The Journal of Animal & Plant Sciences, 26(1): 2016, Page: 17-24
ISSN: 1018-7081

PRODUCTIVE RESPONSE AND PROGESTERONE CONCENTRATION IN HOLSTEIN HEIFERS SUPPLEMENTED WITH *SACCHAROMYCES CEREVISIAE*^{1077} OR *SACCHAROMYCES BOULARDII*^{1079}

U. Coronel-Robles[1], M.E. Ortega-Cerrilla[1*], G.D. Mendoza-Martínez[2], P. Zetina-Córdoba[3], M.T.S. Torres-Esqueda[1], G. Munguía-Ameca[1] and M.V. Teco-Jácome[3].

[1]Colegio de Postgraduados, Campus Montecillo, Texcoco. Estado de México, [2]Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana, Campus Xochimilco, D.F. México.
[3]Programa de Ingeniería en Biotecnología. Universidad Politécnica de Huatusco. Veracruz, México
Corresponding author e-mail: meoc@colpos.mx

ABSTRACT

The objective of this study was to determine the effect of supplementating 10 g/animal/day of *Saccharomyces cerevisiae*^{1077} (SC^{1077}) or *Saccharomyces boulardii*^{1079} (SB^{1079}) on dry matter intake (DMI), daily weight gain (DWG), feed efficiency (F:G), *in vivo* digestibility, pH, and rumen ammonia nitrogen (NH_3-N) concentration in rumen liquor, as well as progesterone concentration (P_4). Twenty four, six-month old, Holstein heifers were randomly assigned to three treatments (n=8): (T_1: Control; T_2:10 g/animal/day of SC^{1077}; T_3:10 g/animal/day of SB^{1079}). Samples of rumen liquor were taken at 60, 120 and 180 d of the experiment to determine pH and ammonia nitrogen. Determination of *in vivo* digestibility of DM, NDF, ADF and N was carried out 14 days before the of 60, 120, and 180 d periods. Blood samples were taken to measure P_4 concentration. No differences ($P > 0.05$) were found for DMI, DWG, and F:G from supplementing with *S. cerevisiae*. Similarly, no differences were reported for rumen pH and rumen NH_3-N concentration were not affected ($P > 0.05$), and *in vivo* digestibility of DM, NDF, ADF and N ($P > 0.05$). The P_4 concentration was similar ($P > 0.05$) among the treatments. It is concluded that supplementing Holstein heifers with SC^{1077} and SB^{1079} did not improve heifers performance nor modified the progesterone concentration.

Keywords: *Saccharomyces*, replacement heifers, digestibility.

INTRODUCTION

The incorporation of microbial additives especially yeast in diets of dairy cows, has been used to manipulate rumen fermentation and increase animal production (Bruno *et al*., 2009). Several studies have reported that the addition of *S. cerevisiae* increased DMI and microbial protein synthesis through higher ammonia nitrogen concentration reduction and consequent increase in the population number of viable and total bacteria (Lascano *et al*., 2009b). It also stimulates dry matter intake (Marden *et al*., 2008), has a positive effect on feed digestibility and reduces somatic cell count in milk (Oliveira *et al*., 2010), A higher rumen pH has been reported to increase the production of ruminal ammonia and VFA production (Bach *et al*., 2007). However, contradictory results have been found regarding fiber digestibility, dry matter intake, weight gain, milk production and composition (Mikulec *et al*., 2010), and pH stability (Erasmus *et al*., 2005). Therefore in order to determine if *S. cerevisiae* affects the productive performance and some ruminal variables in Holstein heifers, two strains. of *S. cerevisiae* were supplemented to Holstein heifers to evaluate DMI, F:G, body weight gain, ruminal pH, ruminal ammonia nitrogen concentration, *in vivo* digestibility of DM, NDF, ADF, N, and serum progesterone concentration in plasma.

MATERIALS AND METHODS

Experimental site, animals, and diets: The present study was conducted in the experimental farm of the Universidad Autonoma Chapingo, Mexico. Twenty four six-month old Holstein heifers with a mean initial body weight of 151.42±19.30 kg were used in this experiment. Animals were kept in individual pens with *ad libitum* availability of clean and fresh water. Heifers were randomly distributed into three treatments with n=8 animals per treatment. Diet was formulated according to the recommendations of the NRC (1988) (Table 1). Feed was offered at 07:30 and 14:00 h, corresponding to 3% of live weight for 180 days. Additionally, *Saccharomyces cerevisiae*^{1077} (SC^{1077}), ruminant specific yeast, CNCM I-1077 strain of *S. cerevisiae* (Levucell®, 2.0×10^{10} UFC/g, Lallemand Animal Nutrition, USA) and *Saccharomyces boulardii*^{1079} (SB^{1079}), monograstric or young ruminant specific yeast, CNCM I-1079 strain of *S. cerevisiae*, (Levucell®, 2.0×10^{10} UFC/g, Lallemand Animal Nutrition, USA) were wrapped in tissue paper, covered with molasses and given to each animal twice a day at the same time as the feed. The dietary treatments were:

T_1: Control, T_2: Addition of 10 g/d SC^{1077}, T_3: Addition of 10 g/d SB^{1079}.

Evaluated parameters, sampling, and chemical analysis: Dry matter intake (DMI) was estimated individually every day, through the difference between the offered and rejected diet. To determine daily weight gain (DWG), the animals were weighed at the beginning of the experiment, and then every 15 d. Four heifers were randomly selected from each treatment to collect rumen fluid 4 hours after the morning feeding, using an esophageal tube at 60, 120, and 180 d of the experiment, Ruminal pH of samples was determined using a pH meter (Fisher Accument, Pittsburgh, PA. USA); whereas, rumen ammonia nitrogen (N-NH_3) was measured using the methods described by McCullough (1967).

In vivo digestibility of dry matter (DMD), neutral detergent fiber (NDFD), acid detergent fiber (ADFD), and nitrogen (ND) were calculated using the chromium oxide (Cr_2O_3) technique (Williams *et al*., 1962). Chromium oxide was orally administered, 2 g/animal/day, at 07:00 and 14:00 h (1 g doses), 14 d before the day 60, 120, 180 of experiment. On the last 4 d after administering Cr_2O_3, rectal grab fecal samples were taken according to the methodology proposed by Stock *et al*. (1987). The determination of Cr_2O_3 concentration was performed using an atomic absorption spectrophotometer (Varian model Espectronic AA-10 plus) at 425.4 nm wavelength.

From the 12 heifers selected, blood samples were collected from jugular vein using 5 ml Vacutainer tubes added with EDTA anticoagulant, twice weekly starting at second month till the end of experiment. The blood samples were centrifuged at 3000 rpm for 15 min, plasma was removed and stored at -20ºC until assayed. Plasma was analyzed for progesterone (P_4) concentration using a commercial kit (COAT-ACOUNT) according to the methodology proposed by Srikandakumar *et al*. (1986).

The experimental diet and refusals were analyzed for dry matter (DM), organic matter (OM), ash, and total nitrogen (TN) (AOAC, 1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest *et al*. (1991).

Statistical Analysis: Data on DMD, DWG, F:G, total weight gain (TWG), and P_4 concentration in the blood were analyzed using repeated measure analysis of variance (ANOVA) technique in a completely random design (PROC MIXED of SAS, 2000). For the variables rumen pH, rumen N-NH_3 concentration, DMD, ND, NDFD, and ADFD, a split-plot model was used where the large plot was the treatment and the small plot was the month of sampling (8, 10, and 12 months of age).

RESULTS

Dry matter intake, weight gain, and feed efficiency: In the six months evaluated, DMI showed no differences ($P > 0.05$) among treatments (Table 2). However, there were differences ($P \leq 0.0001$) for time. There was no effect from the interaction of time and the treatments ($P > 0.05$). There were no differences in DWG ($P > 0.05$) due to the supplementation of SC^{1077} and SB^{1079}. Similar results were observed for the time x treatment interaction ($P > 0.05$). There were differences ($P \leq 0.0001$) due to time, however there was no evidence that they were caused by the addition of the strains of *S. cerevisiae*, With regard to F:G, data indicated that there were no differences among treatments ($P > 0.05$), or for time x treatment interaction.

Effect on pH and ruminal NH_3-N: Supplementing growing heifers with SC^{1077} and SB^{1079} did not affect ($P > 0.05$) rumen pH, with regard to the control treatment in the six months of evaluation (Table 3). The same behavior was observed when evaluating the effect of the animal within the treatment in the three different samplings ($P > 0.05$). Ruminal N-NH_3 concentration showed no changes ($P > 0.05$) with both strains of *Saccharomyces* and the control. However, during the different times of samplings in the heifers, there were variations ($P \leq 0.0024$) in the concentration of N-NH_3, at 8, 10, and 12 months of age (11.31, 14.04, 14.84 mg/100 mL, respectively).

***In vivo* digestibility of DM, NDF, ADF, and N:** The addition of *S. cerevisiae* (SC^{1077} and SB^{1079}) to the diets of growing heifers did not improve ($P > 0.05$) DMD with regard to the control (Table 3), at the different sampling periods ($P > 0.05$). However, there was an increase in digestibility through time (73.9, 79.49, 80.46 %) in the control diet, in contrast to both treatments with *S. cerevisiae* strains, where from the eighth to the tenth months DMD increased, but decreased by the twelfth month of age. Regarding NDFD and ADFD (Table 3), no differences ($P > 0.05$) were found among treatments; the same was observed through time (8, 10, and 12 months of age) ($P > 0.05$).

No differences were found ($P > 0.05$) for ND due to treatments, and although in the control increased as the age of the heifers increased (8, 10, 12 months), the results obtained indicate that the treatment*time interaction was not significant ($P > 0.05$) (Table 3). Nevertheless, it was seen a slight increase with both strains of *S. cerevisiae* in ND at 10 months, but it decreased by month 12.

Progesterone (P_4) concentration: Figure 1 shows P_4 concentration for all three treatments. The results indicate that supplementing with two strains of *S. cerevisiae* (SC^{1077} and SB^{1079}) had no effect on this variable with

regard to the control. There were no differences (P > 0.05) for time x treatment interaction. However, there were differences (P ≤ 0.002) among the sampling periods. It is important to mention that in the control treatment, one of the heifers showed estrus on the tenth month of age, another one on the eleventh month and two did not show estrus. In T_2, one heifer showed estrus on the tenth month, two on the eleventh, and the last one showed no estrus. In T_3, two heifers presented estrus on the tenth month, one on the eleventh, and the last one showed no estrus.

Table 1. Ingredients and chemical composition of the experimental diet

Ingredient	(g/kg dry matter)	Chemical composition	(%)
Alfalfa hay	53.89	Dry matter (%)	88.27
Corn Straw	4.89	As % of dry matter	
Soybean meal	19.13	Neutral detergent fiber	43.27
Ground sorghum	11.80	Acid detergent fiber	27.10
Commercial concentrate feed*	9.79	Crude protein (N*6.25)	22.63
Mineral mix	0.50	Ash	5.45

*Crude protein: 11.5%, crude fat: 1.0%, crude fiber: 15.0%, ash: 11.5%, nitrogen free extract: 48.0%, moisture: 13.0%.

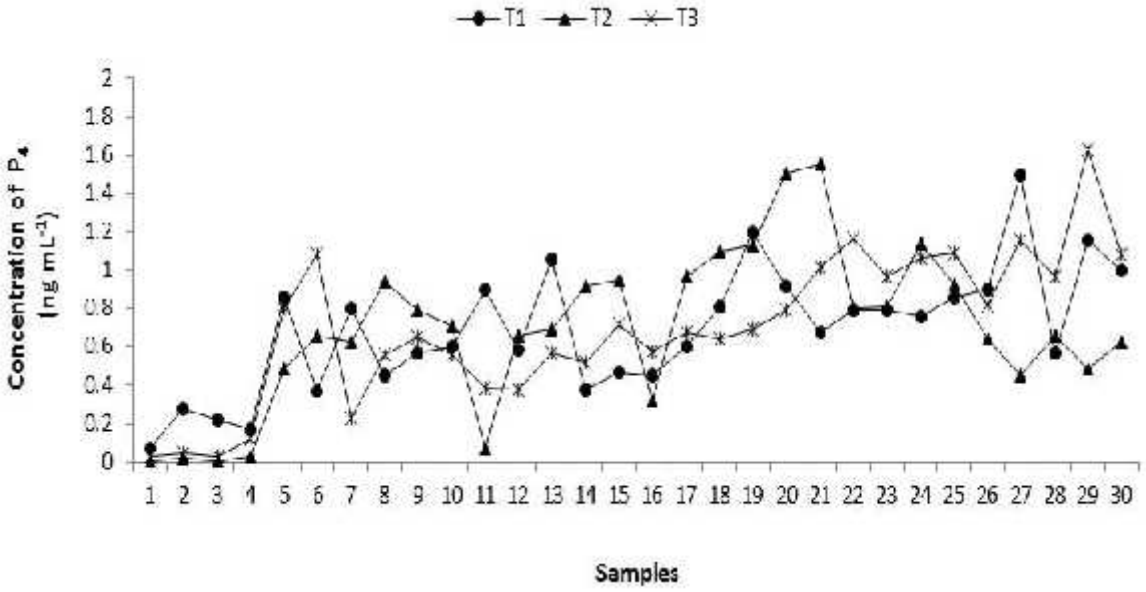

Figure 1. Progesterone (P_4) concentration in heifers supplemented with *S. cerevisiae*. T_1: control, T_2: addition of 10 g/d SC[1077], T_3: addition of 10 g/d SB[1079].

Table 2. Dry matter intake, daily and total body weight gain and feed efficiency **of heifers supplemented with *S. cerevisiae***

		Fortnight													*p-value*		
		1	2	3	4	5	6	7	8	9	10	11	12	SEM	Treat	Time	Treat*time
DMI (kg/d)	T_1	5.72	7.42	7.40	7.51	7.50	8.15	9.99	10.21	9.18	9.94	11.37	11.78				
	T_2	5.81	6.78	7.38	8.61	8.10	9.02	10.24	10.26	10.38	10.99	11.66	11.13				
	T_3	6.61	6.74	7.57	8.43	8.33	8.93	10.08	10.57	11.19	10.93	10.53	10.66	1.91	0.579	0.001	0.285
DWG (kg/d)	T_1	1.06	0.96	0.92	0.77	0.72	0.83	1.34	1.21	1.16	1.09	1.08	1.09				
	T_2	1.09	0.73	0.90	0.94	0.86	1.10	1.26	1.20	1.10	0.94	0.98	1.03				
	T_3	0.88	0.88	0.70	1.05	1.14	1.05	1.05	1.07	1.05	0.81	0.78	0.71	0.14	0.539	0.039	0.265
F:G	T_1	7.71	8.94	9.88	10.05	10.92	11.41	8.25	8.92	8.35	10.72	14.03	14.20				
	T_2	5,41	11.58	8,64	9.34	9.48	9.08	9.38	9.26	9.88	15.27	15.01	13.96				
	T_3	7.81	8.06	13.71	10.02	8.52	8.67	10.99	11.02	11.45	16.96	19.77	22.94	9.24	0.386	0.006	0.716
TWG (kg/d)	$^aT_1$	166.4	180.9	194.8	206.4	217.3	229.9	250.0	268.3	285.7	302.1	318.3	334.7				
	$^bT_2$	160.4	171.3	184.9	199.1	212.1	228.6	247.6	265.7	282.3	296.4	311.2	326.7				
	$^cT_3$	173.0	186.2	196.7	212.6	229.7	245.5	261.3	277.5	293.4	305.6	317.4	328.2	85.86	0.506	0.001	0.292

DMI: Dry matter intake (daily average), DWG: Daily weight gain, F:G: Feed efficiency, TBW: Total body weight. T_1: Control, T_2: with *S. cerevisiae* (SC^{1077}), T_3: with *S. cerevisiae* (SB^{1079}). ^{a,b,c}Initial weight: T_1: 150.5 kg, T_2: 144.0 kg, T_3: 159.7 kg.

Table 3. Rumen pH, NH_3 concentration and *in vivo* digestibility of DM, NDF, ADF, N de NH_3-N in heifers supplemented with *S. cerevisiae.*

Heifers age (months)	**T_1**			**T_2**			**T_3**			**SEM**	*p-value*		
	8	**10**	**12**	**8**	**10**	**12**	**8**	**10**	**12**		**Treat**	**Time**	**Treat* time**
Rumen pH	6.72	6.95	6.80	6.62	6,90	7.10	6.95	7.05	6.97	0.072	0.4142	0.1346	0.3474
NH_3-N (mg/100 mL)	11.39	12.39	13.32	10.60	14.51	15.61	11.95	15.21	15.58	4.78	0.6973	0.0069	0.6301
DMD (%)	73.39	79.49	80.46	79.92	81.36	80.37	75.09	79.97	76.97	19.13	0.1904	0.1333	0.7570
NDFD (%)	70.58	75.12	76.45	74.98	77.18	76.67	73.50	75.42	71.06	24.90	0.4199	0.3535	0.5799
ADFD (%)	64.28	70.20	64.58	70.54	72.84	72.94	64.23	70.41	71.99	41.68	0.1558	0.2347	0.3642
ND (%)	81.58	82.48	83.25	85.27	85.31	83.33	78.77	84.33	82.11	15.55	0.2033	0.3816	0.4263

NH_3-N: Ammonia nitrogen, DMD: Dry matter digestibility, NDF: Neutral detergent fiber digestibility, ADF: Acid detergent fiber digestibility, ND: Nitrogen digestibility. T_1: Control, T_2: with *S. cerevisiae* (SC^{1077}), T_3: with *S. cerevisiae* (SB^{1079}).

DISCUSSION

Dry matter intake, feed conversion, and weight gain: The reports regarding the effects on productive variables due to supplementation to growing heifers and dairy cows with different strains of *S. cerevisiae* have been inconsistent. In this study DMI, DWG, TWG, and F:G showed no differences between the control and the supplementation with SC^{1077} and SB^{1079} on Holstein growing heifers. This agrees with reports from other authors (Hippen *et al*., 2010; Yalcin *et al.*, 2011) using SC (Diamond V XPC®) and SC^{1026}, respectively. Pinos *et al.* (2008) and Ramírez *et al*. (2003), supplemented 1 (SC^{1077} and SB^{1079}), and 5 (SC^{1077} and SB^{1079}) g/anim/d, to Holstein calves and growing heifers, respectively, and found no positive response in DMI, DWG, and FC. They mention that this could be associated with the interaction with the fiber source, the amount of fiber in the diet, and its effects on digestibility, which can increase the rate of passage. Zeljko *et al*. (2010) mention that possibly the composition of the diet, the feeding strategy, the animal health status, and the amount of *S. cerevisiae* given and the number of its viable cells, can influence the response. In this experiment, the diet used was the same for all three treatments, which could explain that the degree of forage, protein availability, or diet composition did not affect the analyzed variables, which makes it possible that the viability of the strains of *S. cerevisiae* used and the amount administered were not adequate.

Rumen fermentation: There were no differences in rumen pH values among treatments. These results agree with data observed by Hippen *et al*. (2010) when using SC (Diamond V XPC®) in Holstein cows, however, this pH values are higher than those reported by Dolezal *et al*. (2005) when supplementing SC^{47} in heifers. On the other hand, Dolezal *et al*. (2011) observed that supplementing Holstein cows with SC^{1077} caused an increase and stabilization of the rumen pH. Nisbet and Martin (1991) and Chaucheyras *et al*. (1996) mention that SC^{1077} can prevent a decrease of pH in the rumen from a lower production of lactic acid due to the increase of rumen microorganisms, especially *Megasphaera elsdenii* or *Selenomonas ruminatum* which use lactate as an energy source. However, the diet used in this study contained 60% forage, which could explain that the action of the yeast was not as efficient as with diets with lower fiber content.

According to the results obtained, supplementation with SC^{1077} and SB^{1079} did not affect the N-NH_3 concentration when compared to the control. This is similar to the results of Guedes *et al*. (2008) and Dolezal *et al*. (2011) when using SC^{1077} in dairy cows, but they differ from the conclusions mentioned by Moallem *et al*. (2009) and Dolezal *et al*. (2005) who observed a decrease of NH_3-N when supplemented SC^{47} to dairy cows. The different results found in these studies are probably due to diverse strains of *S. cerevisiae* used. Small variations, though not significant, in the N-NH_3 concentration were observed 4 h after the feed was given. This could be due to an increase in proteolysis and deamination by the microorganisms (Wallace *et al*., 1992), and although many cellulolytic bacteria require and use NH_3 as their main source of N, high concentrations in the rumen do not always favor an efficient bacterial growth (Lehloenya *et al*., 2008). The modifications in rumen fermentation due to the addition of *S. cerevisiae*, also depend on how many times the feed is offered daily (Enjalbert *et al*., 1999), as it happens when it is done twice a day.

***In vivo* digestibility**. All the diets showed a high DMD (>72.5%). Lascano *et al*. (2009b) report similar values when adding 1g of SC^{1026} kg/DM to diets with a forage-concentrate ratio of 40:60 and 80:20 for growing Holstein heifers. The digestibility of the diet with more concentrate was significantly higher to that of the diet with a greater amount of forage. In this study there was no evidence to suggest that SC^{1077} and SB^{1079} supplementation improves DMD, with a diet with a forage-concentrate ratio of 60:40. This could explain, in part, the null response observed, probably due to that the level of supplementation was not optimal for the strains used (Ramirez *et al*., 2003). Other authors have found similar results in DMD after supplementing SC^{KA500} and SC (Diamond V XPC®) to Holstein dairy cows (Oliveira *et al*., 2010; Schingoethe *et al*., 2004). Fiber digestibility (NDFD and ADFD) was not affected by the addition of the yeast, which agrees with the data of Cabrera *et al*. (2000), when adding 10 g de SC^{1077} to the concentrate supplied to grazing calves. Moallem *et al*. (2009) report a positive effect on fiber digestibility when using SC^{47}, they attribute this effect to an increase of total and cellulolytic bacteria with a relatively stable pH. However, the null response on fiber digestibility, as suggested by Paryad and Rashidi (2009), could be an effect caused by the degree of yeast used, which if it is not optimal, will reduce microbial activity and rumen fermentation. On the other hand, Roa *et al*. (1997) mention that the response of digestibility to the supplementation of *S. cerevisiae* depends on the quality of the forage used in the diet, being higher with good quality forages.

Paryad and Rashidi (2009) suggest that supplementation with *S. cerevisae* can provide factors that stimulate proteolytic bacteria, resulting in an increase in protein digestion. However, in this study, the addition of SC^{1077} and SB^{1079} did not affect ND. This agrees with reports by Lascano *et al*. (2009a) when adding SC^{1026} in Holstein heifers, which could indicate, according to Ramirez *et al*. (2003), that perhaps the strains used were not capable of modifying rumen fermentation.

Progesterone concentration. Only P_4 measurements throughout time were different, reasonable considering that the heifers used in this study were nearing puberty, and the diet covered the requirements for an adequate growth (NRC, 1988). Puberty is regulated by a series of changes in the endocrine system, among these a transitory increase of ovary P_4, which functions as a regulator of endocrine physiological events that release this phenomenon. If feeding is one of the limiting factors for the onset of puberty, underfeeding during pre-puberty affects metabolic pathways to express the genetic potential for reproduction, as well as the adequate weight to reach puberty (Sejrsen and Purup, 1997). On the other hand, increasing nutrient intake in heifers results in high concentrations of the growth factor insulin-I (GF-I) associated with early onset of puberty (Yelich *et al*., 1996), which stimulates and increases production of estradiol and progesterone (Spicer, 1993). If we consider that dry matter intake, weight gain, and digestibility of fiber and N were not affected by the addition of SC^{1077} and SB^{1079}, it is congruent that P_4 concentrations had a similar behavior among treatments. Shriver-Munsch (2011) and Lehloenya *et al*., (2008) report similar results in Holstein cows, not finding differences in P_4 concentration in the plasma due to supplementation with SC (Diamond V XPC®). There are few studies on the effect of supplementing with *S. cerevisiae* on P_4 concentration, and even fewer in growing heifers. According to Kalmus *et al*., (2009), more studies are necessary to determine the influence of supplementation with yeast cultures on reproductive performance.

Conclusions: Supplementation with two strains of *Saccharomyces* (SC^{1077} and SB^{1079}) had no effect on dry matter intake, daily and total weight gain, or feed conversion in growing Holstein heifers. Similar results were observed for rumen pH and N-NH_3 concentration. *In vivo* digestibility of dry matter, neutral detergent fiber, acid detergent fiber, nitrogen and progesterone concentration in plasma was not altered by the addition of these yeasts.

REFERENCES

AOAC (1995). Association of Official Analytical Chemists. Official Methods of Analysis, 15th edition. Arlington, VA, USA.

Bach, A., C. Iglesias and M. Devant (2007). Daily rumen pH pattern of loose-housed dairy cattle as affected by feeding pattern and live yeast supplementation. Anim. Feed Sci. Technol. 136(1-2):146–153.

Bruno R.G.S., H.M. Rutigliano, R.L. Cerri, P.H. Robinson AND J.E.P. Santos (2009). Effect of feeding *Saccharomyces cerevisiae* on performance of dairy cows during summer heat stress. Anim. Feed Sci. Technol. 150:175–186.

Cabrera, E.J.I., M.G.D. Mendoza, I.E. Aranda, C. Garcia-Bojalil, G.R. Bárcena and J.J.A. Ramos (2000). *Saccharomyces cerevisiae* and nitrogenous supplementation in growing steers grazing tropical pastures. Anim. Feed Sci. Technol. 83:49–55.

Chaucheyras, F., G. Fonty, G. Bertin, J.M. Salmon and P. Gouet (1996). Effects of a strain of *Saccharomyces cerevisiae* (Levucell® SC1), a microbial additive for ruminants, on lactate metabolism *in vitro.* Can. J. Microbiol. 42(9), 927–933.

Dolezal, P., J. Doležal and J. Třináctý (2005). The effect of *Saccharomyces cerevisiae* on ruminal fermentation in dairy cows. Czech J. Anim. Sci. 50(11):503–510.

Dolezal, P., J. Dvořáček, J. Doležal, J. Čermáková, L. Zeman and K. Szwedziak (2011). Effect of feeding yeast culture on ruminal fermentation and blood indicators of Holstein dairy cows. Acta Vet. Brno 80(2):139–145.

Enjalbert, F., J.E. Garrett, R. Moncoulon, C. Bayourthe and P. Chicoteau (1999). Effects of yeast culture (*Saccharomyces cerevisiae*) on ruminal digestion in non-lactating dairy cows. Anim. Feed Sci. Technol. 81(3-4):3214–3221.

Erasmus, L.J., P.H. Robinson, A. Ahmadi, R. Hinders and J.E. Garrett (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(2):19–239.

Guedes, C.M., D. Goncalves, M.A.M. Rodrigues and A. Dias da Silva (2008). Effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fibre degradation of maize silages in cows. Anim. Feed Sci. Technol. 145(1-4):27–40.

Hippen, A.R., D.J. Schingoethe, K.F. Kalscheur, P.L. Linke, D.R. Rennich, M.M. Abdelqader and J. Yoon (2010). *Saccharomyces cerevisiae* fermentation product in dairy cow diets containing dried distillers grains plus solubles. J. Dairy Sci. 93(6):2661–2669.

Kalmus, P., T. Orro, A. Waldmann, R. Lindjärv and K. Kask (2009). Effect of yeast culture on milk production and metabolic and reproductive performance of early lactation dairy cows. Acta Vet. Scand. 51(1):32–38.

Lascano, G.J., G.I. Zanton, F.X. Suárez-Mena and A.J. Heinrichs (2009a). Effect of limit feeding high- and low-concentrate diets with *Saccharomyces cerevisiae* on digestibility and on dairy heifer

growth and first-lactation performance. J. Dairy Sci. 92(10):5100–5110.

Lascano, G.J., G.I. Zanton and A.J. Heinrichs (2009b). Concentrate levels and Saccharomyces cerevisiae affect rumen fluid-associated bacteria numbers heifers. Livestock Sci. 126(1-3):189–194.

Lehloenya, K.V., D.R. Stein, D.T. Allen, G.E. Selk, D.A. Jones, M.M. Aleman, T.G. Rehberger, K.J. Mertz and L.J. Spicer (2008). Effects of feeding yeast and propionibacteria to dairy cows on milk yield and components and reproduction. J. Anim. Physiol. Anim. Nutr. 92(2):190–202.

Marden, J.P., C. Julien, U.V. Monteils, E. Auclair, R. Moncoulon and C. Bayourthe (2008). How does live yeasts differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cow?. J. Dairy Sci. 91:3528-3535.

McCullough, H. (1967). The determination of ammonia in whole blood by direct colorimetric method. Clin. Chim. Acta 17(2):297–304.

Mikulec, Ž., T. Mašek, B. Habrun and H, Valpotić (2010). Influence of live yeast cells (*Saccharomyces cerevisiae*) supplementation to the diet of fattening lambs on growth performance and rumen bacterial number. Vet. Arhiv. 80(6):695–703.

Moallem, U., H. Lehrer, L. Livshitz, M. Zachut and S. Yakoby (2009). The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. J. Dairy Sci. 92(1):343–351.

NRC, 1988. Nutrient Requirements of Dairy Cattle. 6th ed. National Research Council. Washington, USA. 159 p.

Nisbet, D.J. and S.A. Martin (1991). Effect of a *Saccharomyces cerevisiae* culture on lactate utilization by the ruminal bacterium *Selenomonas ruminantium*. J. Anim. Sci. 69(11):4628–4633.

Oliveira, B.M.L., L.L. Betancourt, J. R.M. Silva, G.S. Dias Junior, I.C.C. Branco, R.A.N. Pereira and M.N. Pereira (2010). Supplementation of dairy cows with *Saccharomyces cerevisiae* strain KA500. Arq. Bras. Med. Vet. Zootec. 62(5):1174–1182.

Paryad, A. and M. Rashidi (2009). Effect of yeast (*Saccharomyces cerevisiae*) on apparent digestibility and nitrogen retention of tomato pomace in sheep. Pakistan J. Nutr. 8(3):273–278.

Pinos-Rodríguez, J.M., P.H. Robinson, M.E. Ortega, S.L. Berry, G.D. Mendoza and R. Bárcena (2008). Performance and rumen fermentation of dairy calves supplemented with *Saccharomyces cerevisiae*[1077] or *Saccharomyces boulardii*[1079]. Anim. Feed Sci. Technol. 140(3):223–232.

Ramírez, A., M.E. Ortega, S. González, C. Becerril and J. Ayala (2003). Effect of supplementation of two *Saccharomyces cerevisiae* strains on the performance of growing cows. Cuban J. Agric. Res. 37(2):131–137.

Roa, M.L., G.R. Bárcena, M.S. González, M.G.D. Mendoza, C.M.E. Ortega and B.C. García (1997). Effect of fiber source and yeast culture (*Saccharomyces cerevisiae* 1026) on digestion and the environment in the rumen of cattle. Anim. Feed Sci. Technol. 64(2):327–336.

SAS, 2000. SAS; User´s Guide: Statistic. Version 8.1. SAS Institute Inc., Cary, NC, USA.

Schingoethe, D.J., K.N. Linke, K.F. Kalscheur, A.R. Hippen, D.R. Rennich and I. Yoon (2004). Feed efficiency of mid-lactation dairy cows fed yeast culture during summer. J. Dairy Sci. 87(12):4178–4181.

Sejrsen, K. and S. Purup (1997). Influence of prepubertal feeding level on milk yield potential of dairy heifers: a review. J. Anim. Sci. 75(3):828–835.

Shriver-Munsch, C.M. (2011). Effect of feeding various dosages of *Saccharomyces cerevisiae* fermentation product on health, reproduction, and costs in multiparous dairy cows. MSc Diss. Oregon State Univ. 53 p.

Spicer, L.J., E. Apizar and S.E. Echternkamp (1993). Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and or insulin-like growth factor I production *in vitro*. J. Anim. Sci. 71(5):1232–1241.

Srikandakumar, A., R. H. Ingrehan, M. Ellanworth, F. Archbald, A. Liao and R. Gogke (1986). Comparasion of a solid phase, no extraction radioimmunoassay for progesterone with an extraction assay for monitoring luteal function in the mare, bith and cow. Theriogenology 26(6):779–793.

Stock, R.A., D.R. Brink, R.A. Britto, F.K. Goedeken, M.H. Sindt, K.K. Kreikemeir, M.L. Bauer and K.K. Smith (1987). Feeding combinations of high moisture corn and dry rolled grain sorghum to finishing steers. J. Anim. Sci. 65(1):290–302.

Van Soest, P.J., J.B. Robertson and B.A. Lewis (1991). Methods for dietary fiber, neutral detergent fiber and nonstach polysacharides in relation to animal nutrition. Symposium: carbohydrate methodology, metabolism and nutritional implications in dairy cattle. J. Dairy Sci. 74(10):3583–3597.

Wallace, R.J. and C.J. Newbold (1992). Probiotics for ruminants. *In* Probiotics. The Scientific Basis. R.

Fuller, ed. Chapman and Hall, London, England. 317–353.

Williams, C.H., D.J. David and O. Lisma (1962). The determination of chromic oxide in feces samples by atomic absorption spectrophotometry. J. Agric. Sci. 59(3):381–385.

Yalçın, S., S. Yalçın, P. Can, A.O. Gürdal, C. Bağci and Ö. Eltan (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 Australasian J. Anim. Sci.. 24(10):1377–1385.

Yelich, J.V., R.P. Wettemann, T.T. Marston and L.J. Spicer (1996). Luteinizing hormone, growth hormone, insulin-like growth factor-I, insulin and metabolites before puberty in heifers to at two rates. Domest. Anim. Endocrin.. 13(4):325–338.

Željko, M., T. Mašek, B. Habrun and H. Valpotić (2010). Influence of live yeast cells (*Saccharomyces cerevisiae*) supplementation to the diet of fattening lambs on growth performance and rumen bacterial number. Vet. Arhiv. 80(6):695–703.