Int.J.Curr.Microbiol.App.Sci (2018) *7*(10): 2332-2340

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 10 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.710.270

Effect of Probiotic and Synbiotic Feeding on Hematological and Biochemical Parameters of Sahiwal Cattle

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Keywords

Biochemical, Hematological, Probiotic, Sahiwal, Synbiotic

Article Info

Accepted:
18 September 2018
Available Online:
10 October 2018

A B S T R A C T

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 was 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. No significant effect of probiotic and synbiotic supplementation was evident on different hematological parameters viz. total leukocyte count of cows and the values of neutrophils, lymphocytes, eosinophils and monocytes were within normal physiological range. The supplementation of probiotic and synbiotic did not have any significant effect on different biochemical parameters serum cholesterol, glucose, calcium and phosphorus levels.

Introduction

Animal husbandry and dairying along with agriculture forms the backbone of the rural Indian economy. 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 per cent, of which 48 per cent 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. A lot of research has been done on the various feed additives for livestock feeding from early years. 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.

The joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) working group defined probiotics as “live micro-organisms, which when administered in adequate amounts confer a health benefit on the host” (FAO/

WHO, 2001). Adding probiotics to feed for dairy cows leads to an increase in their milkiness and to improved nutrient utilization, due to 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 main aim for using a synbiotic is that a true probiotic, without its prebiotic food, does not survive well in the digestive system. So, taking into consideration the above facts, the present study has been planned to evaluate the effect of Probiotic and Synbiotic feeding on hematological and biochemical parameters 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 in arid region of Rajasthan. All the work related to this study was carried out at the Livestock Research Station, Kodamdesar and laboratories of Rajasthan University of Veterinary and Animal Sciences, Bikaner.

Selection of animals

Eighteen healthy Sahiwal cows in their second month of lactation were selected and randomly divided into three groups of six animals each: Group-I was given only basal diet without any supplementation and acted as control group. Group-II and Group-III were supplemented with probiotic and synbiotic, respectively. The data for various observations were recorded after two weeks of acclimatization where treatment groups were fed supplements and continued till the end of third month of experiment. All cows were 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.

Experimental feeding

Each animal in control and supplemented 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 the other two experimental groups were additionally supplemented with probiotic and synbiotic, respectively.

Amount of probiotic and synbiotic feeding

The animal were fed with probiotic and symbiotic as given in Table 1.

Composition of basal diet

The diets of cows were based on green maize, wheat straw and concentrate mixture with proximate principles have been given in Table 2.

The proximate composition of concentrate mixture has been presented in Table 2. Same dry roughage, green and concentrate feed was offered to animals of control and supplemented groups.

Experimental designs

Time period for sample collection

All the experimental animals were randomly assigned to three groups having 6 animals in each group as given in Table 3.

Parameters studied

Blood parameters

Total Leukocyte count and Differential leukocyte count.

Serum parameters

Serum Cholesterol, Glucose, Calcium and Phosphorus.

Collection of samples

To estimate blood parameters, blood samples were collected on the 15th, 30th, 45th, 60th, 75th and 90th day respectively after start of experiment period. Blood samples were collected from Jugular vein using sterile needle. Blood samples for serum analysis was collected in clean sterile blood collection vials and those for blood cell counts were collected in vials containing EDTA.

Analysis of the sample

The samples were brought to the laboratory immediately after collection and blood cell count was carried out. The total leukocyte count was done using a Neubauer chamber/ hemocytometer. Differential leukocyte count was done by preparing a blood smear followed by staining with Geimsa stain and counting the leukocytes under oil immersion lens.

Biochemical analysis

For serum biochemical analysis, the blood samples were collected fortnightly. The blood for serum analysis was collected in clean sterile blood collection tubes and was immediately placed on ice. After completion of sample collection, the samples were centrifuged at 3000 rpm for 15 minutes, the serum so separated was then collected in clean sterile serum tubes and stored in deep freezer till further analysis. The serum samples were subjected to biochemical analysis for estimation of serum glucose, cholesterol, calcium and phosphorus. The serum analysis for these parameters was done by Idexx chemistry analyzer machine using vet test kits supplied by Idexx laboratories, as per procedure outlined by the manufacturers.

Results and Discussion

Serum parameters

The various serum parameters studied and recorded during the experimental period has been presented in the following subsections:

Serum cholesterol

The overall average serum cholesterol levels recorded during the duration of experiment were 77.81 ± 2.302, 78.80 ± 1.591 and 78.89 ± 1.415 in control, probiotic and synbiotic supplemented groups, respectively. In this study, both the supplemented groups had higher cholesterol levels in serum compared to the control cows. But the increase in serum cholesterol level was statistically non-significant as evident from Table 4 and 5.

Our results concur with the findings of Morsy *et al.,* (2014) and Dehghan-Banadaky *et al.,* (2012).

Serum glucose

The overall average serum glucose levels of control, probiotic and synbiotic supplemented groups during the course of experiment were 59.64 ± 1.037, 62.88 ± 2.079 and 62.35 ± 1.457, respectively. The difference in overall average serum glucose levels between control and supplemented groups was non-significant [Table 4 and 5] during the course of this study. Highest serum glucose level (62.88 mg/dl) was recorded in probiotic fed group followed

by synbiotic fed group (62.35mg/dl), while control cows had the lowest (59.64 mg/dl) serum glucose level. But the differences in glucose levels were statistically not significant and the glucose levels were within normal physiological values for all groups.

Similar to our findings were reported by Morsy *et al.,* (2014), El-Din (2015), Yalçın *et al.,* (2011) and Bruno *et al.,* (2009) who also reported no significant effects of probiotic on serum glucose levels.

Serum calcium

The overall average serum calcium levels (Table 4) of control, probiotic and synbiotic supplemented groups during the course of experiment were 9.64 ± 0.385, 9.53 ± 0.335 and 9.34 ± 0.266, respectively. Statistical analysis (Table 5) revealed non-significantly lower values of overall average serum calcium level in both supplemented groups with the synbiotic supplemented group having lowest serum calcium level.

Similar findings were obtained by Kocabagli *et al.,* (2001) and Sretenovic *et al.,* (2008).

Serum phosphorus

The overall average serum phosphorus levels as shown in Table 6 during the experimental period were 5.81 ± 0.288, 5.87 ± 0.304 and 5.78 ± 0.334 in control, probiotic and synbiotic supplemented groups, respectively. Statistical analysis (Table 7) revealed non-significantly higher overall average serum phosphorus levels in both the supplemented groups compared to control groups.

Zaworski *et al.,* (2014) reported a significant increase in serum phosphorus levels of cows fed probiotic supplemented diets.

Table.1 Supplementation of probiotics and synbiotic in experimental animals

Groups	No. of Animals	Treatment	Dose/Animal/day
Control	6	Nil	Control
Supplemented With Probiotic	6	Probiotic	15g/animal/day
Supplemented With Synbiotic	6	Synbiotic	10g/animal/day

Table.2 Proximate analysis of feeds given to experimental animals

Nutrients (Per cent)	Wheat straw	Green maize	Concentrate mixture
DM	91.50	27.80	89
CP	3.20	6.54	20
CF	29.82	27.52	10
EE	0.30	1.27	4.31
Ash	12.31	11.82	4.92

Table.3 To study the effect of supplementation of probiotic and symbiotic on the blood parameters of Sahiwal cow

GROUP 1 (control cows) n = 6	GROUP 2 (supplemented cows) n = 6	GROUP 3 (supplemented cows) n = 6
Basal diet only	Basal diet + Probiotic	Basal diet + Synbiotic

Table.4 Mean (±SE) of fortnightly serum cholesterol (mg/dl), serum glucose and serum calcium in control and supplemented cows

	Serum cholesterol			Serum glucose			Serum calcium		
FORTNIGHTS	CONTROL	PROBIOTIC	SYNBIOTIC	CONTROL	PROBIOTIC	SYNBIOTIC	CONTROL	PROBIOTIC	SYNBIOTIC
1	77.45 ± 2.667	78.15 ± 1.669	78.51 ± 1.420	58.26 ± 1.274	61.92 ± 2.163	61.96 ± 1.610	9.53 ± 1.125	9.52 ± 1.054	9.29 ± 0.754
2	77.31 ± 2.247	78.87 ± 1.619	78.82 ± 1.365	59.20 ± 1.278	62.87 ± 2.017	62.16 ± 1.586	9.45 ± 0.842	9.56 ± 1.031	9.30 ± 0.592
3	77.87 ± 2.260	78.88 ± 1.619	79.34 ± 1.462	59.76 ± 1.315	63.00 ± 2.154	62.48 ± 1.441	9.94 ± 1.076	9.73 ± 0.824	9.34 ± 0.747
4	77.97 ± 2.201	79.02 ± 1.555	78.94 ± 1.335	60.36 ± 1.089	63.09 ± 2.055	62.98 ± 1.439	9.60 ± 0.926	9.53 ± 0.824	9.48 ± 0.609
5	78.41 ± 2.234	79.16 ± 1.622	79.10 ± 1.484	60.19 ± 0.834	63.62 ± 2.089	62.32 ± 1.432	9.66 ± 0.895	9.48 ± 0.800	9.35 ± 0.696
6	77.88 ± 2.302	78.75 ± 1.540	78.62 ± 1.444	60.09 ± 0.832	62.79 ± 2.097	62.18 ± 1.328	9.65 ± 1.049	9.34 ± 0.600	9.29 ± 0.682
OVERALL	77.81 ± 2.302	78.80 ± 1.591	78.89 ± 1.415	59.64 ± 1.037	62.88 ± 2.079	62.35 ± 1.457	9.64 ± 0.385	9.53 ± 0.335	9.34 ± 0.266

Table.5 Analysis of variance of serum cholesterol, serum glucose and serum calcium in control and supplemented cows

Source	DF	MS	F	MS	F	MS	F
Treatment	2	2.149765	NS	18.09032	NS	0.136462	NS
Error	15	19.67114		15.04828		0.665706	
Total	17						

Table.6 Mean (±SE) of fortnightly Serum Phosphorus, Total Leukocyte Count (x10^3)/mm^3 and blood neutrophil counts (x10^3)/mm^3in Control and Supplemented Cow

	Serum phosphorus			Total Leukocyte Count (x10^3)/mm^3			blood neutrophil counts (x10^3)/mm^3		
FORTNIGHTS	CONTROL	PROBIOTIC	SYNBIOTIC	CONTROL	PROBIOTIC	SYNBIOTIC	CONTROL	PROBIOTIC	SYNBIOTIC
1	5.75 ± 0.859	5.82 ± 0.767	5.74 ± 0.847	10.90 ± 1.824	11.35 ± 1.590	9.61 ± 1.862	3.01 ± 0.693	3.68 ± 0.246	3.56 ± 0.598
2	5.75 ± 0.776	5.90 ± 0.740	5.71 ± 0.839	10.57 ± 1.898	11.61 ± 1.832	9.41 ± 1.998	3.16 ± 0.832	3.86 ± 0.266	3.96 ± 0.600
3	5.97 ± 0.698	5.86 ± 0.754	5.82 ± 0.869	10.98 ± 1.579	11.35 ± 1.384	9.30 ± 1.620	3.70 ± 0.180	4.01 ± 0.157	4.05 ± 0.388
4	5.83 ± 0.741	5.91 ± 0.734	5.81 ± 0.804	10.16 ± 1.320	9.83 ± 1.371	9.35 ± 1.254	3.95 ± 0.251	3.93 ± 0.098	3.65 ± 0.076
5	5.81 ± 0.643	5.88 ± 0.751	5.82 ± 0.778	9.88 ± 1.163	10.38 ± 0.823	9.61 ± 0.831	3.40 ± 0.315	3.58 ± 0.190	3.76 ± 0.154
6	5.75 ± 0.606	5.87 ± 0.738	5.77 ± 0.787	9.58 ± 1.103	10.30 ± 0.724	9.91 ± 0.952	3.83 ± 0.280	4.16 ± 0.135	3.73 ± 0.181
OVERALL	5.81 ± 0.288	5.87 ± 0.304	5.78 ± 0.334	10.34 ± 1.460	10.80 ± 1.263	9.53 ± 1.364	3.51 ± 0.388	3.87 ± 0.142	3.78 ± 0.290

Table.7 Analysis of variance of serum phosphorus, total leukocyte count and blood neutrophil counts in control and supplemented cows

Source	DF	MS	F	MS	F	MS	F
Treatment	2	0.014508	NS	2.482758	NS	0.216991	NS
Error	15	0.57633		11.18318		0.511633	
Total	17						

Table.8 Mean (±SE) of fortnightly blood lymphocyte counts (x10^3)/mm^3, blood monocyte counts (x10^3)/mm^3 and blood eosinophil counts (x10^3)/mm^3 in control and supplemented cows

	blood lymphocyte counts (x10^3)/mm^3			blood monocyte counts (x10^3)/mm^3			blood eosinophil counts (x10^3)/mm^3		
FORTNIGHTS	CONTROL	PROBIOTIC	SYNBIOTIC	CONTROL	PROBIOTIC	SYNBIOTIC	CONTROL	PROBIOTIC	SYNBIOTIC
1	3.98 ± 0.486	3.41 ± 0.425	5.33 ± 0.834	0.33 ± 0.066	0.51 ± 0.101	0.51 ± 0.083	1.16 ± 0.230	0.83 ± 0.088	0.71 ± 0.297
2	3.88 ± 1.156	4.51 ± 1.070	5.70 ± 1.814	0.41 ± 0.055	0.56 ± 0.055	0.51 ± 0.074	0.56 ± 0.152	1.68 ± 0.107	0.95 ± 0.172
3	5.56 ± 1.061	6.26 ± 0.835	6.20 ± 0.794	0.45 ± 0.056	0.38 ± 0.065	0.40 ± 0.057	1.10 ± 0.1	0.88 ± 0.208	0.88 ± 0.213
4	5.76 ± 0.412	6.56 ± 0.488	6.70 ± 0.403	0.41 ± 0.060	0.38 ± 0.040	0.36 ± 0.055	0.91 ± 0.079	1.13 ± 0.111	1.03 ± 0.178
5	6.01 ± 0.430	6.66 ± 0.229	6.53 ± 0.343	0.33 ± 0.033	0.38 ± 0.047	0.45 ± 0.076	0.86 ± 0.080	1.05 ± 0.133	0.96 ± 0.088
6	5.11 ± 0.317	6 ± 0.193	6.08 ± 0.222	0.35 ± 0.042	0.41 ± 0.030	0.45 ± 0.022	0.91 ± 0.074	0.98 ± 0.060	1.01 ± 0.087
OVERALL	5.05 ± 0.391	5.57 ± 0.301	6.091 ± 0.391	0.38 ± 0.032	0.44 ± 0.016	0.45 ± 0.032	0.92 ± 0.056	1.09 ± 0.087	0.92 ± 0.152

Table.9 Analysis of variance of blood lymphocyte counts, blood monocyte counts and blood eosinophil counts in control and supplemented cows

Source	DF	MS	F	MS	F	MS	F
Treatment	2	1.610293	NS	0.007917	NS	0.057469	NS
Error	15	0.794892		0.004843		0.068333	
Total	17						

Blood parameters

Total Leukocyte count

The overall means for Total leukocyte count as evident from Table 6 were 10.34 ± 1.460, 10.80 ± 1.263 and 9.53 ± 1.364 in the control, probiotic and synbiotic supplemented groups, respectively. Statistical analysis (Table 7) revealed that the total leukocyte count did not differ significantly between control and supplemented groups and between probiotic and synbiotic supplemented groups.

Similar findings were reported by Agazzi *et al.,* (2014) and Kim *et al.,* (2011).

Differential leukocyte count

The differential leukocyte count of control and supplemented groups has been presented under following sub-sections:

Neutrophil count of blood

The overall average neutrophil counts were 3.51 ± 0.388, 3.87 ± 0.142 and 3.78 ± 0.290 $(x10^3)/mm^3$ in control, probiotic and synbiotic supplemented groups during the duration of this study (Table 6). Statistical analysis shown in Table 7 revealed non-significant difference in the overall average neutrophil counts between control and supplemented groups and between probiotic and synbiotic supplemented groups.

Consistent with our findings, Agazzi *et al.,* (2014) also reported statistically non-significant difference in the values of Neutrophils in probiotic fed cows.

Lymphocyte count of blood

The overall average lymphocyte counts (Table 8) during the experimental period were 5.05 ± 0.391, 5.57 ± 0.301 and 6.091 ± 0.391 in control, probiotic and synbiotic supplemented groups, respectively. The overall average lymphocyte count did not differ significantly (Table 9) between the control and supplemented groups and between the supplemented groups.

Similar results was reported by Agazzi *et al.,* (2014) also reported statistically non-significant difference in the values of Lymphocytes in probiotic fed cows, which concurs with our results.

Monocyte count of blood

The overall average monocyte counts (Table 8) were recorded as 0.38 ± 0.032, 0.44 ± 0.016 and 0.45 ± 0.032 in control, probiotic and synbiotic supplemented groups, respectively.

Statistical analysis (Table 8) revealed non-significant difference in overall average monocyte counts among control and supplemented groups and between both the supplemented groups.

Consistent with this result was reported by Agazzi *et al.,* (2014).

Eosinophil count of blood

The eosinophil counts during the experimental period were 0.92 ± 0.056, 1.09 ± 0.087 and 0.92 ± 0.152 in control, probiotic and synbiotic supplemented groups, respectively (Table 8). Statistical analysis (Table 9) revealed that the difference between overall average eosinophil counts was non-significant between control and supplemented groups and between both the supplemented groups.

However, Contradictory findings were reported by Agazzi *et al.,* (2014) and Ghazanfer *et al.,* (2015).

The supplementation of probiotics and synbiotics did not reveal any significant effects on blood cell counts and serum biochemistry. However, the result revealed non-significant decline in serum calcium it may be due to enhanced milk yield by feeding of probiotic and symbiotic supplements in Sahiwal cattle.

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How to cite this article:

Gujar, G., V. Kumar, S.C. Goswami and Jhirwal, A.K. 2018. Effect of Probiotic and Synbiotic Feeding on Hematological and Biochemical Parameters of Sahiwal Cattle. *Int.J.Curr.Microbiol.App.Sci.* 7(10): 2332-2340. doi: https://doi.org/10.20546/ijcmas.2018.710.270