

Influence of Probiotics on Dairy Cows Diet

V. A. Vieira, M. P. Sforcini, V. Endo, G. C. Magioni, M. D. S. Oliveira

Abstract—The main goal of this paper was evaluate the effect of diets containing different levels of probiotic on performance and milk composition of lactating cows.

Eight Holstein cows were distributed in two 4x4 Latin square. The diets were based on corn silage, concentrate and the treatment (0, 3, 6 or 9 grams of probiotic/animal/day). It was evaluated the dry matter intake of nutrients, milk yield and composition.

The use of probiotics did not affect the nutrient intake ($p>0.05$) neither the daily milk production or corrected to 4% fat ($p>0.05$). However, it was observed that there was a significant fall in milk composition with higher levels of probiotics supplementation.

These results emphasize the need of further studies with different experimental designs or improve the number of Latin square with longer periods of adaptation.

Keywords—Dairy cow, milk composition, probiotics.

I. INTRODUCTION

THE use of living organisms as additives has been used for many years, however, in Brazil, producers search for alternatives to increase production to obtain improve in milk quality.

In the case of dairy cattle, probiotics are commonly used to improve ruminal and intestinal microflora populations in an effort to improve animal performance and health, giving them conditions to improve the synthesis of proteins and vitamins, milk production and quality.

Probiotic is whole food based supplement of live microorganisms, which benefits the host animal by improving its intestinal microbial balance [1].

Typically, they consist mostly of a combination of fungi (e.g. Yeast) and / or rumen and intestinal bacteria and aims to promote a balance of the microbial flora, providing a more efficient digestion of nutrients and then improving the processing of food transformation in milk and meat without these microorganisms are adsorbed and retained in the tissue [2].

To be classified as probiotic supplement, should present some characteristics such as resistance to digestible enzymes and acid stomach pH, being a living culture (bacteria or yeast), ability to maintain their viability after storage and be able to stay in intestinal ecosystem.

The main effect of probiotics is to maintain the rumen pH stable (6 to 7), preventing oscillations caused by changes in feed composition. A reduction in this variation potencializes the rumen's operation.

There was an improvement in fiber digestibility, increased in microbial protein production and optimization in forage utilization with consequently greater weight gain, gain in milk and fertility [3], [4].

Another mechanism that may explain the gains in milk's production with the addition of live yeast is the fact that they stimulate the bacteria's growth which digests cellulose and hemicellulose as *Fibrobacter succinogens* and *Ruminococcus* spp. Furthermore, increasing fiber digestion in the rumen may result in higher consumption of organic matter and consequently increases the milk's production [5].

Therefore, the addition of live yeast to cows that consumed diets rich in fiber may be a very promising strategy to increasing the digestibility and consequently the milk production.

Reference [6] evaluated the effect of yeast supplementation in diets for Jersey cows, primiparous or multiparous, the past 21 days pre-calving until 140 days post-calving on dry matter intake and milk yield and composition. Supplementation with yeast increased dry matter intake in the last seven days of gestation and in the first 42 days of lactation. Supplementation also resulted in less weight loss and less use of body energy reserves for milk production of cows during early lactation.

Although supplemented cows have peaked early lactation, there wasn't increase of total production or changes in milk composition.

Yeast supplementation may be more effective in the transition period at the beginning of lactation and when the animal is more stressed.

Reference [7] supplemented multiparous cows with 10 g / head / day of yeast culture for a period of 16 weeks (mean beginning 80 days post-partum) and found no effect on CMS (16.24 kg against 16). There was an increase in adjusted milk for 4% fat (18.30 against 17.30 kg / day) ($P < 0.05$), but there was no change in the percentage of milk fat.

Reference [8] observed that Holstein cows with an average production of 29 kg of milk / cow / day, supplemented with a diet based on corn silage, citrus pulp and ripe corn finely ground with *Saccharomyces cerevisiae* strain KA500 (10 g / cow / day) resulted in a gain in feed efficiency with the same result in milk's production and lower dry matter intake.

The present investigation was conducted with major objective to study the effect of probiotics on milk production and composition of lactating cows.

II. MATERIALS AND METHODS

The trial was conducted in the Dairy Cattle sector of Faculdade de Ciências Agrárias e Veterinárias da UNESP, Jaboticabal Campus, SP, Brasil.

Vieira, V. A., Sforcini, M. P., Endo, V., Magioni, G. C., and Oliveira, M. D. S. Are with the Universidade Estadual Paulista, Jaboticabal-SP, Brasil (phone: +55 16 3209- 2600; e-mail: vanessaamaro9@ yahoo.com.br, sforcini@hotmail.com, endo_vica@hotmail.com, gabriela.magoni@hotmail.com, mauro@ fcav.unesp.br).

Eight Holstein cows were assigned according to first calving, lactation stage and level of production in two Latin squares. All cows were kept in tie stall with access to water and troughs.

Cows were fed with diets a 60:40 corn silage and commercial concentrate containing 24% crude protein, providing always remains up to 10%. Each cow received 0, 3, 6 and 9 grams / day for probiotics (selenium premix of vitamins and micro-organisms) produced by the company Biosan Chemistry and Biotechnology Ltda. The treatments are identified in Table I.

TABLE I
TREATMENTS IDENTIFICATION

Treatments	Identification
R1	CS* + concentrate
R2	CS + concentrate + 3g probiotic/cow/day
R3	CS + concentrate + 6g probiotic/cow/day
R4	CS + concentrate + 9g probiotic/cow/day

* CS: corn silage.

The experiment consisted in four periods of 15 days each (10 days for adaptation and 5 days for data collection).

The chemical composition of the probiotic is in Table II.

TABLE II
CHEMICAL AND MICROBIOLOGICAL COMPOSITION OF THE PROBIOTIC

Ingredients	Quantity*	Unit
Vitamin A	7.500.000,00	UI/Kg
Vitamin D3	500.000,00	UI/Kg
Vitamin E	3.000,00	UI/Kg
Biotin	5.000	mg/Kg
Selenium	60,00	mg/Kg
BHT	125,00	mg/Kg
<i>Bacillus cereus</i>	7,0 x 10 ¹⁰	UFC/Kg
<i>Bacillus subtilis</i>	7,0 x 10 ¹⁰	UFC/Kg
<i>Lactobacillus acidophilus</i>	7,0 x 10 ¹⁰	UFC/Kg
<i>Bifidobacterium bifidum</i>	7,0 x 10 ¹⁰	UFC/Kg
<i>Enterococcus faecium</i>	7,0 x 10 ¹⁰	UFC/Kg
<i>Saccharomyces cerevisiae</i>	7,0 x 10 ¹⁰	UFC/Kg
<i>Ruminobacter amylophilum</i>	7,0 x 10 ¹⁰	UFC/Kg
<i>Ruminobacter succinogenes</i>	7,0 x 10 ¹⁰	UFC/Kg
<i>Manooglossacarideo</i>	20,80	g/Kg

* Minimum values

Milk production was recorded on the last 5 days of each experimental period by mechanized milking. The animals were milked twice a day, 5am and 3pm.

Milk production was corrected to 4% fat by 4% PL formula $L = (0.4 + 0.15 \times \text{fat in milk}) \times \text{milk production}$ [9].

On the thirteenth day of each experimental period, samples proportional to the yield obtained in both milking were analyzed in the Clinica do Leite / ESALQ laboratory in Piracicaba - SP.

The contents of fat, protein, ether extract, total solids and lactose and nonfat dry extract were determined. Data were subjected to analysis of variance and Tukey test at 5% significance and polynomial regression analysis, using the Agroestat program [10].

III. RESULTS AND DISCUSSION

No differences were observed by using probiotic in the diet of dairy cows ($p > 0.05$) in dry matter intake, crude protein, ether extract, neutral detergent fiber and acid detergent fiber as shown in Table III.

TABLE III
RATINGS OF DRY MATTER INTAKE (DMI), CRUDE PROTEIN (CP), ETHER EXTRACT (EE), DETERGENT FIBER NEUTRAL (NDF) AND ACID DETERGENT FIBER (ADF) IN PERCENTAGE OF DRY MATTER

Parameters	Treatments ¹					F
	R1	R2	R3	R4	CV,%	
DMI, kg/day/cow	14,71 ^a	13,75 ^a	13,67 ^a	14,64 ^a	10,34	1,18 ^{ns}
CP, kg/day/cow	3,06 ^a	2,77 ^a	2,77 ^a	2,90 ^a	11,21	1,45 ^{ns}
EE, kg/day/cow	1,14 ^a	1,08 ^a	1,06 ^a	1,18 ^a	10,67	1,53 ^{ns}
NDF, kg/day/cow	14,46 ^a	13,65 ^a	13,37 ^a	14,69 ^a	10,73	1,40 ^{ns}
ADF, kg/day/cow	6,44 ^a	6,04 ^a	5,90 ^a	6,46 ^a	10,54	1,53 ^{ns}

Means followed by the same letter in the same row, do not differ by Tukey test. Ns = not significant. ¹ R1 = corn silage (CS) + concentrate (C); R2 = C + CS + 3 g of probiotic; R3 = C + CS + probiotic 6 g; R4 = CS + C + 9 g of probiotic.

The dry matter intake was similar at the treatments. May influence show the same ratio of composition of corn silage and concentrate used in the diet. This has been emphasized by [11] and [12], since the varying responses are probiotic, and depend on the offered amount and type of diet consumed by the animal.

The average intake (Table II) protein, ether extract, neutral detergent fiber and acid detergent fiber were similar ($p > 0.05$). In the present work, the cows produced an average production of 17 kg milk / cow / day and received moderate amount of concentrate.

No differences were observed in milk chemical composition by using probiotic in diet, such that mean values were similar (Table IV).

TABLE IV
RATINGS OF THE LEVELS OF FAT, PROTEIN, LACTOSE, SOLIDS TOTAL, SOLIDS NONFAT (ESD), DENSITY, AND COEFFICIENT VARIATION (CV) VALUES OF F AND, ACCORDING TO THE DIFFERENT TREATMENTS

Parameters %	Treatments ¹					F
	R1	R2	R3	R4	CV,%	
Protein	3,05 ^a	3,00 ^a	3,12 ^a	3,05 ^a	5,04	0,73 ^{ns}
Fat	3,16 ^a	3,28 ^a	3,22 ^a	2,85 ^a	11,15	2,38 ^{ns}
Total Solids	11,59 ^a	11,64 ^a	11,51 ^a	1,21 ^a	2,85	2,71 ^{ns}
ESD	8,43 ^a	8,36 ^a	8,29 ^a	8,36 ^a	2,96	0,40 ^{ns}
Lactose	4,43 ^a	4,39 ^a	4,20 ^a	4,34 ^a	8,04	0,66 ^{ns}

¹R1 = corn silage (CS) + concentrate (C); R2 = C + CS + 3 g of probiotics; R3 = C + CS + 6 g of probiotics; R4 = CS + C + 9 g of probiotic. ² PL 4% G = $(0.4 + 0.15 \times \text{fat in milk}) \times \text{milk production}$, according to NRC (1989).

Possibly, in this work conditions, based on the action of probiotics, alteration in rumen's microbial flora, changing patterns of rumen fermentation, increased passage rate of nutrients in the intestine, increasing the digestibility of the diet, there wasn't suitable conditions for the performance of the same.

Among the various factors that affect the response of dairy cows supplemented with probiotic, stand out from the stage of

lactation, type of forage provided, the feeding strategy and forage: concentrate ratio of the diet [13], [14].

In this paper, due to the Latin square design, the cows were used in the post peak lactation, and usually the effect of probiotic occurs in the pre peak, when milk production is higher. Reference [15] found that cows in final lactation producing 19 kg milk / day and supplemented with yeast (*Saccharomyces cerevisiae*), did not respond to microbial additive.

According to [16], increases in milk production ranging between 3-5%, and might reach 6% without statistical significance. However, in this study, possibly the similarity in production was due to the small number of cows used in the experiment, according to the experimental design.

Generally increases in milk production occur with high producing dairy cows, ranging from 4 to 17% or from 0.3 to 2.9 kg / cow / day, showing a better effect in the rumen, particularly in diets with high levels of concentrated and greater dry matter intake. In this study, the cows were of average production (around 17 kg of milk / cow / day) and received moderate amount of concentrate.

Milk composition was changed statistically ($p < 0.05$), whereas the extent that increased the amount of probiotics in the diet (Fig. 1) there was a linear decrease in total solids. The milk of cows R4 treatment showed lower total solids compared to cow milk treatment R1. There was a linear effect on the total solids of the milk of cows of different treatments ($Y = 11.684 - 0.04241667 X$, $R^2 = 0.73$).

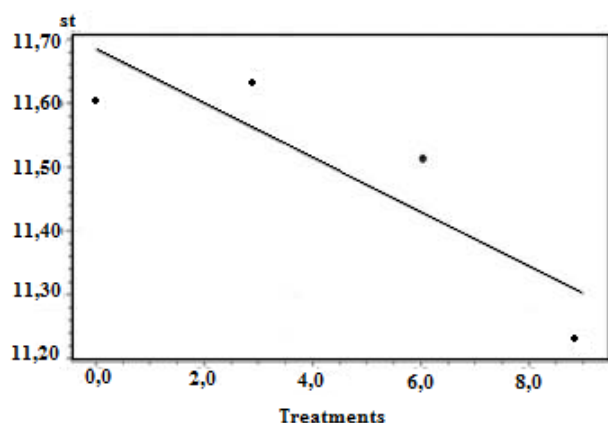


Fig. 1 Linear quantities of probiotic (g / cow / day) in relation to content (%) of total milk solids Regression

Reference [17] found that the use of *Saccharomyces cerevisiae* in the diet of dairy cows did not increase milk production, did not affect the physicochemical characteristics of milk except for the percentage of fat was reduced.

The authors found in relation to ruminal fermentation, probiotic increased the population of protozoa *Holotríquios*, did not affect the physical aspects of rumen fluid, but increased the pH ensuring better conditions for cellulolytic bacteria.

Reference [18] provided 0.3 grams of viable spores of *Bacillus subtilis* C-3102 (3×10^9 cfu / day) for dairy cows and

found no difference in daily milk yield, total solids and milk urea nitrogen ($p > 0.05$).

In this work, statistically, due to the characteristics of the Latin square design, possibly the period of adaptation of cows to receive the treatments was not enough. It might be interesting a new study with longer periods of adaptation and more cows for the Latin squares. This fact is related to the characteristics of a probiotic, or, in the case of colonization because the rumen is a product containing various microorganisms.

IV. CONCLUSION

Based on performed research and presented results it can be concluded that the probiotic supplementation to 9.00 grams/cow/day does not have an influence in milk production and milk production corrected to 4% fat, but decreases the total solids content of milk of Holstein cows with an average production of 17.40 kg / day. We emphasize the need for further studies with different experimental designs or larger number of Latin square and longer periods of adaptation.

REFERENCES

- [1] Newbold, C. J.; Wallace, R. J.; McIntosh, F. M. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. *British Journal of Nutrition*, v.76, p. 249-261, 1996.
- [2] López, J. Probiotics in animal nutrition. *Asian Australasian Journal Animal Science*, v.13, p.12-26, 2000.
- [3] Piva, G.; Belladonna, S.; Fusconi, G. et al. Effects of yeast on dairy cow performance, ruminal fermentation, blood components, and milk manufacturing properties. *Journal of Dairy Science*, v.76, p.2717-2722, 1993.
- [4] Raeth-Knight, M. L. et al. Effect of Direct-Fed Microbials on Performance, Diet Digestibility, and Rumen Characteristics of Holstein Dairy Cows. *Journal Dairy Science*, v.90, p.1802-1809, 2007.
- [5] Bitencourt, L.L., J.R.M. Silva, B.M.L. Oliveira, G.S. Dias JR., F. Lopes, S. Siecola JR., O.F. Zacaroni and M.N. Pereira, 2011. Diet digestibility and performance of dairy cows supplemented with live yeast. *Sci. Agric.* 68:301-307.
- [6] 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.
- [7] Garg, M.R., Siddiqui, M.U., Singh, D.K., Bhandari, B.M. Effect of supplementing YEA SACC-1026 in the ration of Holstein Friesian cows on milk production. *Indian J. Anim. Nutr.*, v.17, n.2, p.175-177, 2000.
- [8] Oliveira, B.M.L.; Bitencourt, L.L.; Silva, J.R.M.; Dias JR, G.S.; Branco, I.C.C.; Pereira, R.A.N.; Pereira, M.N. Suplementação de vacas leiteiras com *Saccharomyces cerevisiae* cepa KA500. *Arquivo Brasileiro Medicina Veterinária e Zootecnia*, v.62,n.5, p. 1174-1182, 2010.
- [9] National Research Council - NRC. Nutrient requirements of the dairy cattle. 6.ed. Washington, D.C., 1989. 158p.
- [10] Barbosa, J.C.; Maldonado JR, W. *AgroEstat Sistema para Análises Estatísticas de Ensaios Agronômicos*, versão 1.0., 2010.
- [11] Wallace, R.J. Ruminal microbiology, biotechnology, and ruminant nutrition: progress and problems. *Journal of Animal Science*, v.72, p.2992-3003, 1994.
- [12] Newbold, C.J. Proposed mechanisms for enzymes as modifiers of ruminal fermentation. Disponível em: <http://www.animal.ufl.edu/dairy/pubs>, capturado em Agosto de 2009.
- [13] Piva, G.; Belladonna, S.; Fusconi, G. et al. Effects of yeast on dairy cow performance, ruminal fermentation, blood components and milk manufacturing properties. *Journal of Dairy Science*, v.76, p.2717-2722, 1993.
- [14] Raeth-Knight, M.L. et al. Effect of Direct-Fed Microbials on Performance, Diet Digestibility and Rumen Characteristics of Holstein Dairy Cows. *Journal Dairy Science*, v.90, p.1802-1809, 2007.

- [15] Santos , F.A.P. et al. Desempenho de vacas em lactação recebendo dietas com diferentes teores de amido total, acrescidas ou não de levedura (*Saccharomyces cerevisiae*). 2006.
- [16] Bernard, J.K. Influence of supplemental yeast on the performance of hosltein cows during early lactation. J. Anim. Sci., v.75, Suppl. 1, p.332, 1992.
- [17] Antunes, A.P.S. Efeito da inclusão de probiótico a base de *Saccharomyces cerevisiae* na dieta de vacas leiteiras sobre o desempenho produtivo, características físico-químicas do leite e parâmetros ruminais. Universidade Estadual de Montes Claros, MG, 45p. Monografia programa de Graduação em Zootecnia, 2006.
- [18] Souza, V.L. Desempenho e utilização de nutrientes por vacas leiteiras suplementadas com *Bacillus subtilis*. Universidade Federal do Paraná, Curitiba, (Dissertação de Mestrado), 63p., 2011.