Journal of Veterinary Medical Research (2022); 29(1): 21-26
https://doi.org/10.21608/jvmr.2022.123351.1051

JVMR

ORIGINAL ARTICLE

Comparing the Efficacy of *Saccharomyces* and *Lactobacillus* Probiotics on Feeding Behavior and Milk Production of Dairy Cattle

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Received: 22 March 2022 I **Accepted:** 11 April 2022 I **Published online:** 13 April 2022

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Abstract

Probiotics are widely used in livestock animals to improve their health and performance. Feeding behavior is an important sign of health and has an influence on animal productivity. Hence, this study was conducted to compare the efficacy of two commonly used probiotics (*Saccharomyces cerevisiae; SAC and Lactobacillus acidophilus; LA*) in dairy livestock on the feeding behavior and milk yield of cows. Thirty Holstein-Friesian cows were equally subdivided into three groups. The control group received no feed supplement, the second group received 25 gm *SAC*/head daily for six weeks and the third group was supplied with 25gm *LA*/head daily for successive six weeks. Then, the feeding behavior was videotaped every two weeks and the milk yield and composition were measured. Ruminal mobility and score, body condition score, and fecal total colony count were calculated. As a result, *SAC* increased the feeding frequency of cows in the sixth week of supplementation. *SAC* increased the milk yield in the fourth and sixth weeks, while *LA* increased the milk yield in the fourth week of supplementation. In the sixth week of supplementation, *LA* decreased the total colony count. Both probiotics did not affect the milk composition, rumen mobility, and score as well as body condition score. The obtained data suggested that the use of *SAC* improved the feeding and milk yield while *LA* increased the milk yield in addition to a reduction of the gut pathogens that enhances the mucosal immunity.

Keywords

Feeding Behavior, *Lactobacillus*, Milk Yield, *Saccharomyces*, Total Colony Count

1. Introduction

Probiotics are widely used in livestock animals for improving their health and production **(Hossain et al., 2017).** A probiotic is a live microorganism that enhances the host' health when it is supplemented in sufficient amounts according to the used strain of microorganism and species **(Hill et al., 2014; Rossoni et al., 2020).** Probiotics modulate the gut environment and metabolic activity by alteration of the microbiota composition **(Uyeno et al., 2015).** The gut's microbiota is considered a biosystem that is included in the physiological activities controlling the immune system development and functioning **(Raabis et al., 2019).** *Saccharomyces cerevisiae (SAC)* and *Lactic acid bacteria (LA)* are the commonly used probiotics in dairy cattle live stocks. *Saccharomyces cerevisiae (SAC)* is typically administered to dairy cows as a live yeast or a yeast culture that contain live and dead cells and the fermentation products or a combination of both to alter the ruminal fermentation that causes nutrient digestion improvement, stabilizing of ruminal pH, and improve immunity and nutrient absorption of mucosa **(Timmerman et al., 2005; Uyeno et al., 2015).** Therefore, probiotics enhance animal performance **(Desnoyers et al., 2009).** *LA (Lactobacillus, Streptococci, bifidobacterial)*, are desirable microflora of the gut **(Tannock, 1997; Morrow et al., 2012)**. These bacteria produce lactic acid-reducing pH to provide an undesirable environment to pathogenic bacteria **(Lambo et al., 2021).** In addition, some probiotic strains produce bacteriocins that maintain intestinal health **(Chiquette, 2009)**. The time spent for eating, and the pattern of meals can have important effects on the total daily intake of dairy cattle **(Grant and Albright, 2000).** Hence, recent studies on the management and nutrition of dairy cows highlighted not only on the feed intake alteration but also on the feeding behavior alterations **(DeVries et al., 2003)**. The effect of *Saccharomyces cerevisiae (SAC)* on the feeding behavior and milk yield of dairy cattle was reported **(Dias et al., 2018; Perdomo et al., 2020).** However, no available published data is comparing the effect of *SAC* and *LA* on the feeding behavior, milk production, and milk composition. Thus, the current study aimed to compare the role of *SAC* and *LA* in improving feeding behavior, milk yield, milk constituents, ruminal score, rumen motility, and body condition score (BCS) of dairy cattle.

2. Materials and Methods

2.1. Experimental Design

A thirty Holstein-Friesian cows were housed in a convenient barn (tie-stall) on a concrete floor in a dairy cattle farm. Cows were fed with a specialized dairy ration for mid-lactation. The ration composition consists of crude protein (CP) of not less than 16%, a crude fat of not less than 2%, a crude fiber (CF) of not more than 15%, crude ash of not more than 12%, and moisture of not more than 12% so that the sum of the digested nutrients (TDN) is not less than 65%. Cows were divided randomly into three equal groups (n=10). Group (1); control group: received a basal diet without feed supplementation; group (2); *SAC* group: received a basal diet plus 25gm *Saccharomyces cerevisiae*; group (3); *LA* group: received a basal diet plus 25gm *Lactobacillus acidophilus* for six weeks. *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* were used in the form of a lyophilized powder. The viable counts were $1x10^7$ CFU/g. Cows were milked twice daily by milking machine after washing and cleaning of udder and bellies.

2.2. Feeding Behavior Observation

Feeding behavior was recorded from outside the stall without causing any disturbance to the animals **(Martin et al., 1993)**. Four cows from each group were videotaped starting ten minutes before feeding and continuous recording for thirty minutes during feeding. Feeding behavior was analyzed by focal observation (the number of animals' visits to a feeder being recorded in frequency and duration) according to **Nielsen (1999)** every two weeks for two consecutive days and for a three hours later to evaluate ruminal motility, rumen scoring, and body condition scoring **(Dias et al., 2018).**

2.3. Measurement of the Milk Yield and Milk Constituents

The milk yield of all cows was recorded twice daily after the milking process for each group to evaluate the effect of administered supplementation on the milk production. In addition, the samples' collections were at zero time and every 2 weeks for six weeks. Individual milk samples were collected (100ml of well-mixed milk samples were obtained from each cow into clean screw-capped tubes) and kept at 4ºC for milk composition analysis (Fat, Protein, Total Solids (TS), Solid Non-Fat (SNF) and Ash percentages) using milkoScan analyzer **(FOSS, Hillerod, Denmark).**

2.4. Body Condition Scoring (BCS)

Evaluation of BCS was estimated by using two scoring systems. The first system depends on a manual palpation of certain animal points such as loins, pelvis, tail head and gives a score from 1-5 according to **Edmonson et al., (1989)**, and the second system depends on animals' lactation stage according to **Manzoor et al., (2018)** as shown in **Table (1).**

2.5. Rumen Score

The rumen score was determined by examining the rumen fill, while the cows were standing on all four legs and without rumen contractions using a visual inspection of the paralumbar fossa and rating from 1 to 5. According to **Zaaijer and Noordhuizen, (2003)** scoring system **(Table 2).**

2.6. Ruminal motility

Ruminal motility was detected by auscultation by placing a stethoscope on the middle third of the left flank region at the time of cows' rumination and counting motility sounds for two minutes **(Constable et al., 1990).**

Table (1). Body condition scoring system.

Score	Palpation indication	Score	Lactation stage indication
1	Extremely thin	2.5-3	One month postpartum
2	Thin	3	Mid lactation
3	Moderate	3-4	Late lactation
4	Fat	3.5	First lactation heifers at calving
5	Very fat	3.5-4	Calving
		3.5-4	Drying off

Table (2). Rumen scoring system

Score	Indication
1	Inside, the transverse processes, there seem to measure a hand's breadth wide.
2	Inside, the transverse processes, there seem to measure a half hand's breadth wide.
3	Measure quarter hand's breadth behind transverse processes.
4	Arches appear immediately beneath transverse processes, directly bulging out and covered with skin.
5	The transverse processes and the 13^{th} rib are not visible and nearly erases by the rumen.

2.7. Fecal Samples

Fresh fecal samples were collected in clean plastic bottles from the cows' rectum and samples were examined at the same time for the total colony count. Standard plate count (SPC) **(APHA 1992; Choudhry et al. 2009).** Firstly, for dilution, one gram of the fecal samples was taken from the examined groups individually in a sterile labeled test tube containing 1mL of normal saline, the samples were thoroughly mixed using a vortex followed by centrifugation at 700x g for 10min. Then the supernatant of each sample was prepared as 10-fold serial dilution and reaching seven dilutions were operated. From each dilution tube, 1mL was poured into a single sterile Petri dish followed by 15mL standard plate count agar. The inoculated plates were incubated at 35ºC for 24-48 h. After incubation, plates containing 25-250 colonies were counted. SPC was calculated according to the following formula.

SPC= Number of colonies/plate X dilution.

2.8. Statistical Analysis

Data analysis was performed using one way ANOVA test. Scores were analyzed using chi-square by SPSS software (SPSS V22, 2013, Ltd., IL, USA). Data are expressed as mean ± SD. The significance of findings was assessed at a p-value < 0.05.

3. Results

Fig. (1), showed the alterations in feeding behavior caused by probiotic treatment throughout the experiment. A significant (P<0.05) increase in the feeding frequency of cows treated by *SAC* with that of control group was observed at the 6th week of treatment. However, the *LA* group showed no significant difference in feeding frequency during the experimental period. In addition, both probiotics induced no significant alterations in feeding duration in relation to control groups. Moreover, A significant (P<0.05) increase in the milk yield of cows treated with SAC at the 4th and 6th week of treatment was noted **(Fig. 2)**. The cow's milk yield was increased at the 4th week of the experiment only significantly (p<0.05) with *LA* **(Fig 2)**. The milk composition of cows treated by both probiotics was not significantly different than control **(Table 3)**. **Table (4)** showed that *SAC* and *LA* did not significantly affect ruminal motility, rumen score, and body condition score. Total colony count was decreased in *LA* (1.8 ± 0.55) group than control (2.55 ± 0.42) at the 6th week of treatment (**Table 4**).

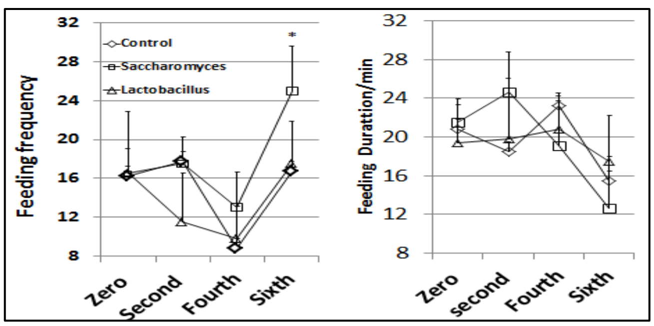

Fig 1. Effect of probiotics supplementation o on feeding behavior of dairy cows. Data presented as Means ± SD (n= 4). *Asterisks on the bars show a significant difference at P<0.05.

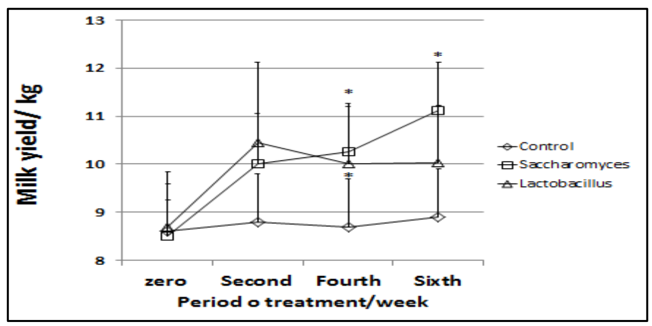

Fig 2. Effect of probiotic supplements on dairy production. Data is available as Means ± SD (n =10). *Asterisk on the bars denotes a statistically significant difference at P<0.05.

Table (3). Effect of probiotics supplementation on cow milk yield and constituents.

Time (Week)	Item	Control	SAC	LA
Zero	Milk yield (Kg)	8.60 ± 0.82^a	8.50 ± 0.75^a	8.70 ± 1.00^a
	Fat %	3.31 ± 0.21^a	3.17 ± 0.13^a	3.24 ± 0.18^a
	Protein%	3.23 ± 0.21^a	3.27 ± 0.16^a	3.18 ± 0.16^a
	Total solids%	11.84 ± 0.33^a	11.86 ± 0.02^a	11.90 ± 0.35^a
	Solids-non-fat%	8.53 ± 0.12^a	8.69 ± 0.11^a	8.67 ± 0.18^a
	Ash	0.74 ± 0.08^a	0.69 ± 0.08^a	0.73 ± 0.08^a
Second	Milk yield (Kg)	8.80 ± 0.94^a	10.00 ± 1.00^a	10.45 ± 1.60^a
	Fat %	3.34 ± 0.28^a	3.06 ± 0.25^a	3.21 ± 0.18^a
	Protein%	3.19 ± 0.13^a	3.32 ± 0.16^a	3.13 ± 0.13^a
	Total solids%	11.97 ± 0.50^a	11.82 ± 0.42^a	11.85 ± 0.40^a
	Solids-non-fat%	8.63 ± 0.23^a	8.76 ± 0.17^a	8.64 ± 0.22^a
	Ash	0.72 ± 0.03^a	0.71 ± 0.04^a	0.75 ± 0.05^a
Fourth	Milk yield (Kg)	8.50 ± 1.00^a	10.25 ± 1.00^b	10.25 ± 1.17^b
	Fat %	3.42 ± 0.36^a	3.23 ± 0.33^a	3.24 ± 0.32^a
	Protein%	3.28 ± 0.13^a	3.24 ± 0.16^a	3.20 ± 0.12^a
	Total solids%	11.98 ± 0.21^a	11.84 ± 0.18^a	11.79 ± 0.14^a
	Solids-non-fat%	8.57 ± 0.15^a	8.62 ± 0.15^a	8.56 ± 0.18^a
	Ash	0.72 ± 0.02^a	0.67 ± 0.06^a	0.69 ± 0.01^a
Sixth	Milk yield (Kg)	8.70 ± 1.10^a	11.40 ± 0.90^b	10.00 ± 1.20^a
	Fat %	3.57 ± 0.44^a	3.11 ± 0.33^a	3.16 ± 0.37^a
	Protein%	3.17 ± 0.16^a	3.31 ± 0.10^a	3.08 ± 0.12^a
	Total solids%	12.07 ± 0.74^a	11.68 ± 0.47^a	11.89 ± 0.48^a
	Solids-non-fat%	8.50 ± 0.30^a	8.58 ± 0.13^a	8.74 ± 0.11^a
	Ash	0.70 ± 0.04^a	0.69 ± 0.06^a	0.66 ± 0.06^a

SAC, Saccharomyces cerevisiae; LA, Lactobacillus acidophilus. Data are represented as mean ± SD.
$^{a, b}$ Means within a row with different letter differ significantly (P≤0.05).

Table (4). Effect of probiotics supplementation on rumen motility, rumen score, body condition score, and fecal total colony count of dairy cows.

	Control	*SAC*	*LA*	Significance
Rumen motility	2.75 ± 1.75	3.50 ± 0.58	4.25 ± 0.56	No significance
Rumen Score	3.50 ± 0.57	3.25 ± 0.2	3.4 ± 0.28	
Body condition Score	3.25 ± 0.26	3.3 ± 0.57	4.25 ± 0.95	
Fecal sample analysis (total colony count X 10^7)	2.55 ± 0.42	2.9 ± 0.82	1.8 ± 0.55	

Data are represented as mean ± SD

4. Discussion

In intensive farming practices, there is an excessive administration of high fermentable carbohydrate supply to enhance the performance and production of animals. This leads to disturbance of rumen microbial balance that results in severe metabolic disorders and production impairment. Hence, the use of probiotics as feed supplements for livestock animals became in great demand.

The results of this study highlighted the marked role of probiotic supplements in the improvement of feeding behavior and milk production of cows.

The obtained data revealed that *SAC* markedly improved the feeding frequency of dairy cows. Similar to this result, **DeVries and Chevaux, (2014)**; **Bach et al., (2018)**; **Dias et al., (2018) and Perdomo et al., (2020)** reported that *SAC* potentially improves dairy cows feeding behavior patterns. The increased feeding may be attributed to the capability of probiotics in improving ruminal microbial fermentation and feed digestibility **(Retta, 2016).**

Our results indicated that the addition of *Saccharomyces cerevisiae* or *Lactobacillus acidophilus* into cows' diet for 4 weeks increased the milk yield significantly ($P<0.05$). While, the prolonged addition of these probiotics into cows' diet for 6 weeks indicated that *Saccharomyces cerevisiae* significantly ($P<0.05$) increased the milk yield than *Lactobacillus acidophilus*, these results were supported by **Zhu et al., (2016)** who found that *SAC* induced a prominent increase in milk yield of cows more than that feed on *LA* at the 4th to 8th week of probiotic administration. The combination of *L. acidophilus*, *L. casei and Enterococcus faecium* improved the milk yield of dairy cattle **(Tesfaye and Hailu, 2019)**. In addition, **Nocek et al., (2003)** found that the milk yield (2.3 kg/day) of cows fed on a combination of probiotics (*Enterococccus faecium* and *S. cerevisiae*) supplied for three weeks prepartum to ten weeks post-partum was markedly increased. Meanwhile, some early studies found that probiotics did not affect the milk production of cows **(Spaniol et al., 2015; Ambriz-Vilchis et al., 2017)**. These variable results may be owing to the kind of probiotics, dose, and lactation period of cows. For example, **Tristant and Moran (2015)** found that *S. cerevisiae* supplementation induced more increase in milk production during the early lactation period than the later stage. The observed increase in milk production may be attributed to the capability of *SAC* in stabilizing rumen fermentation **(Callaway and Martin, 1997)** that led to enhance the fiber-digesting bacteria growth **(Harrison et al., 1988)**, resulting in fiber-digestion and rumen fermentation improvement **(Mao et al., 2013; Retta, 2016)**.

Our findings indicated that both *SAC* and *LA* did not alter milk composition. There are variable effects of probiotics/on milk composition and usually, the effect is expressed as an increase in the percentage of fat **(Chiquette, 2009)**. Similar to our data, **Oetzel et al., (2007)** found no effect of *Enterococccus faecium* and *S. cerevisiae* on milk composition of cows fed on the combination. In addition, **Xu et al., (2017)** revealed that the addition of 50gm/day of probiotics mixture composed of 1.3x109 cfu/g of *Lactobacillus casei* and *Lactobacillus plantarum* didn't affect the milk composition significantly. On the other hand, **Chiquette (1995)** found that the mixture (10g/head/day) of *Saccharomyces cerevisiae* and *Aspergillus oryzae* increased dry matter of milk efficiency. Moreover, **Stein et al., (2006)** found that administration of Propionibacterium ($6x10^{10}$ cfu/cow) had no effect on the milk fat percentage through 25 weeks of lactation.

Our data revealed that *LA* has a decrement effect on total colony count at the 6th week of administration. This result was in agreement with **Peterson et al., (2007)** who observed that *Lactobacillus acidophilus* strains decreased the shedding of *Escherichia coli* O157:H7 in cattle. However, **Beauchemin et al., (2003)** found an increase in the total coliform count due to the high colon acidification that occurred due to lowering minimum pH. The observed decrease in total colony count may be due to the inhibitory effect of lactic acid and hydrogen peroxide produced by *LAC* on enteropathogens **(Fuller, 1977; Ratcliffe et al., 1986).**

The gut and rumen microbiota are complicated environments that are affected by different factors, such as genetics, age, disease, and diet **(Faniyi et al., 2019; Morshedi et al., 2019)**. In addition, probiotic has to be supplemented in adequate amounts to improve the host's health **(Hill et al., 2014; Rossoni et al., 2020)**. Thus, the lack of significant ruminal motility, ruminal and body condition score in our study may be due to insufficient probiotics dose to induce a significant alteration. The variations in the effects of probiotics reported in different studies may be owing to the different design of the included studies, probiotics dose; turnover of ruminal content as well as, production of saliva is usually variable between animals. It is worth noting that *SAC* had marked improvement to feeding and milk production while *LA* induced a more potent decrement effect on enteropathogens. Therefore, further studies are highly required to determine the effect of both probiotics and their mixture on different behavioral patterns for longer periods of supplementation and different doses.

5. Conclusion

Supplementation of *Saccharomyces cerevisiae* for six weeks induced a marked improvement in feeding and milk yield of the cows than *Lactobacillus bacterium*. However, *Lactobacillus bacterium* decreased the fecal total colony count. Both probiotics had no effect on milk composition, ruminal motility, ruminal, and body condition score.

6. Authors Contributions

All authors contributed equally to study design methodology, interpretation of results and preparing of the manuscript.

7. Conflict of Interest

The authors declare no conflict of interest.

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How to cite this article:

Khalil F, Korany AM, Abdel Aziz SA, Emeash HH, Abdelghany AK. Comparing the Efficacy of *Saccharomyces* and *Lactobacillus* Probiotics on Feeding Behavior and Milk Production of Dairy Cattle. J Vet Med Res., 2022; 29(1): 21–26. https://doi.org/10.21608/jvmr.2022.123351.1051