Original Research

Effect of Probiotic and Synbiotic Supplementation on Different Milk Constituents in Sahiwal Cattle

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Rec. Date:	Feb 21, 2018 18:09
Accept Date:	Apr 09, 2018 16:35
DOI	10.5455/ijlr.20180221060958

Abstract

This study was conducted to evaluate the effect of probiotic and synbiotic supplementation on production performance of lactating Sahiwal cattle in arid region of Rajasthan. Eighteen apparently healthy Sahiwal cows in their second month of lactation were randomly assigned to three groups. One group acted as control and were given basal diet only and the cows in other two groups were supplemented with probiotic and synbiotic at the dose of 15g/animal/day and 10g/animal/day, for three months. The supplementation of probiotic and synbiotic supplementation resulted in significantly (P<0.01) higher milk fat percentage (4.62 and 4.54 percent against 3.99 percent) in supplemented cows. Highly significant (P<0.01) increase in the milk lactose percentage (4.92 percent) and total solids (13.35 and 14.25 percent against 12.51) was evident in probiotic supplemented cows. However, milk lactose percentage though higher in synbiotic supplemented group (4.78 percent) compared with control cows (4.75 percent), the difference was not statistically significant. No significant effect of probiotic and synbiotic supplementation was evident on milk protein (3.31 and 3.33 percent against 3.35 percent), and solids not fat (8.73 and 8.70 percent against 8.51) percentage during the course of supplementation. It can be concluded that the supplementation of probiotic and synbiotic at the dose of 15g/animal/day and 10g/animal/day can effectively increase different milk constituent (Milk fat percentage, Milk lactose percentage and total solids) significantly.

Key words: Milk Fat Percentage, Milk Lactose Percentage, Milk Protein, Probiotic, Sahiwal, Solids Not Fat, Synbiotic

How to cite: Gujar, G., Kumar, V., & Goswami, S. (2018). Effect of Probiotic and Synbiotic Supplementation on Different Milk Constituents in Sahiwal Cattle. International Journal of Livestock Research, 8(9), 87-97. doi: 10.5455/ijlr.20180221060958

Introduction

Animal husbandry and dairying along with agriculture forms the backbone of the rural Indian economy. Milk is India's single largest agricultural commodity in value terms and is more than the combined value of paddy and wheat put together. The milk production has increased from 146.3 million tonnes in 2014-15

to 155.5 in 2015-16 registering a growth of 6.27 percent, of which 48 percent is contributed by cattle alone. Sahiwal is one of the established milch cattle breed of tropical regions, with large population concentrated in India and Pakistan. The average lactation yield ranges from 1600-2750 Kg per lactation with average fat percentage ranging from 4.8-5.1(AGRIS-IS). The major bottleneck in exploiting the full potential of animals is the feeding practices adopted in our country and the scarcity of fodder, both qualitative and quantitative further exacerbates the problem of low output. Most of the feed additives used for ruminants are aimed at rumen manipulation mainly to enhance rumen microbial fermentation, predominantly fibre degradation by incorporating such additives which make the ruminal environment favourable for the useful cellulolytic microbes to proliferate and suppress the harmful microbial population. Probiotics are defined as "live micro-organisms, which when administered in adequate amounts confer a health benefit on the host" (FAO/ WHO, 2001). Feeding of probiotics to dairy cows lead to an increase in their milkiness and improve nutrient utilization by the stabilization of bacterial microflora in the rumen (Semeniuk *et al.*, 2008). Synbiotics may be defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract (Gibson and Roberfroid, 1995). The combined action of probiotics and prebiotics on the animal body is much more effective than the probiotics or prebiotics alone (Radzikowski, 2017). The reason behind using a synbiotic is that without its prebiotic food, a true probiotic does not work well in the digestive system. It has been suggested that a combination of a probiotic and a prebiotic, *i.e*. synbiotics, might be more effective than either a probiotic or prebiotic alone (Hamasalim, 2016). So, assuming the above consideration, the present study was planned to evaluate the effect of supplementation of probiotic and synbiotic on production performance of Sahiwal cattle in the arid area of Rajasthan.

Materials and Methods

The present study was conducted to evaluate the effects of probiotic and synbiotic supplementation on the production performance of Sahiwal cattle at the Livestock Research Station, Kodamdesar and laboratories of Rajasthan University of Veterinary and Animal Sciences, Bikaner Rajasthan.

Selection of Animals

Eighteen healthy Sahiwal cows were selected and randomly divided into three groups of six animals in each: Group-I (control group) was given only basal diet without any supplementation and Group-II (treatment 1) and Group-III (treatment 2) were supplemented with probiotic and synbiotic, respectively. All the animals were in their second month of lactation and free from physiological, anatomical and infectious diseases. Animals were housed in well ventilated and protected shed and were allowed to acclimatize for a period of seven day for experimental feeding. The data for various observations were recorded after two weeks of acclimatization where treatments were continued till the end of three month of experiment.

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Experimental Feeding

Each animal in control and treatment groups were offered green fodder *ab lib.* and 2.5 kg concentrate mixture daily. 1 kg extra concentrate mixture was given for every 2.5 kg milk production. The control group (Group-I) cows daily received the basal diet formulated on the guidelines of NRC, 2001 based on pelleted concentrate feed, green maize and wheat straw and the cow in treatment groups were additionally supplemented with probiotic and synbiotic, respectively.

Amount of Probiotic and Synbiotic Feeding

Probiotic

Commercial preparation of probiotic was supplemented at the dose of 15g/animal/day.

Composition *Saccharomyces cerevisiae*	500 Billion cfu (Per 125g)

Synbiotic

Commercial preparation of synbiotic was fed supplemented at the dose of 10g/animal/day.

Composition (Per 125g)	*Saccharomyces cerevisiae*	1000 Billion cfu
	Lactobacillus acidophilus	30000 million cfu
	Lactobacillus sporogenes	30000 million cfu
	Bacillus subtilis	60000 million cfu
	Bacillus licheniformis	60000 million cfu
	Fructo oligosaccharide	5000 mg
	Mannan Oligosaccharide	5000 mg

Composition of Basal Diet

The diets of cows were based on green maize, wheat straw and concentrate mixture as their proximate composition have been presented in Table 1 and same feed and fodder was offered to animals of control and treatment groups.

Table 1: Proximate analysis of feeds given to experimental animals

Nutrients (Per cent)	Wheat Straw	Green Maize	Concentrate Mixture
DM	91.5	27.8	89
CP	3.2	6.54	20
CF	29.82	27.52	10
EE	0.3	1.27	4.31
Ash	12.31	11.82	4.92

Parameters Studied

Production Parameters

Milk Composition: Milk fat, protein, lactose, SNF and total solids.

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Collection of Samples

A total 70 ml of milk from individual animals after each milking was collected weekly in a sterile milk sample collection tube. Milk sample was kept for two hours after milking and then stirred well for minimum 5 minutes by vertical and circular slow movements. The sample so collected was then kept at 4°C till analysis was done.

Preparation of Sample for Analysis

The milk sample was pre warmed at 39-40^0 C before analysis. Then the milk sample was poured slowly from one vessel to another vessel by slightly tilting the vessel and using the side walls of the vessel to avoid formation of foams for the equal distribution of fat within the sample.

Analysis of the Sample

The samples were analyzed, for milk composition i.e. fat, protein, lactose, SNF and total solids, by automatic milk analyzer at Urmul Dairy Bikaner.

Accuracy of Milk Analyzer

The accuracy of milk analyzer was checked by comparing the data obtained by analyzing milk samples by the analyzer for some of the measuring parameters with the corresponding reference method of analysis (Gerber for fat, Kjeldhal for protein etc.).

Statistical Analysis

The properly classified and tabulated data collected during the experimental period were subjected to statistical analysis by adopting appropriate methods of analysis of variance as described by Snedecor and Cochran (1994). Wherever, the variance ratio (F-values), were found significant at 5 per cent and 1per cent level of probability. The significance of mean difference was tested by Duncan's New Multiple range test (Duncan's range test) as modified by Kramer (1957). Statistical analysis was carried out by using SPSS software, version 23.0.

Results and Discussion

Milk Fat Percentage

The overall means of milk fat percentage recorded were 3.99 ± 0.019, 4.619 ± 0.038 and 4.54 ± 0.019 in control, probiotic and synbiotic supplemented groups, respectively (Table 2). The significant (P<0.01) increase (Table 3) in milk fat percentage was seen in treatment groups. The probiotic supplemented group had the highest milk fat percentage followed by synbiotic supplemented and control groups. The milk fat percentage between the supplemented groups was not significantly differing. The increased production of milk fat might be due to the changes in rumen fermentation as a result of feeding probiotics which may lead

 DOI 10.5455/ijlr.20180221060958

to increased supply of glucogenic, aminogenic and lipogenic substrates as reported by Erasmus *et al.* (2005).

Table 2: Mean ± SE of weekly milk fat (%) in control and supplemented cows

	Treatments		
Weeks	**Control**	**Probiotic**	**Synbiotic**
1	4.05 ± 0.108	4.33 ± 0.042	4.29 ± 0.051
2	4.10 ± 0.057	4.38 ± 0.048	4.285 ± 0.071
3	3.92 ± 0.030	4.46 ± 0.034	4.44 ± 0.024
4	4 ± 0.057	4.63 ± 0.066	4.42 ± 0.067
5	4.18 ± 0.060	4.69 ± 0.050	4.52 ± 0.030
6	4.02 ± 0.094	4.77 ± 0.072	4.55 ± 0.020
7	4.03 ± 0.071	4.83 ± 0.047	4.62 ± 0.013
8	3.88 ± 0.070	4.79 ± 0.043	4.66 ± 0.010
9	3.95 ± 0.056	4.73 ± 0.038	4.69 ± 0.013
10	3.96 ± 0.042	4.66 ± 0.032	4.72 ± 0.022
11	3.92 ± 0.060	4.59 ± 0.030	4.73 ± 0.019
12	3.88 ± 0.030	4.55 ± 0.032	4.66 ± 0.020
Overall	3.99^{a} ± 0.019	4.619^{b} ± 0.038	4.54^{b} ± 0.019

Table 3: Analysis of variance of milk fat percentage in control and supplemented cows

Sources	df	MSS	F value
Treatment	2	0.710721	154.20**
Error	15	0.004609	
Total	17		

***(P<0.01)*

Our results are consistent with the findings of Azzaz *et al.* (2015), as they reported an increase in milk fat yield when buffaloes were supplemented with yeast culture (*Saccharomyces cerevisiae*) alone or in combination with *Propionibacterium freudenreichii* strain P169. Further, the studies of El-Din (2015), Yuan *et al*. (2015) and Dehghan-Banadaky *et al.* (2012) also showed similar trends of increase in milk fat percentage with supplementation of probiotics, which concurs with our findings.

Contrary to our findings, the works of Xu *et al.* (2017) and Nocek *et al.* (2011) revealed that probiotic supplementation did not have any effect on milk fat percentage. Further, Rossow *et al.* (2017) and Lehloenya *et al.* (2007) revealed that probiotic supplementation tended to decrease milk fat percentage, which is not compliant with our findings.

Milk Protein Percentage

The overall means for milk protein percentage recorded during the trial period were 3.35 ± 0.036, 3.31 ± 0.033 and 3.33 ± 0.009 for control, probiotic supplemented and synbiotic supplemented groups, respectively (Table 4).

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Table 4: Mean ± SE of weekly milk Protein (%) in control and supplemented cows

	Treatments		
Weeks	**Control**	**Probiotic**	**Synbiotic**
1	3.30 ± 0.0704	3.37 ± 0.048	3.28 ± 0.010
2	3.36 ± 0.051	3.36 ± 0.047	3.30 ± 0.013
3	3.36 ± 0.042	3.33 ± 0.046	3.33 ± 0.009
4	3.36 ± 0.027	3.36 ± 0.042	3.36 ± 0.009
5	3.39 ± 0.035	3.39 ± 0.036	3.41 ± 0.015
6	3.36 ± 0.051	3.36 ± 0.034	3.44 ± 0.013
7	3.39 ± 0.044	3.32 ± 0.033	3.43 ± 0.008
8	3.42 ± 0.037	3.27 ± 0.032	3.38 ± 0.012
9	3.38 ± 0.026	3.25 ± 0.029	3.33 ± 0.013
10	3.34 ± 0.024	3.23 ± 0.026	3.27 ± 0.012
11	3.30 ± 0.018	3.22 ± 0.018	3.23 ± 0.011
12	3.27 ± 0.019	3.22 ± 0.009	3.19 ± 0.008
Overall	3.35 ± 0.036	3.31 ± 0.033	3.33 ± 0.009

It was found that there was non-significant effect (Table 5) of treatment on milk protein percentage as the control and supplemented groups recorded almost similar milk protein percentage during the experiment, with little or no changes over the trial period.

Table 5: Analysis of variance of milk protein percentage in control and supplemented cows

Source	**DF**	**MSS**	**F**
Treatment	2	0.003078	0.612^{NS}
Error	15	0.005023	
Total	17		

NS- Non-significant

Our findings are in agreement with Xu *et al.* (2017), Hagg *et al.* (2012), Peng *et al.* (2011) and Hippen *et al.* (2010) who also found no difference in milk protein percentage following supplementation of probiotics. The work of Rossow *et al.* (2014) revealed decrease in milk protein percentage following probiotic supplementation in cows, contrary to our results. Studies of Rossow *et al.* (2017), Azzaz *et al.* (2015), Bernard (2015), Yuan *et al*. (2015) and Zaworski *et al.* (2014) reported an increase in the milk protein percentage following supplementation of probiotics, which is inconsistent with our results.

Milk Lactose Percentage

The overall means for milk lactose percentage of control, probiotic and synbiotic supplemented cows were 4.75 ± 0.013, 4.92 ± 0.018 and 4.78 ± 0.009, respectively during the experimental period (Table 6). The statistical analysis of milk lactose percentage shown the highly significant (P<0.01) difference (Table 7) between control and probiotic supplemented groups. However, the milk lactose percentage did not differ

DOI 10.5455/ijlr.20180221060958

significantly between control and synbiotic supplemented group and between probiotic and synbiotic supplemented groups.

Table 6: Mean ± SE of weekly milk lactose (%) in control and supplemented cows

	Treatments		
Weeks	**Control**	**Probiotic**	**Synbiotic**
1	4.85 ± 0.057	4.80 ± 0.011	4.79 ± 0.010
2	4.85 ± 0.023	4.86 ± 0.006	4.81 ± 0.014
3	4.85 ± 0.025	4.88 ± 0.004	4.83 ±0.011
4	4.82 ± 0.011	4.91 ± 0.011	4.82 ± 0.016
5	4.82 ± 0.011	4.93 ± 0.006	4.82 ± 0.010
6	4.81 ± 0.018	4.96 ± 0.006	4.82 ± 0.012
7	4.79 ± 0.018	5.09 ± 0.055	4.83 ± 0.015
8	4.75 ± 0.017	5.33 ± 0.084	4.80 ± 0.014
9	4.71 ± 0.016	4.90 ± 0.074	4.75 ± 0.015
10	4.66 ± 0.016	4.83 ± 0.066	4.72 ± 0.013
11	4.60 ± 0.016	4.79 ± 0.063	4.69 ± 0.011
12	4.55 ± 0.016	4.75 ± 0.060	4.66 ± 0.012
Overall	4.75^{a} ± 0.013	4.92^{b} ± 0.018	4.78^{a} ± 0.009

Means bearing different superscripts differ significantly

Table 7: Analysis of variance of milk lactose percentage in control and supplemented cows

Source	DF	MSS	F Value
Treatment	2	0.047436	39.074**
Error	15	0.001214	
Total	17		

**- *Highly significant (P<0.01)*

Rigout *et al*. (2002) reported that increased glucose appearance rates favour increased glucose uptake by the mammary gland and therefore, increased lactose production. This enhanced level of milk lactose may be due to increased propionate production in rumen following supplementation of yeast culture as reported by Miller-Webster *et al*. (2002) which in turn increases glucose-6-P levels in milk (Rigout *et al.*, 2003). Similar to our findings, higher milk lactose percentage on supplementing probiotics were also reported in the works of Azaz *et al.* (2015), Zhang *et al.* (2013) and Peng *et al.* (2011) with the addition of probiotic in diets of animals whereas Maamouri *et al.* (2014), Dehghan-Banadaky *et al.* (2012) and Hagg *et al.* (2012) revealed a non-significant effect of probiotic supplementation on milk lactose percentage.

Milk Total Solids Percentage

Overall means of 14.66 ± 0.025, 14.68 ± 0.030 and 14.67 ± 0.027 for milk total solids percentage were recorded in control, probiotic and synbiotic supplemented groups, respectively (Table 8).

DOI 10.5455/ijlr.20180221060958

Table 8: Mean ± SE of weekly total solids in control and supplemented cow

	Treatments		
Weeks	**Control**	**Probiotic**	**Synbiotic**
1	12.55 ± 0.043	12.99 ± 0.077	12.74 ± 0.043
2	12.61 ± 0.035	13.05 ± 0.043	12.75 ± 0.038
3	12.55 ± 0.036	13.14 ± 0.035	12.98 ± 0.031
4	12.57± 0.032	13.35 ± 0.026	13.01 ± 0.021
5	12.70 ± 0.029	13.4 ± 0.027	13.15 ± 0.028
6	12.58 ± 0.026	13.52 ± 0.023	13.22 ± 0.030
7	12.56 ± 0.022	13.61 ± 0.031	13.34 ± 0.028
8	12.38± 0.019	13.62 ± 0.031	13.44 ± 0.027
9	12.43 ± 0.020	13.54 ± 0.024	13.52 ± 0.026
10	12.43 ± 0.020	13.45 ± 0.022	13.61 ± 0.023
11	12.39± 0.017	13.33 ± 0.019	13.69 ± 0.023
12	12.35 ± 0.017	13.21 ± 0.017	13.59 ± 0.014
Overall	**12.51^{a} ± 0.025**	**13.35^{b} ± 0.030**	**13.25^{b} ± 0.028**

Statistical analysis of milk total solids percentage data revealed (Table 9) highly significant (P<0.01) difference in milk total solids percentage between control and supplemented groups.

Table 9: Analysis of variance of total solids in control and supplemented cows

Source	DF	MSS	F Value
Treatment	2	0.054441	**3.72****
Error	15	0.014621	
Total	17		

***P<0.01*

Over the experimental period, the control and supplemented groups recorded almost equal total solids percentage, with little or no change due to treatments. El-Din (2015), Morsy *et al.* (2014) and Dehghan-Banadaky *et al.* (2012) also reported the non-significant effect of probiotic supplementation on milk total solid percentage. However, the study conducted by Alshaikh *et al.* (2002) revealed a significant increase in milk total solids percentage with yeast cultures supplementation. Zaworski *et al.* (2014) also found increased total solids percentage on *Saccharomyces cerevisiae* supplementation, inconsistent to our result finding.

Milk SNF Percentage

Overall means of milk SNF percentage were 8.51 ± 0.147, 8.73 ± 0.102 and 8.70 ± 0.017 in control, probiotic and synbiotic supplemented groups, respectively (Table 10). The probiotic supplemented group recorded the highest SNF percentage, closely followed by synbiotic supplemented group. Statistical

DOI 10.5455/ijlr.20180221060958

analysis revealed that milk SNF percentage did not differ significantly (Table 11) between control and supplemented groups and also between supplemented groups.

Table 10: Mean ± SE of weekly SNF (%) in control and supplemented cows

	Treatments		
Weeks	**Control**	**Probiotic**	**Synbiotic**
1	8.50 ± 0.181	8.66 ± 0.093	8.45 ± 0.024
2	8.51 ± 0.183	8.67 ± 0.101	8.47 ± 0.025
3	8.60 ± 0.087	8.68 ± 0.087	8.54 ± 0.022
4	8.57 ± 0.168	8.72 ± 0.109	8.59 ± 0.023
5	8.52 ± 0.131	8.71 ± 0.097	8.63 ± 0.021
6	8.56 ±0.154	8.75 ± 0.111	8.67 ±0.017
7	8.53 ± 0.135	8.78 ± 0.113	8.72 ± 0.015
8	8.5 ± 0.132	8.83 ± 0.117	8.78 ± 0.014
9	8.48 ± 0.128	8.81 ±0.118	8.83 ± 0.014
10	8.47 ± 0.123	8.79 ± 0.110	8.89 ± 0.018
11	8.47 ± 0.121	8.74 ± 0.106	8.96 ± 0.029
12	8.47 ± 0.132	8.66 ± 0.078	8.93 ± 0.007
Overall	8.51 ± 0.147	8.73 ± 0.102	8.70 ± 0.017

Table 11: Analysis of variance of SNF in control and supplemented cows

Source	DF	MS	F Value
Treatment	2	0.085078	1.296 ^{NS}
Error	15	0.065622	
Total	17		

NS- Non-significant

El-Din (2015), Maamouri *et al.* (2014), Morsy *et al.* (2014) and Diler *et al.* (2014) also reported findings similar to our results and no effect of probiotic supplementation was evident on milk SNF following probiotics supplementation in animals. Inconsistent with our results, Alshaikh *et al.* (2002) reported a significant increase in the SNF percentage following supplementation of yeast culture to the diets of lactating Holstein cows. Bruno *et al.* (2009) also reported an increase in milk solids not fat percentage post probiotic supplementation, which is contradictory to our findings.

Conclusion

The present study was conducted to evaluate the effect of probiotics and synbiotic supplementation in Sahiwal lactating cows on their milk quality parameters and it was found that the supplementation of these feed additive had the positive effect on milk fat and lactose while other component were invariable. Therefore it is concluded on the basis of present study that supplementation of probiotics and synbiotic as a feed additive will be beneficial to enhance the milk quality in term of their energy content.

DOI 10.5455/ijlr.20180221060958

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