



Effects of the Inclusion of Yeast Culture (*Saccharomyces cerevisiae*) in the Diet of Holstein Cows on Milk Yield and Composition in Early Lactation

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ABSTRACT

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The effect of including a yeast culture (*Saccharomyces cerevisiae*; 10¹⁰ cfu/g; YC) in diets for lactating Holstein cows was evaluated. One hundred thirty-eight cows in early lactation were randomly assigned into two groups of 69 cows each, according to their days in milk and parity and were fed a basal diet without (CTRL diet) or with the addition of 15 g YC/cow/d (yeast diet) for 7 weeks. The basal diet contained 17.7% CP and 31.8% NDF on DM basis. The inclusion of YC increased (P=0.04) feed intake compared to the CTRL diet. The YC did not affect (P>0.05) serum total protein, albumin and globulin concentration, as well as albumin/globulin ratio, and urea-N. The YC increased serum glucose concentration (P=0.002) and decreased serum cholesterol (P=0.001) levels compared to CTRL group. Addition of YC did not affect (P>0.05) actual and energy corrected milk yields and daily milk total solids, solids-not-fat, protein, and milk energy content and output; however, YC increased milk fat (P=0.04) and lactose (P=0.028) daily yields. Increased milk fat concentration (P=0.027) and lactose (P=0.004) were observed in cows fed the yeast diet. It was concluded that YC at 15 g/cow/d during the first 7 weeks of lactation increased feed intake, and milk fat concentration and lactose without affecting daily milk yield and milk efficiency.

Keywords: Globulin, Feed utilization, Milk yield, Yeast.

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INTRODUCTION

In developing countries, there are many challenges facing the animal production industry; one of them is the low production efficiency and poor feed utilization. Inclusion of feed additives from natural sources, such as phytochemical extracts (Cedillo *et al.*, 2014, 2015), exogenous enzymes (Rojo *et al.*, 2015; Morsy *et al.*, 2016), and yeast (Ahmed *et al.*, 2016; Hassan *et al.*, 2016) seems to be a good alternative to increase feed utilization and production. Besides, antibiotic and ionophores have been banned in the European Union since 2006 and are restricted as feed additives in other countries (Martin *et al.*, 2010), because their use increase antimicrobial resistance in bacteria (Langford *et al.*, 2003) and their residues and metabolites appear in milk and meat (Russell and Houlihan, 2003).

Live yeast is a microbial alternative to antimicrobial feed additives that could enhance health and performance of animals (Hassan *et al.*, 2016). *Saccharomyces cerevisiae* has been generally recognized as safe for human and animal consumption. Dietary addition of live yeast (*S. cerevisiae*) alters the rumen environment enhancing microbial activity (Ahmed *et al.*, 2015) and causing changes in ruminal short-chain fatty acids production, which results in an increased milk production and higher milk fat, and protein content in lactating cows (Poppy *et al.*, 2012). Moreover, *S. cerevisiae* (i.e., YC) has the ability to remove traces of oxygen, swallowed during eating, that may be toxic to rumen microorganisms. This increases the number of anaerobic microorganisms such as cellulolytic bacteria (Jouany, 2001). In addition, yeast culture provides various growth factors, pro-vitamins, and other stimulants for growth and activity of ruminal microorganisms (Newbold *et al.*, 1995; Miller-Webster *et al.*, 2002), and balance of the ruminal fluid redox potential for optimal fermentation conditions for the rumen microflora (Chaucheyras-Durand *et al.*, 2008).

Yeast feeding to dairy animal increased feed intake in some experiments (Stella *et al.*, 2007; Moallem *et al.*, 2009) and decreased it in others (Schingoethe *et al.*, 2004). Moreover, some studies (Bruno *et al.*, 2009; Ramsing *et al.*, 2009) observed significant effects of yeast on milk production, while others reported no effect on milk yield (Schingoethe *et al.*, 2004; Bagheri *et al.*, 2009). Inconstancy between experiments is problematic to nutritionists and dairy farmers as they have contradictory information to decide whether to use YC to improve milk production and composition. Regarding this issue, possible reasons for the inconstancy between experiments are the lack of statistical power (Egger *et al.*, 2001), different study locations by different researchers and different experimental designs (Lean *et al.*, 2009), and the type of yeast products (Elghandour *et al.*, 2017). Besides, differences between active ingredients with different modes of action of different tested yeast products are another reason of inconsistent results. Therefore, the present experiment aimed to study the effect of feeding live *S. cerevisiae* at 15 g/cow/d to early lactating Holstein cows under commercial condition on feed intake, blood chemistry and milk production and composition.

MATERIALS AND METHODS*Cows, feeding and management*

One hundred thirty-eight Holstein cows in early lactation were randomly allocated according to parity and days in milk (DIM) into two groups of 69 cows each and fed a basal diet without (CTRL diet; DIM 39 ± 22 , parity 2 ± 1) or with the addition of yeast at 15 g/cow/d (YEAST diet; DIM 39 ± 22 , parity 2 ± 1). Ingredients and nutrient contents of the basal diet are shown in Table 1. The yeast product (Actisaf Sc 47, Lesaffre, France) is live *S. cerevisiae* yeast NCYC Sc 47 containing 10^{10} cfu/g of the product. The experiment lasted 7 weeks. Cows were divided into two groups and placed in shaded free-stall pens. The daily amount of yeast was mixed individually in the ration and offered at 04:00 h to each cow. Water was available constantly and feed was available separately *ad libitum* to each animal with weighed amounts of fresh feed offered daily.

The amount of feed delivered was registered by electronic scales fixed to the mixer-feeder wagon. The diet was mixed and fed using a Delaval mixer wagon (Delaval, Tumba, Sweden). The diet was formulated using Gavish computer operated cattle feeding system 2008 to meet the requirements of 650-kg cows according to National Research Council recommendations (NRC, 2001). Daily feed intake was determined daily through the experiment.

During the entire experiment, the diet offered was sampled weekly and stored at -20°C until chemical analysis. Dried feed, orts and fecal samples were ground to pass a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA,

Table 1. Ingredient and chemical composition of the total mixed ration (% DM bases) fed to cows (CTRL diet)

Ingredients	g/kg DM
Alfalfa hay	5.07
Alfalfa fresh	25.3
Corn silage	35.5
Corn grain	17.7
Soybean meal	9.50
Sunflower meal	1.27
Wheat straw	2.53
Limestone	0.25
Sodium chloride	0.25
Minerals	0.13
Vitamins	0.06
Protected fats [†]	1.77
Sodium bicarbonate	0.51
Disodium phosphate	0.13
<i>Chemical composition (%)</i>	
Dry matter	95.7
Organic matter	90.5
Crude protein	17.7
Neutral detergent fiber	31.8
Acid detergent fiber	19.4
Cellulose	15.9
Hemicellulose	12.4
Acid detergent lignin	3.5
Neutral detergent insoluble crude protein	2.5
Acid detergent insoluble crude protein	1.4
Ether extract	4.9
Non-structural carbohydrates	36.1
<i>Nutritive value</i>	
Total digestible nutrients [‡] (%)	71.5
Digestible energy [‡] (MJ/kg)	13.6
Metabolizable energy [‡] (MJ/kg)	10.8
Net energy lactation [‡] (MJ/kg)	6.8

[†]Calcium soaps protected fats (Megalac, Volac Int., Orwell, Royston, Hertfordshire, UK).

[‡]Total digestible nutrients (TDN), digestible energy (DE), metabolizable energy (ME) and net energy for lactation (NE_L) were calculated using the NRC (2001) equations.

USA), and analyzed for DM (method 930.15), ash (method 942.05), nitrogen (method 954.01) and ether extract (method 920.39), according to AOAC (1997) official methods. Neutral detergent fiber (NDF) was determined by the procedure of Van Soest *et al.* (1991) without use of alpha-amylase but with sodium sulfite and expressed exclusive of residual ash. Acid detergent fiber (ADF) was analyzed according to AOAC (1997; method 973.18) and expressed exclusive of residual ash. Lignin was analyzed by solubilization of cellulose with sulfuric acid in the ADF residue according to Van Soest *et al.*, (1991). Total nitrogen of NDF and ADF residues were analyzed for neutral (NDICP) and acid (ADICP) detergent insoluble detergent crude protein respectively (Licitra *et al.* 1996). Non-structural carbohydrates, cellulose, hemicelluloses and organic matter were calculated. The nutritive values of the diet expressed as total digestible nutrients (TDN), digestible energy (DE), metabolizable energy (ME) and net energy for lactation (NE_L) were calculated using the NRC (2001) equations.

Sampling and analysis of blood serum

Ten mL blood samples were collected at 10-d intervals 4 h after feeding, from the jugular vein into non-heparinized, silicone coated tubes from 23 cows of each treatment group. Blood samples were centrifuged at $3,000 \times g$ for 20 min within one hour after collection. Serum was separated into 2-mL Eppendorf tubes and frozen at -20°C until analysis. By using specific kits (Stanbio Laboratory, Boerne, Texas, USA) and following manufacturer instructions, blood serum samples were analyzed for of total protein, albumen, urea-N, glucose, total cholesterol and iron concentrations. Globulin concentration was calculated by subtracting albumin values from their corresponding total protein values.

Milk sampling and analyses

Daily and throughout the experiment, cows were milked 3 times daily at 04:00, 12:00 and 20:00 h in a double-20 parallel milking parlor equipped with automatic cow identification, milk recording system, and automated detacher milker units. Samples (100 g/kg of recorded milk yield) were collected at each milking. A mixed sample of milk (proportional to amounts produced in the 3 milking) was collected and analyzed biweekly. Milk samples were analyzed for total solids, fat, protein, and lactose using infrared spectrophotometry (Milko tester Instruments Inc, Bulgaria). Average yields (g/d) of each milk component were calculated for individual cows by multiplying milk yield by the component content (g/kg) of milk. The gross energy content in milk was calculated according to Tyrell and Reid (1965) as:

Milk energy content (MJ/kg) = $4.184 \times [(41.63 \times \text{fat (g/kg)} + 24.13 \times \text{protein (g/kg)} + 21.60 \times \text{lactose (g/kg)} - 117.2) / 10000] \times 2.204$. The milk energy output (MJ/d) was then estimated as milk energy (MJ/kg) \times milk yield (kg/d). Energy-corrected milk (ECM) was calculated according to Sjaunja *et al.*, (1991) as: ECM (kg/d) = milk (kg/d) \times $[38.3 \times \text{fat (g/kg)} + 24.2 \times \text{protein (g/kg)} + 16.54 \times \text{lactose (g/kg)} + 20.7] / 3140$.

Statistical analysis

Feed intake, blood serum metabolites and milk yield and components in the two groups of cows were analyzed as a completely randomized block design for repeated measures, using the general linear models procedure of SAS (2004). The significance level of the tests was $P < 0.05$.

RESULTS AND DISCUSSION*Feed intake*

Feeding YEAST increased ($P=0.04$) feed intake compared to CTRL by about 8% (Table 2). In agreement with this result, Stella *et al.* (2007) and Moallem *et al.* (2009) observed improvement in feed intake in yeast-fed lactating dairy goats and cows. Many factors can affect feed intake including feed palatability, fiber digestion and digesta flow rate (Kholif *et al.*, 2014, 2015). *S. cerevisiae* contains a flavoring effect due to its content of glutamic acid, which apparently enhances the taste of feed (Wallace and Newbold, 1995). However, we did not determine the fermentation kinetics and it is believed that yeast enhanced rumen fermentation (Newbold *et al.*, 1996) and improved fiber digestion causing a decreased rumen fill (Patra, 2012), which apparently improved feed utilization. At least for a short time, live *S. cerevisiae* are metabolically active within the rumen (Kung *et al.*, 1997), affecting ruminal fermentation and stimulating microbial growth (Al Ibrahim *et al.*, 2010).

Blood parameters

Blood metabolites determined in the present study were within the reference ranges reported by Boyd (2011). Addition of YC did not affect serum total protein albumin,

Table 2. Feed intake and blood chemistry of lactating Holstein cows in early lactation fed a diet supplemented with yeast (*Saccharomyces cerevisiae*; 10^{10} cfu/g)

	Diets [†]		SEM	P value
	CTRL	YEAST		
Feed intake (kg/cow/d)	21.7	23.4	0.40	0.040
<i>Serum metabolites</i>				
Total proteins, g/dL	7.94	7.80	0.256	0.412
Albumin, g/dL	4.11	4.09	0.164	0.369
Globulin, g/dL	3.83	3.71	0.190	0.078
Albumin/globulin ratio	1.07	1.10	0.166	0.052
Urea, g/dL	29.8	31.0	1.19	0.473
Glucose, mg/dL	61.6	70.2	1.17	0.002
Total cholesterol, mg/dL	157	136	1.1	0.001
Iron, μ g/dL	211	223	6.2	0.449

[†]The basal diet without (CTRL) or with addition of yeast (YEAST) at 15 g/cow/day.

globulin, albumin/globulin ratio, and urea-N concentration (Table 2). All these metabolites are indicators of the nutritional status of animals, protein status and protein catabolism, as well as kidney function (Hosten, 1990). Besides, similar values between groups of cows indicate that kidney function was not affected by treatments. Yalçın *et al.* (2011) observed unaffected blood total protein, albumin, and urea-N in Holstein cows offered a diet containing yeast.

The inclusion of YC increased ($P=0.002$) serum glucose concentration compared to CTRL. The higher serum glucose may be due to greater propionate production in the rumen (Kholif *et al.*, 2015). This assumption is supported by the increased milk lactose content. Serum glucose concentration depends mainly on energy consumption and utilization by tissues, and organic matter digestibility. As previously mentioned, *S. cerevisiae* increased intake, which could have favored the production of propionate in the rumen; thus, increasing glucose availability from gluconeogenesis (Hassan *et al.*, 2016). Kholif and Khorshed (2006) observed that feeding Egyptian lactating buffaloes on diet supplemented with yeast increased serum glucose concentration compared to the control group.

Serum cholesterol concentration in the yeast-fed cows was lower ($P=0.001$) compared to CTRL. This observation is very important from the nutritional standpoint. Kowalik *et al.* (2012) who observed that heifers fed diets with *S. cerevisiae* had decreased serum cholesterol concentration obtained similar results. Hassan *et al.* (2016) explained that the decreased blood cholesterol concentrations of animals receiving yeast is due to some positive changes in rumen fermentation and population of ruminal bacteria and protozoa with *S. cerevisiae* supplementation. Pysera and Opalka (2001) showed that the change in rumen short chain fatty acids is responsible for the decreased synthesis of cholesterol in the liver. The content of yeast cell wall of β -glucans can be another reason for the reduction of serum cholesterol as β -glucans can reduce this blood metabolite (Nicolosi *et al.*, 1999).

Milk production and composition

Fig. 1 shows daily milk production throughout the 7 weeks of the experimental period. Actual and ECM milk yields, daily yields of total solids, solids-not-fat, protein, and milk energy content and energy output were not affected ($P>0.05$) by the YC in the diet (Table 3). Daily milk fat ($P=0.04$) and lactose ($P=0.028$) yields were increased with the inclusion of YC to the diet. Milk total solids, solids-not-fat, and protein contents were not affected ($P>0.05$) by the addition of the YC, while milk fat ($P=0.027$) and lactose ($P=0.004$) contents increased with the YEAST diet compared to CTRL. Milk efficiency (milk yield/feed intake) and ECM/feed intake was not affected ($P>0.05$) by the addition of the YC (Table 3). Despite the fact that YEAST in the present study increased feed intake, YC did not affect the actual and fat-corrected milk yields. Bagheri *et al.* (2009) reported no effects on milk production when early-lactation Holstein cows were offered diets containing yeast. Increased milk fat and lactose daily yields were observed in cows fed the YEAST diet, as a result of increased milk concentration of fat

Effects of dietary *Saccharomyces* on milk yield

Table 3. Milk production and composition of lactating Holstein cows in early lactation fed a diet supplemented with yeast (*Saccharomyces cerevisiae*; 10^{10} cfu/g)

Attributes	Diets [†]		SEM	P value
	CTRL	YEAST		
<i>Production, kg/d</i>				
Milk	34.8	34.7	0.35	0.915
Energy corrected milk	31.7	32.4	0.64	0.113
Total solids, kg/d	4.12	4.14	0.421	0.747
Solids not fat, kg/d	2.97	2.96	0.303	0.717
Fat, kg/d	1.14	1.17	0.102	0.040
Protein, kg/d	1.11	1.13	0.121	0.085
Lactose, kg/d	1.69	1.73	0.114	0.028
Milk energy content, MJ/kg	2.83	2.91	0.114	0.444
Milk energy output, MJ/d	99	101	1.1	0.142
<i>Milk composition, g/kg</i>				
Total solids	118	119	0.4	0.305
Solids not fat	85.5	85.2	0.26	0.387
Fat	32.9	33.8	0.26	0.027
Protein	31.8	32.7	0.56	0.287
Lactose	48.7	50.0	0.25	0.004
<i>Milk efficiency</i>				
Milk yield/unit feed intake	1.60	1.48	0.25	0.629
ECM/unit feed intake	1.46	1.39	0.27	0.520

[†]The basal diet without (CTRL) or with addition of yeast (YEAST) at 15 g/cow/d.

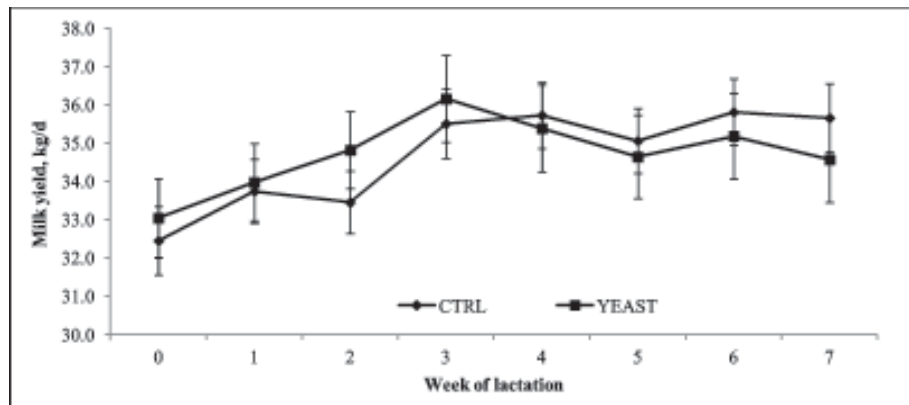


Fig. 1. Milk production of Holstein cows in early lactation fed a diet supplemented with *Saccharomyces cerevisiae*.

and lactose. Increased milk fat concentration may be due to altered rumen fermentation by the yeast, possibly by increasing acetate production in the rumen. Milk fat concentration depends on dietary feed, animal and environmental factors, with the major effect being feed (Nuddaet al., 2014). Giger-Reverdin et al. (1996) reported increased milk fat concentration in animals fed a diet containing yeast. Increased milk lactose content may be due to increased ruminal propionate with the YC. Propionate is the precursor for gluconeogenesis and lactose synthesis, and increasing glucogenic precursors has a favorable effect on milk lactose content. In line with the present results, Moallem et al. (2009) observed a greater lactose concentration in cows fed live yeast compared to control cows.

CONCLUSION

Under the condition of this experiment, the addition of *S. cerevisiae* at 15 g/cow/d increased feed intake, and milk fat and lactose concentration without affecting milk production (actual and energy corrected milk) or milk efficiency. The inclusion of live yeast in diets for lactating Holstein cows early in lactation does not improve milk yield but increased milk components.

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