# The effects of using saccharomyces cerevisiae in the dairy cows feeding on their long-term performances

**Marinela Enculescu, Maria Voiculescu, S. Sertu, Ioana Nicolae**

*Research & Development Institute for Bovine Breeding Balotesti, 077015 Balotesti, Sos. Bucuresti-Ploiesti, km. 21, Romania*

SUMMARY

It is well known that Saccharomyces cerevisiae is an important source of energy and proteins in animal feeding. The purpose of this paper was to study the effects of the fresh yeast used in the dairy cows' food, on productive and reproductive performances as well as on animals health. The efficiency testing of the fresh yeast was carried out at the Experimental Farm of the Research and Development Institute for Bovine Balotesti, on a number of 40 Romanian Black Spotted heads: n=20 cows as experimental group (E) and n=20 cows as control group (M). During a long period (2013-2016) we introduced 50 g fresh yeast/head/day (group E), as a source of protein, amino acids, minerals and vitamins, for the energetic-protein balance of the ration. Results were expressed as a mean (±standard deviation). The Student's test was applied to obtain the significance of difference. The recorded values showed a higher average daily milk production with 2.53±0.16 l/head/day ($P<0.05$) for the experimental group (E) comparative with the control group (M) and a short calving interval, at the end of the experiment. The haematological profile parameters (red blood cells, haemoglobin, haematocrit, total white blood cells, lymphocytes, monocytes, neutrophil) were in the normal physiological limits with no influence of the fresh yeast addition ($P>0.05$) during the three years of the experiment. The means values recorded for asparagine aminotransferase (GOT) and alkaline phosphatase (PAL) were within normal physiological limit for the both groups. In terms of protein profile parameters (total proteins, urea), significantly differences ($P<0.05$) at the end of the experiment have been observed. Our results confirm that fresh yeast is a valuable ingredient in the dairy cows` food with long-term positive effects on their performances.

Keywords: yeast, dairy cows, productive performances, metabolic profile.

## INTRODUCTION

The genus Saccharomyces, sp. *Saccharomyces cerevisiae*, have been used for many years as supplements in ruminants to improve productive performances and health status of the animals (Piva et al., 1993; Swartz et al., 1994; Newbold et al., 1996; Dawson, 2000; Li et al., 2016). Yeasts are a taxonomic group and heterogeneous of eukaryotic unicellular microorganisms, having the main characteristic of simple carbohydrates fermentation in anaerobiosis, with the releasing of the $CO_2$ and ethylalcohol (Yong, 2012). Mode of action of yeast depends on the rumen microbial population. Arambel and Kent, (1990) reported that *Saccharomyces cerevisiae* addition increased the nutritional value of poor quality forages and high grain diets. The *Saccharomyces cerevisiae* can optimize the rumen function, improve the microbial activity by increasing the number of beneficial bacteria in the rumen of the animals (Robinson and Erasmus, 2009; Ayad et al., 2013; Maamouri et al., 2014).

Nocek et al. (2011) reported that the effects of the yeast were enhanced when the animals consumed diets with a higher proportion of concentrate. Saccharomyces cerevisiae cell wall components contribute to the release of cytokines from macrophages (Majtan et al., 2005). Yeast, can also, improve performance and change of the metabolism. Many studies showed that dairy cows diet supplemented with *Saccharomyces cerevisiae* displayed less volatile fatty acid (VFA) concentrations in the rumen comparative to control animals, decreased duration of acidosis, increased dry matter intake (DMI) and milk yield in dairy cows (Thrune et al., 2009). Data from an *in vitro* study suggested that *Saccharomyces cerevisiae* supplements may also affect rumen pH and nutrient digestibility (Callaway and Martin, 1997). Yeast cells also can improve digestibility and absorption of minerals such as phosphorus, magnesium, calcium, copper, potassium, zinc and manganese (Sontakke, 2012).

The purpose of this paper was to study the effect of the *Saccharomyces cerevisiae* on the milk production and its composition, calving interval and blood parameters.

## MATERIAL AND METHODS

The efficiency testing of the fresh yeast, as a source of protein, amino acids, minerals and vitamins, for energetic-protein balance of the ration, was carried out at the Experimental Dairy Cows Farm of the Research and Development Institute for Bovine Balotesti (44°36′46″N 26°4′43″E), Romania. A number of 40 Romanian Black Spotted heads were randomly divided into two groups: n=20 cows as experimental group (E) and n=20 cows as control group (M), according to age, milk yield and health status.

The effects of the fresh yeast were evaluated during a long period (2013-2016) by introducing 50 g *Saccharomyces cerevisiae*/head/day (group E), in order to improve the structure of mixed feed. The cows were housed in a tie-stall system and feeding was differentiated by season and based on local feedstuffs (in general, during the winter season the diet/head/day consisted of 6-7 kg alfalfa hay, 20 kg corn silage, and 5 kg concentrates or 7 kg alfalfa hay, 15 brewer's yeast, and 6 kg concentrates; during the summer season the diet/head/day consisted of 5 kg alfalfa hay, 40 kg green mass pastures, and 5 kg concentrates). The dairy cows received salt and water *ad libitum*. The chemical composition of the studied fodder was performed using Weende scheme. The nutritive crude value of the feeds was determined by I.B.N.A. methodology (Burlacu, 1998 cited by Stoica and Stoica, 2001). The nutritional value of the feed used in this study varied according to the nature of the feed, floral composition, the vegetation stage as well as the way of harvesting, preservation and storage conditions. The average daily consumption of the dairy cows is presented in Table 1.

**Table 1.** The average daily consumption of the dairy cows' diet.

| | DM, kg | UNL | PDIN, g | PDIE, g | Ca, g | P, g |
|---|---|---|---|---|---|---|
| 1st year | 15.18-15.68 | 13.21-13.56 | 1215-1225 | 1158-1141 | 97.30-99.21 | 54.09-55.50 |
| 2nd year | 13.8-15.52 | 10.60-13.01 | 1204-1231 | 1147-1152 | 89.65-96.32 | 55.43-56.02 |
| 3rd year | 15.28-15.74 | 13.47-13.78 | 1227-1249 | 1193-1189 | 96.08-103.2 | 53.91-58.53 |

* DM=dry mater; UNL= milk nutrition units; PDIN= digestible intestine protein allowed by the nitrogen content of the fodder; PDIE= digestible intestine protein allowed by the energy content of the fodder Ca=calcium; P=phosphorusTable footer
* source Burlacu, 1998.

Milk production was evaluated monthly and the parameters of the milk (fat, protein) were determined using an automated analyser, Lactoscan (Milkotronic Ltd, Bulgaria).

For the haematological examinations, blood samples were collected aseptically (2 samples/animal/year) from the jugular vein of each animal, 2-4 hours after morning feeding. The harvested amount of blood was 1-2 ml for each sample in vacutainer tubes with disodium ethylene diamine tetra acetic acid (EDTA) as anticoagulant. After harvesting, the samples were chilled to +4 °C. Haematological parameters (red blood cells, haemoglobin, haematocrit, total white blood cells, lymphocytes, monocytes, neutrophil) were determined by using automated haematology analyser Abacus Junior Vet 5 (Diatron, Hungary).

For the biochemical examination of blood serum, the samples were collected in dedicated vacutainer tubes under sterile conditions. A blood

sample of approximately 9 ml was taken to perform the biochemical examination. After harvesting, the blood samples were kept at room temperature (approximately 22-24 °C) until the serum was expressed. Serum was separated by centrifugation at 3000 g for 15 min and stored in aliquots at –20 °C until they were analysed. The blood biochemical parameters (glucose, total proteins, urea, total cholesterol, alkaline phosphatase, asparagine aminotransferase, alanine aminotransferase, total calcium, inorganic phosphorus, magnesium) were estimated by using a semiautomated biochemical analyser StarDust MC 15 (DiaSys Diagnostics Systems Gmbh, Germany) and DiaSys reagents in dedicated kits. The analyses were carried out in the Animal Physiology and Biochemistry Laboratory of the I.C.D.C.B. Balotesti.

Results were expressed as a mean (±standard deviation). The Student's test was applied to obtain the significance of differences. The difference of the mean values was considered significant for $P<0.05$.

## RESULTS AND DISCUSSION

The average milk production was 16.67±1.49 l/day for the experimental group (E) and 16.33±1.41 l/day for the control group (M) in the 1st year after the start of the experiment ($P>0.05$). In the 2nd year of the experiment the difference between the experimental group E (17.21±1.46 l/day) and the control group M (16.81±1.70 l/day) was higher (0.4 l/day), but not significant ($P=0.346$) A significant difference was observed in the 3rd year of the experiment: it was recorded a higher average daily milk production, with 1.63±0.16 l/head/day ($P=0.047$) for the experimental group (E) comparative with the control group (M). Also, an increase of the milk fat percentage ($P<0.05$) was observed after three years of the experiment. Our results are presented in Table 2.

The addition of the fresh yeast in the dairy cows` diet, improved the milk protein percentage (Figure 1) during the three years of the experiment, but without statistical significance ($P=0.671$). Also, the addition of the fresh yeast has contributed to a short calving interval at the end of the experiment.

A slight increase in milk yield induced by the addition of yeast in the dairy cow's diet has been reported by numerous authors: Piva et al. (1993); Kung et al. (1997); Robinson and Garrett (1999); Bertin and Andrieu (2005); Formigoni et al. (2005); Nocek and Kautz, (2006); Sinclair et al. (2006); Szusc et al. (2013); Bakr et al. (2015). Yalcin et al. (2011), reported a significant difference between the control group (23.49±1.83 kg/day) and the experimental group (24.97±1.95 kg/day) in dairy cows. Other authors reported only a minor improvement of the milk production (Dann et al.,

2000) or found no effects of yeast on the milk production (Erasmus et al., 2005; Cooke et al., 2007).

**Table 2.** The effects of the fresh yeast on milk production, milk fat, milk protein and calving interval in the dairy cows.

| Parameters | Group E | Group M |
|---|---|---|
| *Milk production, l/day* | | |
| Start | 16.20±1.14 | 16.21±1.15 |
| 1st year | 16.67±1.49 | 16.33±1.41 |
| 2nd year | 17.21±1.46 | 16.81±1.70 |
| 3rd year | 18.57±3.99[a] | 16.94±3.83[b] |
| *Fat, %* | | |
| Start | 3.89±0.17 | 3.88±0.16 |
| 1st year | 3.90±0.14 | 3.82±0.17 |
| 2nd year | 3.83±0.18 | 3.72±0.20 |
| 3rd year | 4.03±0.19[a] | 3.89±0.25[b] |
| *Protein, %* | | |
| Start | 2.80±0.31 | 2.90±0.15 |
| 1st year | 3.10±0.24 | 2.99±0.14 |
| 2nd year | 3.01±0.15 | 2.87±0.33 |
| 3rd year | 3.16±0.22 | 3.09±0.23 |
| *Calving interval, days* | | |
| Start | 460.70±8.07 | 462.00±9.60 |
| 1st year | 450.55±15.20 | 458.9±11.88 |
| 2nd year | 445.38±16.82 | 455.45±17.28 |
| 3rd year | 431.10±16.50 | 441.54±17.56 |

E=experimental group; M=control group; values are expressed as averages standard deviation;
[a, b] = mean values with different letters in the same row are significantly different.

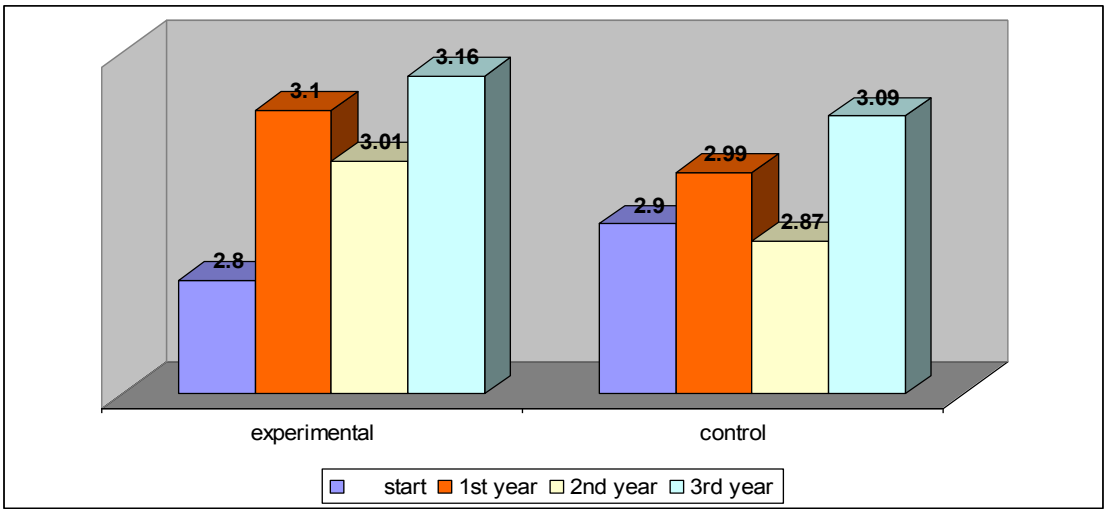


**Figure 1**. Graphical representation of the milk protein percentage in the dairy cows

The responses to addition of the fresh yeast vary upon diets, types and doses of used yeasts, category of the tested animals, stage of lactation or physiological condition of the animals (Dawson, 1989; Williams et al., 1991).

Several studies showed that the increasing of the milk production induced by dietary supplementation with *Saccharomyces cerevisiae* is not always associated with a change in milk fat and milk protein (Soder and Holden, 1999). Our results are in agreement with the results of Nikkhah et al. (2004) who showed an increasing of the fat content and those of Moallem et al. (2009) who found no influence on protein content. This is relevant for the Romanian dairy sector, where the price of milk is set upon fat content only (while the protein content is not considered).

Desnoyers et al. (2009) showed that yeast supplementation diet increased milk yield without any significant effect on milk composition. In the study carried out by Bagheri et al. (2009) the yeast culture addition had no positive effect on milk composition of dairy cows.

Kravale et al. (2005), also, reported that yeast additions significantly improved milk yield of the dairy cows, fat and protein content during the hot season. The obtained results for haematological parameters evaluated are presented in Table 3. In the present study, all the mean values for haematological parameters were situated within the normal physiological limits, without statistically significant differences ($P>0.05$) between experimental group (E) and control group (M), during the three years of the experiment.

Positive effects of the yeast cultures addition on haematological parameters were reported by Heinrichs et al., (2003), Agazzi et al. (2014) in dairy calves and Ghazanfar et al. (2015) in dairy cows` heifers.

The results of the determinations of the serum biochemical parameters are presented in Table 4. The addition of the fresh yeast improved the serum glucose level for the experimental group (E) comparing with control group (M) during all three years of the experiment, but without significance differences ($P>0.05$). The glucose increase in experimental group (E) may be attributed to increased gluconeogenesis, which raises blood glucose levels in ruminants (Huntington and Eisemann, 1988). This explanation is supported by low glucose levels obtained by Antunovic et al., (2005) in treated lambs with probiotics after inhibition of gluconeogenesis by insulin, which inhibits phosphorylase and gluconeogenic enzymes. In terms of protein profile parameters (total proteins, urea), inorganic phosphorus level, significant differences ($P<0.05$) in the 3$^{rd}$ year of the experiment have been observed.

**Table 3.** Results regarding the effects of the fresh yeast on haematological parameters in the dairy cows.

| Haematological parameters[1] | Group E | Group M |
|---|---|---|
| ***RBC, $10^6$/µl*** | | |
| Start | 6.32±0.46 | 6.28±0.37 |
| 1st year | 6.62±0.67 | 6.48±0.54 |
| 2nd year | 6.74±0.89 | 6.51±0.63 |
| 3rd year | 6.84±0.85 | 6.59±1.05 |
| ***HGB, g/dl*** | | |
| Start | 9.60±1.06 | 9.59±1.10 |
| 1st year | 9.75±1.07 | 9.62±1.61 |
| 2nd year | 10.01±1.03 | 9.86±1.21 |
| 3rd year | 10.21±1.12 | 9.87±1.24 |
| ***HTC, %*** | | |
| Start | 31.02±5.42 | 31.99±5.36 |
| 1st year | 32.26±5.49 | 31.66±5.55 |
| 2nd year | 33.24±5.90 | 32.21±5.54 |
| 3rd year | 32.54±6.60 | 31.96±5.75 |
| ***WBC, $10^3$/µl*** | | |
| Start | 8.80±1.78 | 8.89±1.60 |
| 1st year | 8.38±2.88 | 9.06±2.02 |
| 2nd year | 7.95±1.75 | 8.09±1.87 |
| 3rd year | 8.17±1.82 | 8.27±1.90 |
| ***LY, %*** | | |
| Start | 61.23±16.96 | 60.2±17.06 |
| 1st year | 58.9±17.57 | 60.46±18.07 |
| 2nd year | 55.54±15.11 | 55.96±18.37 |
| 3rd year | 55.36±19.16 | 58.42±17.96 |
| ***MO, %*** | | |
| Start | 1.28±0.6 | 1.31±0.67 |
| 1st year | 1.02±0.71 | 1.34±0.68 |
| 2nd year | 1.08±0.87 | 1.43±0.59 |
| 3rd year | 0.98±0.73 | 1.31±0.77 |
| ***NE, %*** | | |
| Start | 35.16±7.26 | 36.31±8.02 |
| 1st year | 34.99±7.96 | 35.04±7.98 |
| 2nd year | 37.04±7.75 | 33.64±7.54 |
| 3rd year | 36.01±8.07 | 34.14±7.89 |

E=experimental group; M=control group.

[1]RBC = red blood cells count, HGB = haemoglobin concentration, HCT = haematocrit percentage, WBC = total white blood cells count, LY = lymphocytes percentage, MO = monocytes percentage, NE = neutrophil percentage,

a, b = mean values with different letters in the same row are significantly different.

**Table 4.** Results regarding the effects of the fresh yeast on serum biochemical parameters in the dairy cows.

| Biochemical parameters[1] | Group E | Group M |
|---|---|---|
| 0 | 1 | 2 |
| ***Glucose, mg/dl*** | | |
| Start | 61.12±5.60 | 60.32±7.80 |
| 1st year | 51.65±7.79 | 50.05±9.19 |
| 2nd year | 49.35±8.02 | 48.55±10.06 |
| 3rd year | 62.4±14.63 | 60.90±15.60 |
| ***Proteins, mg/dl*** | | |
| Start | 6.20±1.30 | 6.30±1.27 |
| 1st year | 6.08±1.08 | 5.40±1.43 |
| 2nd year | 6.96±1.23 | 6.20±1.25 |
| 3rd year | 7.83±1.31[a] | 6.78±1.72[b] |
| ***Urea, mg/dl*** | | |
| Start | 31.67±7.21 | 30.5±8.02 |
| 1st year | 33.45±8.46 | 31.6±9.25 |
| 2nd year | 35.95±7.43 | 33.05±9.36 |
| 3rd year | 38.85±6.89[a] | 34.3±7.58[b] |
| ***Cholesterol, mg/dl*** | | |
| Start | 191.03±48.27 | 199.21±50.08 |
| 1st year | 181.85±49.17 | 196.35±51.16 |
| 2nd year | 189.95±54.68 | 211.35±55.26 |
| 3rd year | 185.40±48.99 | 207.85±59.72 |
| ***GOT, U/L*** | | |
| Start | 59.06±12.60 | 60.11±13.08 |
| 1st year | 52.05±13.73 | 59.20±13.01 |
| 2nd year | 53.20±13.38 | 60.20±12.62 |
| 3rd year | 57.15±13.12 | 61.35±12.27 |
| ***GPT, U/L*** | | |
| Start | 27.30±8.27 | 28.26±9.20 |
| 1st year | 25.15±10.97 | 25.85±10.55 |
| 2nd year | 26.06±9.21 | 26.25±10.36 |
| 3rd year | 24.40±6.40 | 22.35±6.20 |
| ***PAL, U/L*** | | |
| Start | 71.00±2.67 | 72.34±13.19 |
| 1st year | 70.85±13.10 | 72.35±17.89 |
| 2nd year | 68.35±14.22 | 74.35±17.97 |
| 3rd year | 66.85±15.14 | 71.85±18.56 |
| ***Ca, mg/dl*** | | |
| Start | 8.30±1.60 | 9.01±1.20 |
| 1st year | 8.24±0.76 | 7.94±1.01 |
| 2nd year | 8.19±0.55 | 7.85±1.45 |
| 3rd year | 8.60±0.87 | 8.10±1.07 |

| 0 | 1 | 2 |
|---|---|---|
| ***P, mg/dl*** | | |
| Start | 4.20±1.08 | 4.23±1.21 |
| 1st year | 4.68±1.12 | 4.21±1.24 |
| 2nd year | 4.53±1.18 | 4.02±1.25 |
| 3rd year | 4.88±0.89[a] | 4.14±1.21[b] |
| ***Mg, mg/dl*** | | |
| Start | 2.20±1.11 | 2.32±1.16 |
| 1st year | 2.30±1.23 | 2.03±0.97 |
| 2nd year | 2.19±1.03 | 1.98±0.97 |
| 3rd year | 2.58±1.43 | 2.12±0.98 |

E=experimental group; M=control group.
[1]GOT = asparagine aminotransferase, GPT = alanine aminotransferase, PAL = alkaline phosphatase, Ca = total calcium, P = inorganic phosphorus, Mg = magnesium.
a, b = mean values with different letters in the same row are significantly different.

The rest of the blood parameters (total cholesterol, alkaline phosphatase, asparagine aminotransferase, alanine aminotransferase, total calcium, magnesium) were not influenced by the addition of the fresh yeast ($P>0.05$). The mean values recorded for asparagine aminotransferase (GOT) and alkaline phosphatase (PAL) were within normal physiological limit for the both experimental groups, however an improvement of this parameters in experimental group (E) was observed, during all three years of the experiment.

In other studies, the blood parameters were not influenced by live yeast culture supplementation (Piva et al., 1993; Yalcin et al., 2011). Putnam et al. (1997) observed that serum urea and plasma glucose were not influenced by 10 g/day yeast culture addition to the diets of dairy cows. Also, Bagheri et al., (2009) found no effects of the live yeast addition in the diets of early lactation Holstein cows on the levels of glucose and urea nitrogen in blood serum.

Moallem et al. (2009) reported that the effects of the yeast cultures to ruminants can be more beneficial under stress conditions than in the normal conditions. Dobicki et al. (2005) and Galvão et al. (2005) reported that live yeast addition improves the general health status, in calves. The differences between these results and our results may be due to the type and dose of yeast, type of forage, and environmental conditions.

## CONCLUSIONS

The addition of the *Saccharomyces cerevisiae* in the dairy cows` diet led to a general improvement, although not always statistically significant, of milk yield, milk fat content, calving interval and some of the blood

parameters. The overall effects expressed during all three years of the experiment, disregarding the variations in season, feeding, physiological evolution of cows. Overall, the benefits in terms of milk yield, fat content, exceeded the supplementary costs implied by the addition with 50 g yeasts / head / day. Our results confirmed that fresh yeasts are a valuable ingredient in the dairy cows feeding, with positive long-term effects on performances and health status.

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