Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(3): 05-08

Received: 02-03-2020
Accepted: 05-04-2020

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Effect of probiotic (*Saccharomyces cerevisiae*) and prebiotic (*Mannan oligosaccharide*) on Cell-mediated immune response and economics of rearing of *murrah* buffalo calves: Original research paper

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Abstract
The present research work was designed to study the Effect of Probiotic and Prebiotic on Cell-Mediated Immune Response and Economics of Rearing of Murrah Buffalo Calves. Total 18 Murrah buffalo calves with similar body weight of either sex at the age of 15th day were selected and divided into 3 groups randomly and each group contains 6 animals. Group T1 (Control) was fed basal ration, group T2 was fed basal ration with Probiotic (*Saccharomyces cerevisiae*) @4g/animal/day and group T3 was fed basal ration with Prebiotic (*Mannan oligosaccharide*) @4g/animal/day. The duration of experiment was 3 months. The expenditure (#/kg) incurred for milk was 11952, 12096 and 12048 in T1, T2 and T3 groups respectively. The expenditure (#/kg) incurred for calf starter were 299, 276 and 310 in T1, T2 and T3 groups respectively. The expenditure for Probiotic and Prebiotic (#) were 52 and 40 in group T2 and T3 groups respectively. Total expenditure (#/calf) on milk, feed, fodder and feed additives were 12751, 13003 and 12938 in group T1, T2 and T3 groups respectively.

Keywords: Probiotic, prebiotic, murrah buffalo calves, *Saccharomyces cerevisiae*, *Mannan oligosaccharide*, expenditure

Introduction
The intestinal micro biota of calves during the first month of life is extremely unstable [1]. Hence is prone to proliferation by pathogenic microorganisms that frequently lead to gastrointestinal diseases. Antibiotic therapy has been applied to maintain the performance of calves antibiotics in animals feed results in the presence of antibiotic residues in animal products and development of drug-resistant microorganisms in human beings [6]. These demerits of antibiotic therapy have lead to the search for new alternatives. Feed additives as Probiotic (*Saccharomyces cerevisiae*) and Prebiotic *(Mannan oligosaccharide)* are the examples of such alternatives. These are materials that used to enhance the effectiveness of nutrients and exert their effects in the gut or on the gut wall cells to the animal [8].
Probiotic (*Saccharomyces cerevisiae*) are the microorganism which contributes to the intestinal microbial balance. Probiotics have a broad range of beneficial effects such as regulation of intestinal microbial homoeostasis, stabilization of the gastrointestinal barrier function [13], immunomodulatory effects and decrease mortality [7], improving body weight gain (BWG) and feed efficiency [1], protection of young animals against gastrointestinal disorders like diarrhoea [3].
Prebiotics *(Mannan oligosaccharide)* are non-digestible carbohydrates which are not metabolized in the small intestine and fermented in large intestine. Prebiotics *(Mannan oligosaccharide)* are highly effective in times of stress or increased pathogen exposure throughout the calf's lifetime [10], with enhanced immune system [2], and growth performances, decrease faecal coli form count and enhance growth [4], and are found to be most effective in times of stress or increased pathogen exposure throughout the calf's lifetime.

Material and methods
The present experiment on buffalo calves was conducted at Livestock Farm, Adhartal, Department of Livestock Production and Management,

College of Veterinary Science and A.H., N.D.V.S.U., Jabalpur (M.P.). In this experiment 15 days old eighteen buffalo calves were selected and randomly divided into 3 groups with 6 calves in each group as per theTable.1. The calves were housed in a well ventilated calf shed. Individual calf pens with feeding box were provided to the entire experimental animal. All the experimental animals were offered diets as per schedule shown in table.2. The routine of milk feeding was two times a day *i.e* in morning (6.30 am) and evening (5.00 pm). Probiotic & Prebiotic were mixed with milk and offered to the calves daily in the morning up to 3 months of age. Green fodder and calf starter were fed to the all groups of animal with equal opportunity to treatment and control groups. The animals were free access to water throughout the day and night. Calf starter was formulated as per formula depicted by [9], with maize, ground nut cake (GNC), wheat bran, fish meal, mineral mixture, common salt, vitamins (A, B2 &D3) with 22% crude protein (Table.3).

Body weights of all calves were recorded on weekly basis in the morning before feeding with the use of platform type electronic weighing balance. Daily feed consumption was recorded on the basis of measured quantity feed offered and left over. Cell mediated immune response was measured by skin fold response by PHA injected intradermally. The PHA was dissolved in sterile Phosphate Buffer Saline (PBS). 0.1ml of PBS containing 150 µg of PHA was injected intradermally on a base of shaved area of posterior to the scapula using insulin syringes. The skin fold thickness was measure before injection and 2, 6, 12, 24 and 48h after injection using verniercalipper [12]. Economics of production was calculated on the basis of cost of feed and fodder, cost of labour, cost of feed additives (probiotic and prebiotic), body weight gain etc. Data was stated as mean ± standared error. Statistical significance of mean comparisons was calculated using ANOVA test as described by [14]. P value of 0.05 was considered statistically significant.

Result and discussion

Cell-mediated immune response

The results of average difference in skin fold thickness at hrs 0, 2, 6, 12, 24 and 48 hrs after injection of PHA in group T1, T2 and T3 presented in table. 4. Table revealed that there was increase in skin fold thickness in different treatment groups indicated increase in cellular immunity which might be attributed to increase in T lymphocyte stimulation and proliferation or might be due to production of cytokines which mediate different pathway of cellular immunity. The skin test provides a measure of the proliferative response potential of circulating T lymphocytes to an injected mitogen such as PHA. Cell thickness was increase suddenly in all the groups after 2 hr injection of PHA. There was no significant difference between treatments in average skin fold thickness. [12] No significant difference in increase skin fold thickness between probiotic, prebiotic and synbiotic supplemented group.

Economics of rearing of Murrah buffalo calves

Institutional rate of buffalo milk (₹/kg), cost of calf starter and concentrate mixture based on the approved tender rate of feed ingredients at college level, market rate of green fodder and dry roughage, present wages (₹/month) and price of probiotic and prebiotic (₹/kg) were 48.00, 23.00, 2.00, 7500, 144.0 and 110.0, respectively. The expenditure (₹/kg) incurred for milk was 11952, 12096 and 12048 in T1, T2 and T3 groups respectively. The expenditure (₹/kg) incurred for calf starter were 299, 276 and 310 in T1, T2 and T3 groups respectively. The expenditure (₹/kg) incurred for green fodder and dry roughage were 500, 580 and 540 in T1, T2 and T3 groups respectively. The expenditure for probiotic and prebiotic (₹) were 52 and 40 in group T2 and T3 groups respectively. Total expenditure (₹/calf) on milk, feed, fodder and feed additives were 12751, 13003 and 12938 in group T1, T2 and T3 groups respectively. Expenditure on labour/ calf for three months experiment periods @ 1 labour/24 calves and wage ₹7500/month was 937.00 and same for all the groups. Average initial body weights of calves (kg) were 33.33, 33.31 and 33.35 in T1, T2 and T3 groups respectively. Average final body weights of calves (kg) were 51.56, 60.50 and 58.47 in T1, T2 and T3 groups respectively. Average weight gains of calves (kg) were 18.23, 27.19 and 25.12 in T1, T2 and T3 groups respectively. Reduction of recurring expenditure (₹/kg) per kg body gain in comparison to control group was 244 and 206 in T2 and T3 groups. Percent decrease of recurring expenditure per kg body weight gain in comparison to control group 34 and 29.8 in T2 and T3 groups.

Table 1: Grouping of the animal and their treatment

Groups	Number of calves	Treatment
T1	6	Basal diet
T2	6	Basal diet + Probiotic (*Saccharomyces cerevisiae*) @4g per calf/day
T3	6	Basal diet + Prebiotic (*Mannan oligosaccharide*) @4g per calf/day

Table 2: Composition of calf starter

Ingredients (%)	Calf starter
Yellow maize	50%
Groundnut cake	30%
Fish meal	7%
Wheat bran	10%
Mineral mixture	2%
Common salt	1%
Vitamins (A,B &D3)	15gm/ 100 kg feed

Table 3: Feeding schedule of buffalo calves

Age (days)	Colostrum	Milk	Calf starter	Chaffed green
1-3	1/10th of BW in 3 feed	-	-	-
4-7	-	1/10th of BW in 3 feed	-	-
8-15		1/10th of BW	-	-
15-20	-	1/10th of BW	*Ad lib*	*Ad lib*
21-40	-	1/15th of BW	100g	*Ad lib*
41-60	-	1/20th of BW	250g	*Ad lib*
60-90		Milk is gradually reduced	500g	*Ad lib*

Table 4: Effect of Probiotic and Prebiotic on cell-mediated immune response (skin-fold thickness in mm) of Murrah buffalo calves

Hrs	Treatments		
	T_1 (Control)	T_2 (Probiotic)	T_3 (Prebiotic)
0	4.77±0.16	4.81±0.23	4.64±0.36
2nd	8.96±0.22	8.60±0.31	8.69±0.22
6th	7.98±0.21	7.99±0.32	7.97±0.30
12th	5.68±0.29	6.18±0.14	6.56±0.27
24th	4.70±0.14	5.45±0.42	5.41±0.33
48th	4.63±0.14	4.48±0.16	4.18±0.27

Table 5: Effect of Probiotic and Prebiotic on economics of rearing of Murrah buffalo calves

S. No.	Particulars	T1	T2	T3
1	Total milk, feed and fodder consumption per calf during the 3 month experiment			
1.1	Milk consumed (litter)	249	252	251
1.2	Calf starter (kg) consumed	13	15	13.5
1.3	Green fodder and dry roughage (kg) consumed	250	290	270
1.4	Feed additive consumed			
1.4.1	Probiotic (g)		360	
1.4.2	Prebiotic (g)			360
2	Expenditure on milk, feed and fodder per calf during the 3 months experimental period			
2.1	Expenditure on milk (@₹48/litter)	11952	12096	12048
2.2	Expenditure on calf starter (@₹23/kg)	299	276	310.5
2.3	Expenditure on green fodder and dry roughage (@₹2/kg)	500	580	540
2.4	Expenditure on Probiotic (@₹144/kg)		51.84	
2.5	Expenditure on Prebiotic (@₹110/kg)			39.6
	Total expenditure on milk, feed, fodder and feed additives	12751	13003	12938
3	Expenditure on labour/calf for 3 months (@1 labour/24 calves and wage₹7500/month)	937	937	937
4	Miscellaneous expenditure (medicines, deworming, tagging etc.) (@₹40/calf)	40	40	40
5	Total recurring expenditure (₹/kg) during the 3 month experimental period	13728	13980	13915
6	Reduction of total recurring expenditure in comparison to control group	-	252	187
7	Body weight changes during the experimental period	-		
7.1	Average initial weight of calf (kg)	33.33	33.95	33.35
7.2	Average final weight of calf (kg)	51.56	60.50	58.47
7.3	Average weight gain of calf (kg)	18.32	27.19	25.12
8	Recurring expenditure(₹/kg) per kg body weight gain(no.5/no.7.3)	750	514	554
9	Reduction of recurring expenditure (₹/kg) per kg body weight gain in comparison to control group	-	236	196
10	Percent decrease of recurring expenditure per kg body weight gain in comparison to control group	-	31%	26%

Conclusion

It is concluded that there was significant difference ($p<0.5$) found in body weight, average daily gain and feed efficiency due to the different treatment groups. Daily DM intake of *Murrah* buffalo calves higher in different treatments compared to control groups also there was reduction in total recurring expenditure for per kg body weight gain, maximum in group T4(35%) followed by T2(31%),T3(26%) in comparison to T1 group.

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