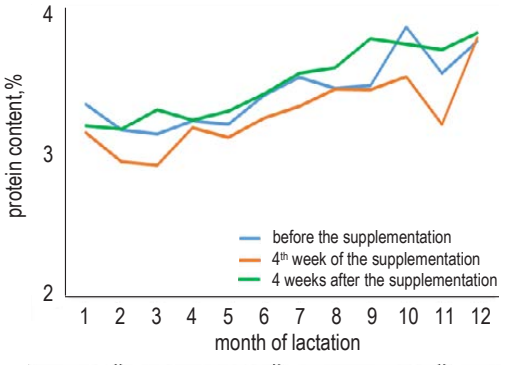

Figure 9. Changes in the protein content in milk from cows used in the experiment

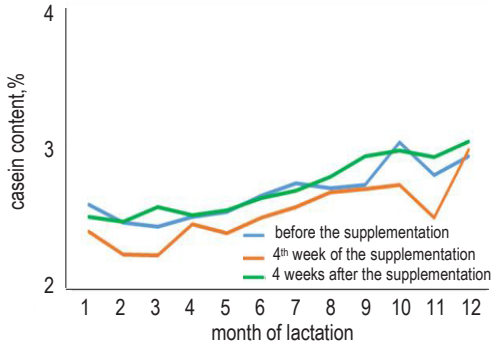

Figure 10. Changes in the casein content in milk from cows used in the experiment

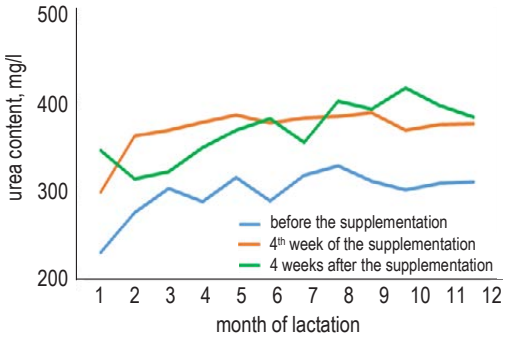

Figure 11. Changes in the urea content in the milk from cows used in the experiment

Figure 9 and 10 illustrate the changes in the protein and casein contents in milk during different months of lactation. The changes in the lactation curve were similar to the fluctuations in milk fat content; however, they were not statistically significant.

It should be emphasized that the average urea content in the milk from each group was elevated (Figure 11). However, the urea content in the milk was the lowest before the experiment.

SCCs during different lactation stages are presented in Figure 12. During yeast supplementation, lactating cows had a more favourable SCC, which increased with lactation time. Cows not receiving yeast supplements had a lower SCC at the beginning of the lactation period.

The SCC values were analysed in compliance with current regulations, and their distribution in the tested cows is presented in Figure 13. Clinical and chronical mastitis cases were observed in 24% of the cows, and SCC in 21% of the cows exceeded the value allowed by EU regulations. Mastitis is a serious problem for producers because it affects the economics of milk production. Only approximately 50% of the cows in the herd showed no udder health problems.

The effect of supplementation with fluidised dried yeast on the percentage of cows with different SCC in milk is presented in Figure 14.

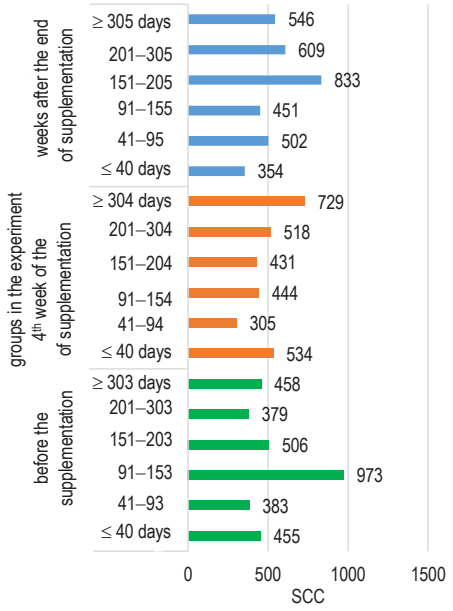

Figure 12. Somatic cell count (SCC) in different lactation stages before, during, and after dietary supplementation with fluidised dried yeast

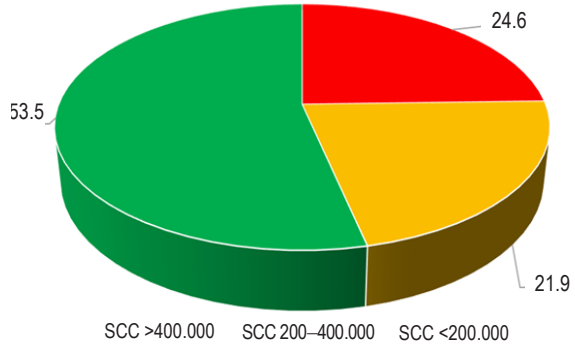

Figure 13. Somatic cell count (SCC) distribution in the herd, %

The highest proportion of cows (21%) with < 200,000 somatic cells occurred in the group of cows receiving yeast supplements. The percentage of cows with < 200,000 somatic cells was lower before the supplementation and after the end of the experiment. This suggested that yeast supplementation exerted a positive effect on SCC.

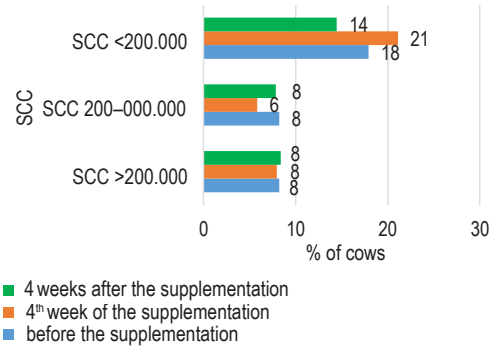

Figure 14. Somatic cell count (SCC) distribution before, during, and after dietary supplementation with fluidised dried yeast

The fattoprotein ratio is a useful indicator as it is associated with conditions such as ketosis and acidosis, which can be common problems in the farming of modern dairy cattle if nutrition is not properly balanced. Figure 15 presents the percentage of cows included in the experiment with different values of the fat-to-protein ratio. A fat-to-protein ratio below 1.0 indicates the presence of acidosis and high values over 1.4 can be associated with ketosis. a fat-to-protein ratio within the range of 1.0 to 1.4 is considered the most favourable for PHF cows.

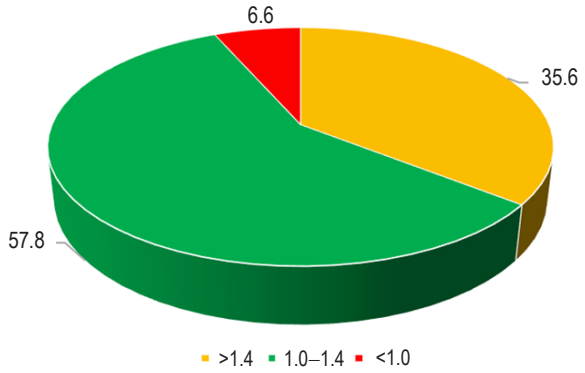

Figure 15. Fat-to-protein ratio distribution in the herd, %

The analysis of data related to the distribution of the fat-to-protein ratio values suggests that fluidised dried yeast supplementation can have a positive influence on cows' health and reduce the risk of metabolic disorders (Figure 16). Yeast supplementation resulted in a 2% increase in the proportion of cows with a favourable fat-to-protein ratio (1.0-1.4), and a consequent decrease of animals with a high fat-to-protein ratio.

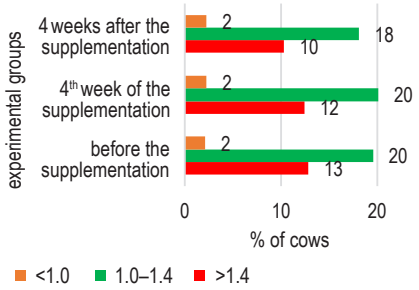

Figure 16. Ratio distribution before, during, and after dietary supplementation with fluidised dried yeast

The final part of the analysis involved the economic effect of yeast supplementation. The additional income was calculated according to the method described in this study. The additional milk production of 1.8 kg/day/cow coupled with changes in milk composition resulted in an extra income of 2.26 PLN/day/cow during supplementation.

This income was calculated taking into account the average price per 1 l of milk during the experiment based on the invoice information regarding the herd. Additionally, the average costs of yeast supplementation, as provided by both the manufacturer and the actual spendings, were calculated at 0.30 PLN per day per cow.

Discussion

Yeasts as a valuable source of protein in dairy cows' diet

Yeasts (*S. cerevisiae*) can be used as a valuable nutritional supplement in the diet of dairy cows, with positive effects on feed intake, milk yield, milk properties and its composition (Lehloenya et al., 2008; Yalcin et al., 2011; Zhao and Qin, 2011; Zaworski et al., 2014). Previous studies have shown that yeast doses can range from 5 g/day/cow to as high as 50 g/day/cow (Yalcin et al., 2011). Findings from these studies consistently support the beneficial outcomes of yeast supplementation (Yalcin et al., 2011; Zaworski et al., 2014).

Other studies also found increases in daily milk yield when cows' diets were supplemented with *S. cerevisiae.* The results from studies conducted by Robinson and Garrett (1990), Huber et al. (1994), and Shaver et al. (1997) have shown that milk yield typically increases by 1 or 2 kg after yeast supplementation, similar to the results obtained in this trial. Some authors have presented data suggesting that daily milk yield of cows receiving yeast supplements during three months of lactation increased by 3.7 kg (Korniewicz et al., 2005). Contrasting results have been reported regarding the effects of yeast supplementation on the various milk components. Some authors, such as Alshaikh et al. (2002), Yalcin et al. (2011), and Zhao and Qin (2011) have presented data suggesting that yeast supplementation increases fat, protein, and lactose contents in milk. On the other hand, in several studies no significant changes were observed in milk composition when cows were administered yeast supplements (Zaworski et al., 2014; Ambriz–Vilchis et al., 2017).

The lactation curve is expected to exhibit an upward trend in milk production from calving to the peak of lactation, followed by a gradual decrease leading to the dry period. However, in this study, cows in all lactation stages showed decreased milk yields at the end of the supplementation period compared to their milk production before the experiment. This decline was clearly visible when examining their lactation curves as depicted in Figure 2. The lactation curves for both the pre-supplementation and post-supplementation groups showed steep declines in milk yield. However, cows that received yeast supplements achieved higher initial productivity, and their decline in milk yield was notably less pronounced compared to cows from the post-supplementation group. Similar findings have been presented in previous studies, supporting the notion that yeast supplementation can extend the lactation peak (Cakiroglu et al., 2010).

Effect of yeast supplementation on milk composition

The urea content in milk should ideally not exceed 280 mg/ml. However, yeast supplementation in this study influenced the urea content in milk, which had already been elevated before the experiment. These high values observed in the analysed individuals are unfavourable for their health, because they overload the detoxifying function of the liver and additionally cause far-reaching negative changes in the reproductive system, resulting in a significant deterioration in reproduction rates (Mimoune et al., 2017). a high urea content in milk (> 340 mg/l) with a proper protein level (3.46%) suggested that cows received an excessive amount of non-protein nitrogen compounds that could be readily degraded by rumen bacteria. The low quality of the feeds (Table 1), particularly their low protein content, prompted the addition of concentrate feeds with a higher protein content. These feeds could have contained too high proportion of non-protein nitrogen compounds, e.g. urea, which could have contributed to the undesired increase in urea content in the cows' diet and subsequently their milk.

The decrease in the fat content was the result of a negative relationship between milk production and the amount of milk components. Similar findings regarding decreased fat content have been reported previously by Hippen et al. (2007). However, a daily milk yield of 25 kg/day during yeast supplementation with a fat content of 4.5% can be considered a satisfactory result for the Holstein-Friesian breed. Conversely, there have also been studies indicating higher fat content during supplementation (Alshaikh et al., 2002). Another interesting phenomenon observed in other studies was the variation in milk fatty acid composition and quantity depending on the yeast strain used. Yeast supplementation has been shown to improve the fatty acid composition in buffalo milk, with a decrease in saturated fatty

acids by 12.2% and an increase of 30.1% in unsaturated fatty acids (Huber et al., 1994).

During the experiment, a decrease in milk fat content was observed, which was consistent with findings of other authors (Zaworski et al., 2014). Changes in the protein and casein content in the milk from cows used in the experiment followed a similar pattern to the changes in the fat content. However, there were also studies reporting an increase in protein content during supplementation (Alshaikh et al., 2002; Erasmus et al., 2005; Hippen et al., 2007). While variations in the protein and casein content in milk samples were similar to the fluctuations in the fat content, they were not statistically significant.

Effect of yeast supplementation on SCC

Udder health is a key factor in maintaining regular lactations. The data presented in the current study indicated that the cows had significant problems with udder condition and clinical mastitis, as the SCC exceeded the recommended levels (150–200,000 cells/ml) set by regulatory standards, and even increased after the experiment. Previous studies have suggested that the addition of *S. cerevisiae* to the cows' diet can lower SCC Zaworski et al., 2014; Ajithakumar et al., 2017). A decreased number of somatic cells is not the only positive effect of yeast supplementation. During the supplementation period, lactating cows showed more favourable SCC distribution. The decrease in SCC during supplementation may suggest that improved rumen function can have a positive impact on udder condition. Udder inflammations (*mastitis*), occurring at the beginning or in the mid-lactation have a highly negative impact on milk production economics. In the experimental group of cows supplemented with yeasts, an increase in SCC was recorded during lactation. Conversely, cows from other groups demonstrated higher somatic cell counts at the beginning of the production cycle. Therefore, mastitis remains a significant concern for producers as it directly affects the economic aspects of milk production. Moreover, it should be noted that only around 50% of cows in the herd showed no udder health problems. In this study, SCC increased by 12% four weeks after the end of supplementation. This may suggest that a more stable functioning of the rumen can affect udder condition.

Economic benefits of yeast supplementation

The fat-to-protein ratio is a valuable indicator that is associated with the occurrence of ketosis and acidosis, which can be a prevalent issue in modern dairy cattle when their nutrition is not properly balanced. The most favourable fat-to-protein ratio ranging from 1.0 to 1.4 is considered optimal for PHF cows. A fat-to-protein ratio below 1.0 suggests the presence of acidosis, while values exceeding 1.4 may be connected to ketosis. In our research, we have analysed data related to the distribution of the fat-to-protein ratio, and the findings indicate that the inclusion of fluidised dried yeast in the cows' diet can have a positive impact on their health and reduce the risk of metabolic disorders. These findings were consistent with previous studies, including the work by Guedes et al. (2008), who also demonstrated similar beneficial effects of yeast supplementation

Various authors, such as Zhao and Qin (2011) and Ajithakumar et al. (2017), have conducted studies to calculate the economic returns of yeast supplementation in dairy cattle. Their research supports the notion that yeast supplementation can be considered a valuable addition to the cows' diet, as it has shown positive effects on various aspects of cattle health and performance. For instance, Cakiroglu et al. (2010) have suggested that supplementation with *S. cerevisiae* can positively stimulate cows' immune systems. Additionally, the beneficial effect of yeast supplementation on dry matter intake and health has been also observed in calves (Finck et al., 2014). However, it is worth noting that not all studies have reported consistent findings. Some authors, such as Bayat et al. (2015), have suggested that yeast supplementation does not affect dry matter intake, rumen fermentation, ruminal gas production, or apparent total-tract nutrient digestibility. The results of the current study have indicated that yeast supplementation exerted a positive effect on SCC, with higher values observed in cows before and after the experiment. However, certain studies have found no statistically significant changes in SCC or protein and fat content (Dann et al., 2000; Schingoethe et al., 2004; Ramsing et al., 2009).

Conclusions

Fluidised dried yeast in the present study exerted a favourable impact on daily milk yield (+1.8 kg/day), milk yield in all lactation stages, udder condition and cow health. We also found that yeast supplementation resulted in a decrease in ruminal pH and ketosis, indicating improved rumen health and a reduced risk of metabolic disorders. This suggests that yeast can be a valuable dietary supplement for cattle of different ages,

including calves and beef cattle. Furthermore, yeast supplementation has improved the benefits in terms of increased income for the farm (+2 PLN/cow/day). However, it is important to note that further analysis is required to assess the long-term effects of yeast supplementation on milk production, milk technological parameters and overall cow health.

Conflict of interest

The Authors declare that there is no conflict of interest.

References

Ajithakumar H.M., Singh M., Sharma S., Punitha M., Khan S.S., Patel B., 2017. Effect of prilled fat and yeast supplementation on milk production, fatty acid profile and economics of feeding in Murrah buffaloes (Bubalus bubalis). Int. J. Curr. Microbiol. Appl. Sci. 6, 1757–1767, https://doi.org/10.20546/ijcmas.2017.610.212

Alshaikh M.A., Alsiadi M.Y., Zahran S.M., Mogawer H.H., Aalshowime T.A., 2002. Effect of feeding yeast culture from different sources on the performance of lactating Holstein cows in Saudi Arabia. Asian – Australas. J. Anim. Sci. 15, 352–356, https://doi.org/10.5713/ajas.2002.352

Ambriz-Vilchis V., Jessop N.S., Fawcett R.H., Webster M., Shaw D.J., Walker N., Macrae A.I., 2017. Effect of yeast supplementation on performance, rumination time, and rumen pH of dairy cows in commercial farm environments. J. Dairy Sci. 100, 5449–5461, https: 477–487, https://doi.org/10.3168/jds.2016-12346

Bayat A.R., Kairenius P., Stefański T., Leskinen H., Comtet-Marre S., Forano E., Chaucheyras-Durand F., Shingfield K.J., 2015. Effect of camelina oil or live yeasts (*Saccharomyces cerevisiae)* on ruminal methane production, rumen fermentation, and milk fatty acid composition in lactating cows fed grass silage diets. J. Dairy Sci. 98, 3166–3181, https://doi.org/10.3168/jds.2014-7976

Bruno R.G., Rutigliano H.M., Cerri R.L., Robinson P.H., Santos J.E., 2009. Effect of feeding *Saccharomyces cerevisiae* on performance of dairy cows during summer heat stress. Anim. Feed Sci. Technol. 150, 175–186, https://doi.org/10.1016/j.anifeedsci.2008.09.001

Cakiroglu D., Meral Y., Pekmezci D., Akdag F., 2010. Effects of live yeast culture (*Saccharomyces cerevisiae*) on milk production and blood lipid levels of Jersey cows in early lactation. J. Anim. Vet. Adv. 9, 1370–1374, https://doi.org/10.3923/javaa.2010.1370.1374

Cakiroglu D., Meral Y., Pekmezci D., Onuk E.E., Kabak Y.B., 2010. Effects of yeast culture (*Saccharomyces cerevisiae*) on humoral and cellular immunity of Jersey cows in early lactation. J. Anim. Vet. Adv. 9, 1534–1538, https://doi.org/10.3923/javaa.2010.1534.1538

Chung Y.H., Walker N.D., McGinn S.M., Beauchemin K.A., 2011. Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in nonlactating dairy cows. J. Dairy Sci. 94, 2431–2439, https://doi.org/10.3168/jds.2010-3277

Dann H.M., Drackley J.K., McCoy G.C., Hutjens M.F., Garrett J.E., 2000. Effects of yeast culture (*Saccharomyces cerevisiae*) on prepartum intake and postpartum intake and milk production of Jersey cows. J. Dairy Sci. 83, 123–127, https://doi.org/10.3168/jds.S0022-0302(00)74863-6

Desnoyers M., Giger-Reverdin S., Bertin G., Duvaux-Ponter C., Sauvant D., 2009. Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. J. Dairy Sci. 92, 1620–1532, https://doi.org/10.3168/jds.2008-1414

Erasmus L.J., Robinson P.H., Ahmadi A., Hinders R., Garrett J.E., 2005. Influence of prepartum and postpartum supplementation of a yeast culture and monensin, or both, on ruminal fermentation and performance of multiparous dairy cows. Anim. Feed Sci. Technol. 122, 219–239, https://doi.org/10.1016/j.anifeedsci.2005.03.004

Finck D.N., Ribeiro F.R.B., Burdick N.C. et al., 2014. Yeast supplementation alters the performance and health status of receiving cattle. Appl. Anim. Sci. 30, 333–341, https://doi.org/10.15232/S1080-7446(15)30125-X

Guedes C.M., Goncalves D., Rodrigues M.A.M., Dias-da-Silva A., 2008. Effects of a *Saccharomyces cerevisiae*yeast on ruminal fermentation and fibre degradation of maize silages in cows. Anim. Feed Sci. Technol. 145, 27–40, https://doi.org/10.1016/j.anifeedsci.2007.06.037

Hippen A.R., Schingoethe D.J., Kalscheur K.F., Linke P., Gross K., Rennich D., Yoon I., 2007. Interactions of yeast culture and dried distillers grains plus solubles in diets of lactating dairy cows. J. Dairy Sci. 90, (Suppl 1), p. 452

Huber J.T., Higginbotham G., Gomez-Alarcon R.A., Taylor R.B., Chen K.H., Chan S.C., Wu Z., 1994. Heat stress interactions with protein, supplemental fat, and fungal cultures. J. Dairy Sci. 77, 2080–2090, https://doi.org/10.3168/jds.S0022-0302(94)77151-4

Jiao P.X., He Z.X., Ding S., Walker N.D., Cong Y.Y., Liu F.Z., Beauchchemin K.A., Yang W.Z., 2018. Impact of strain and dose of live yeast and yeast derivatives on in vitro ruminal fermentation of a high-grain diet at two pH levels. Can J Anim Sci. 98, 477–487, https://doi.org/10.1139/cjas-2017-0079

Kononoff P.J., Heinrichs A.J., Buckmaster D.R., 2003. Modification of the penn state forage and total mixed ration particle separator and the effects of moisture content on its measurements. J. Dairy Sci. 86, 1858–1863, https://doi.org/10.3168/jds.S0022-0302(03)73773-4

Korniewicz A., Kinal S., Korniewicz D., Bialon K., 2005. The influence of Diamond V XP yeast culture feed additive on cows milk production and composition. Acta Scientiarum Polonorum. Zootechnica 1, 81–93

Lehloenya K.V., Krehbiel C.R., Mertz K.J., Rehberger T.G., Spicer L.J., 2008. Effects of propionibacteria and yeast culture fed to steers on nutrient intake and site and extent of digestion. J. Dairy Sci. 91, 653–662, https://doi.org/10.3168/jds.2007-0474

Martorell P., Fernández-Espinar M.T., Querol A., 2005. Molecular monitoring of spoilage yeasts during the production of candied fruit nougats to determine food contamination sources. Int. J. Food Microbiol. 101, 293–302, https://doi.org/10.1016/j.ijfoodmicro.2004.11.014

Mimoune N., Messai C.R., Khelef D., Salhi O., Azzouz M.Y., Kaidi R., 2017. Reproductive parameters and metabolic profile of repeat breeder cows. Livest. Res. Rural Develop. 29, Article 146

Ramsing E.M., Davidson J.A., French P.D., Yoon I., Keller M., Peters-Fleckenstein H., 2009. Effects of yeast culture on peripartum intake and milk production of primiparous and multiparous Holstein cows. Appl. Anim. Sci. 25, 487–495, https://doi.org/10.15232/S1080-7446(15)30739-7

Robinson P.H., Garrett J.E., 1999. Effect of yeast culture (*Saccharomyces cerevisiae*) on adaptation of cows to postpartum diets and on lactational performance. J. Anim. Sci. 77, 988–999, https://doi.org/10.2527/1999.774988x

Sauvant D., Meschy F., Mertens D., 1999. Components of rumen acidosis and acid-forming effect of rations (in French). INRAE Productions Animales 12, 49–60, https://doi.org/10.20870/productions-animales.1999.12.1.3854

Schingoethe D.J., Linke K.N., Kalscheur K.F., Hippen A.R., Rennich D.R., Yoon I., 2004. Feed efficiency of mid-lactation dairy cows fed yeast culture during summer. J. Dairy Sci. 87, 4178–4181, https://doi.org/10.3168/jds.S0022-0302(04)73561-4

Shaver R.D., Garrett J.E., Coppock C., 1997. Effect of dietary yeast culture on milk yield, composition and component yields at commercial dairies. Appl. Anim. Sci. 13, 204–207, https://doi.org/10.15232/S1080-7446(15)31885-4

Suriyapha C., Supapong C., So S., Wanapat M., Cherdthong A., 2022. Bioconversion of agro-industrial residues as a protein source supplementation for multiparous Holstein Thai crossbreed cows. PloS one, 17(9), e0273916, https://doi.org/10.1371/journal.pone.0273916

Torriani S., Zapparoli G., Malacrino P., Suzzi G., Dellaglio F., 2004. Rapid identification and differentiation of *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and their hybrids by multiplex PCR. Lett. Appl. Microbiol. 38, 239–244, https://doi.org/10.1111/j.1472-765X.2004.01468.x

Yalcin S., Yalcin S., Can P., Gurdal A.O., Bagci C., Eltan O., 2011. The nutritive value of live yeast culture (*Saccharomyces cerevisiae*) and its effect on milk yield, milk composition and some blood parameters of dairy cows. Asian-Australas. J. Anim. Sci. 24, 1377–1385, https://doi.org/10.5713/ajas.2011.11060

Zaworski E.M., Shriver-Munsch C.M., Fadden N.A., Sanchez W.K., Yoon I., Bobe G., 2014. Effects of feeding various dosages of *Saccharomyces cerevisiae* fermentation product in transition dairy cows. J. Dairy Sci. 97, 3081–3098, https://doi.org/10.3168/jds.2013-7692

Zhao J., Qin J.Y.X., 2011. Effects of *Bacillus subtilis* and yeast culture for feed intake and milk production. Adv. Mater. Res. 183, 695–699, https://doi.org/10.4028/www.scientific.net/AMR.183-185.695