Indian J. Anim. Nutr. 2020. 37 (3): 206-212
doi: 10.5958/2231-6744.2020.00033.X

Indian Journal of Animal Nutrition

Response of Different Synbiotics on Gut Health, Immunity and Growth Performance of Pre-ruminant Buffalo Calves

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ABSTRACT

The study was conducted to evaluate the response of synbiotics on gut health, immunity and antioxidant status, and growth performance of pre-ruminant Murrah buffalo calves. Twenty-four pre-ruminant Murrah buffalo calves (5-10 d old) having similar body weight (34 to 37 kg), were randomly divided into four groups of six animals in each group. Calves of the control (CON) group were fed basal diet (milk, calf starter and green maize) without any supplementation. Basal diet of the calves were supplemented with 3 g inulin+*L. plantarum* CRD-7 as fermented milk (150 ml) having 10^8 CFU/ml/calf/day, 6 g inulin+ *L. plantarum CRD-7* as fermented milk (100 ml) having 10^8 CFU/ml/calf/day, and 9 g inulin+*L. plantarum* CRD-7 as fermented milk (50 ml) having 10^8 CFU/mL/calf/day in groups, SYN1, SYN2 and SYN3, respectively. A digestibility trial of five days was carried out after 56 day of experimental feeding. The final body weight (kg), average daily gain (g) and chest girth were higher (P<0.05) in SYN3 as compared to control. Crude protein and dry matter digestibility were higher (P<0.05) in SYN3 group than CON group, while digestibility of other nutrients was similar among the groups. Fecal *Lactobacilli* and *Bifidobacterium* count was increased (P<0.05) and coliform count was decreased (P<0.05) in SYN3 groups than CON group. Cell mediated immune response and fecal score was higher (P<0.001) in SYN3 than CON group. It was concluded that supplementation of 9 g inulin with 50 g *L. plantarum* CRD-7 as fermented milk (50 ml) having 10^8 CFU/mL/calf/day to pre-ruminant calves will improve immunity and growth performance.

Key words: Synbiotics, Pre-ruminant calves, Gut health, Performance

INTRODUCTION

Gastrointestinal tract (GIT) of newly born calves is sterile at the time of birth; microbes are introduced from the surrounding environment and also from the dam's birth canal to colonize the tract of the newborns (Ewaschuk *et al.,* 2004). The gastrointestinal microbial population in newborn calves is in extremely sensitive, hence, sudden changes in diet and/ or environment, disease or other stress can cause alteration in this microbial system (Krehbiel *et al.,* 2003) that may lead to diarrhoea. Neonatal diarrhoea is the major cause of death in pre-ruminant life of calves and contributes >50% of calf's death in dairy sector (Uetake, 2013). Health and growth of calf depends mostly upon establishment of GI microbiota within first 7 weeks of life (Oikonomou *et al.,* 2013). To address the issues of diarrhoea, dietary interventions of prebiotic and probiotic have been found to be effective through modulation of gut microbiota or by improving the immunity status (Sharma *et al.,* 2018). Probiotics are "microorganisms that generate small molecular metabolic byproducts that exert beneficial regulatory effect on host biological function and may function as immunomodulators" (Hickey *et al.,* 2012). Synbiotic is a combination of prebiotic and probiotic that might be more effective than either probiotic or prebiotic alone (Panesar *et al.,* 2009). Even though many synbiotics have been tested (Fuller, 1989; Masucci *et al.,* 2011) and recommended, efficacy of the same is not always consistent (Sharma *et al.,* 2018; Zhang *et al.,* 2019). Furthermore, research on synbiotics in buffalo calves are few and far between (Shehta *et al.,* 2019; Sharma *et al.,* 2018). The study was conducted to find out a suitable synbiotics that would improve gut health, immunity, antioxidant status, and growth performance of pre-ruminant Murrah buffalo calves.

MATERIALS AND METHODS

Twenty four pre-ruminant Murrah buffalo calves (5-10 d old) having similar body weight (34 to 37 kg; avg- 35 kg), were randomly assigned into four groups (CON, SYN1, SYN2 and SYN3) with six animals in

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each group. Calves of the control (CON) group were fed basal diet (milk, calf starter and green maize) without any supplementation. Basal diet of the calves were supplemented with 3 g inulin+*L. plantarum* CRD-7 as fermented milk (150 ml) having 10^8 CFU/ml/calf/day, 6 g inulin+ *L. plantarum CRD-7* as fermented milk (100 ml) having 10^8 CFU/ml/calf/day, and 9 g inulin+*L. plantarum* CRD-7 as fermented milk (50 ml) having 10^8 CFU/mL/calf/day in groups, SYN1, SYN2 and SYN3, respectively. A digestibility trial of five days collection period was carried out after 56 day of experimental feeding. Calves were housed in well-ventilated pens and arrangement were made to protect them from cool environment. The calf pens the adjoining open space were cleaned twice a day.

A freeze-dried pure culture of *Lactobacillus plantraum* CRD-7 strain was procured from the Synbiotic Functional Foods Laboratory, Dairy Microbiology Division, National Dairy Research Institute, Karnal for preparation of probiotic. Freeze-dried pure culture of *L. plantraum CRD-7* was activated in De Man, Rogosa and Sharpe agar (MRS) broth that contained 10^8cfu/ml, then one loop of inoculum from this broth use to ferment milk (Dahi). The feeding of milk was carried out twice a day. Whole milk was fed to the calves at $1/10^{th}$ of BW up to two weeks, $1/15^{th}$ of BW third and fourth week, $1/20^{th}$ of BW up to eight weeks of experiment. Calf starter was offered from first week onwards. All the calves were fed concentrate and green fodder (berseem) *ad libitum*. Calf starter consisted of maize grain (31%), oats grain (8%), wheat bran (24%), expeller GNC (2%), mineral mix (2%) and salt (1%). All the calves had 24h access to *ad libitum* fresh water.

Milk samples were analyzed of total solid (TS), fat, protein, lactose, solid not fat (SNF) using an autoanalyzer (Lactoscan, Bulgaria). Proximate composition of calf starter and berseem, residues and samples (feed, orts, faeces) collected during digestion trial estimated methods described by AOAC (2005). Neutral detergent and acid detergent fibre (NDF, ADF) were measured according to the method of Van Soest *et al.* (1991).

Body weight of the calves was on the first day of experiment and then at weekly interval. Skeletal measurements were monitored by measuring body height, hip-height, heart girth and length using measuring tape. Dry matter intake was recorded daily.A digestion trial of 5-day collection period was conducted after 56 days of experimental feeding. For estimation of fecal pH, ammonia, lactic acid, VFA and microbial population (lactobacillus, *Bifidobacterium* and coliform), fecal samples (10-12 g) were collected by manual stimulation from rectum on day 0, 30, and 60. After sample seeding, petri dishes of *Lactobacillus* and coliform were incubated at 38°C for 24 h, those of *Bifidobacterium* and *Clostridium* were incubated in an anaerobic jar. After incubation colony forming unit were counted with the help of colony counter and then expressed in the form of $\log_{10}$cfu/g. Faecal consistency score in calves was recorded every day throughout the experimental period by adopting standard protocol (Larson *et al.,* 1977). Blood sample were collected from jugular vein from each calves on day 0, 30 and 60 in serum collection vacutainer, brought to laboratory, centrifuged at 4000 rpm for 15 min at 4°C to harvest serum, which were collected into small Eppendorf vials (2 ml) and stored at -20°C till further analysis Antioxidant activity was estimated in RBC hemolysate prepared from freshly collected blood. SOD was estimated as per the method described by Madesh and Balasubramanian (1998). Catalase enzyme activity was estimated by spectrophotometric (Specord 200) method of Aebi (1984). Serum concentration of IgG and IgA were measured by using Enzyme-Linked Immuno-sorbent Assay (ELISA) kit provided by Bioassay Technology Laboratory. The humoral immune response was performed by challenging the animal on day 30 with single dose of chicken erythrocyte in 10% phosphate buffer saline (PBS) by intravascular administration at the dose rate of 1 ml/animal. The blood samples were collected before inoculation (0 d) and thereafter at 7, 14- and 21-days post challenge for estimation of antibody response. About 2 ml of blood was collected in sterile serum collection vacationers and the harvested serum was carefully transferred to the clean labeled

plastic vials and stored in deep freeze (–20°C) till further analysis using hemagglutination (HA) test. Cell mediated immune (CMI) response was assessed at the end of the experiment by means of a skin test based on non-specific delayed type hypersensitivity (DTH) reaction against phytohaemaglutinin-P (PHA-P) as described by Masucci *et al.*(2011). The data obtained were analysed using the statistical software program of SPSS (SPSS, Chicago, llinois, USA). Treatments were compared with the Tukey test. Difference between groups were considered significant when P values were <0.05.

RESULTS AND DISCUSSION

The initial body weight was similar in all the groups, and final body weight at eighth week was higher in (P<0.05) in SYN3 as compared to control group, while CON, SYN 1 and SYN2 had similar weight (Table 1). There was improvement in average daily gain (P<0.05) on supplementation of all the three synbiotic formulations, however, the best response was obtained with SYN3. Zhang *et al.,* (2019) also observed improvement in average daily gain in Holstein calves due to *Lactobacillus rhamnosus* GG during pre-weaning stage. Roodposhti and Dabiri (2012) showed that addition of probiotics, prebiotics and combination of both as synbiotic had consistently improved average daily gain as compare to probiotic alone and control. Howver, Monter *et al.,* (2019) observed no change in body weight gain in lamb due Ito inulin and *Lactobacillus casei* supplementation. Intake and digestibility of nutrients were similar among the groups except DM and CP which was higher (P<0.05) in SYN3 group as compared to others.

There was no effect (P>I0.05) of synbiotic formulation on morphometry (height and length) of pre-ruminant buffalo calves (Table 2) except heart girth (P<0.02). Body length, height at withers and hip are the parameters of bone growth while chest girth is measure of development of muscles, bones and fat. It has a direct relationship with the live weight. Stefanska *et al.* (2018) supplemented *Yarrowia Ilipolytica* culture to dairy calves and found improvement growth performance of calves along with morphomety. Similar to our results, Gupta *et al.* (2015) reported that probiotic supplementation had a significant effect on morphometry of crossbreed caves. Riddell *et al.* (2010),

Table 1. Growth performance, nutrient intake and nutrient utilization of pre-ruminant buffalo calves

Parameters	Dietary groups				P-value
	CON	SYN1	SYN2	SYN3	
Nutrient intake and growth performance					
Dry matter intake (g/day)	1009.1±20.5	1012.5±25.7	991.8±24.8	1023.6±19.8	0.71
Protein intake (g/day)	184.5±2.41	186.3±4.22	192.3±2.33	188.7±2.10	0.85
TDN intake (g/day)	998.1±19.2	996.4±17.4	974.6±20.5	1001.1±316.7	0.71
Initial body weight (kg)	35.9±0.99	36.1±0.81	37.8±0.82	37.3±0.68	0.77
Final body weight (kg)	54.2^{a}±1.50	58.1^{ab}±1.31	57.8^{ab}±1.09	59.2^{b}±0.89	0.05
Average daily gain (g)	327.4^{a}±11.5	390.4^{b}±12.8	394.9^{b}±10.7	431.6^{c}±6.02	<0.001
Apparent nutrient digestibility (%)					
Dry matter	72.9^{a}±0.39	74.2^{ab}±0.33	74.4^{ab}±0.3	75.2^{b}±0.55	0.02
Organic matter	74.7±0.31	76.9±0.40	76.8±0.64	75.8±0.71	0.13
Crude protein	73.3^{a}±0.63	75.2^{ab}±0.60	75.5^{ab}±0.82	76.3^{b}±0.67	0.04
Ether extract	80.0±0.94	82.2±0.84	83.7±0.88	84.5±1.09	0.09
Neutral detergent fiber	57.2±0.54	58.1±0.29	58.7±0.60	59.0±0.41	0.12
Acid detergent fiber	48.5±0.89	49.2±1.69	48.9±0.72	48.2±0.84	0.52

[a,b]Means bearing different superscripts in a row differ significantly (P<0.05)

Table 2. Effect of supplementation of synbiotic formulations on morphometry (inches) of pre-ruminant buffalo calves

Parameter	Dietary groups				P-values
	CON	SYN1	SYN2	SYN3	
Height	32.30.37	32.30.62	31.60.44	32.20.39	0.13
Hip Height	34.0±0.37	34.1±0.67	33.3±0.48	34.2±0.40	0.09
Body length	24.6±041	25.0±0.69	23.9±0.46	24.1±0.43	0.12
Girth	35.2^{a}±0.51	35.9^{ab}±0.73	35.7^{ab}±0.54	36.3^{b}±0.50	0.02

[a,b]Means bearing different superscripts in a row differ significantly (P<0.05)

however, did not find any difference on morphometry with probiotic inclusion in the diet of calves. Zhang *et al.* (2019) also not observedany significant effect on morphometry of calves due to supplementation of *Lactobacillus rhamnosus* GG to Holstein calves.

Lactobacillus count was I8.81, I9.06, I9.02 and I9.13 (10^{7} cfu/g of fresh feces) in groups ICON, ISYN1 ISYN2 and ISYN3, respectively. Fecal lactobacillus count was higher (P<0.05) in all supplemented groups as compared CON group. The average *Bifidobacterium* count ($\log_{10}{}^{7}$cfu/g of fresh feces) was higher (P<0.05) SYN3, than CON group. The Bifidobacterium responded positively (P=0.001) Ito synbiotic supplementation The coliform count ($\log_{10}$cfu/g in fresh feces) was lower I (P<0.05) in ISYN3 group, followed by ISYN1 and ISYN2 as compared Ito control group. *Lactobacilli* species have the ability Ito produce antimicrobial substances at gut level I (Gogineni *et al.,* 2013); they can also reduce the proliferation of gram negative bacteria. Monter *et al.* (2019) reported that synbiotics and probiotics individually produce certain metabolites like lactic acid or other antimicrobial substances that have the ability Ito reduce harmful bacteria (coliform, clostridia, *Salmonella* etc) at gut levels. Prebiotics particularly stimulate the growth of *IBifidobacterium* and *Lactobacillus* sp. and also some

Table 3. Fecal microbes, metabolites and VFAs of calves fed different synbiotics

Parameter	Dietary groups†				P-value
	CON	SYN1	SYN2	SYN3	
Lactobacillus	8.81^{a}±0.09	9.06^{b}±0.05	9.02^{b}±0.04	9.13^{b}±0.09	0.003
Bifidobacterium	8.58^{a}±0.11	8.84^{ab}±0.10	8.82^{ab}±0.10	9.01^{b}±0.08	0.011
Coliform	8.97^{b}±0.05	8.81^{ab}±0.07	8.84^{ab}±0.07	8.70^{a}±0.09	0.03
Clostridia	8.88^{a}±0.13	8.69^{ab}±0.06	8.65^{ab}±0.11	8.48^{b}±0.11	0.05
Fecal pH	7.18^{c}±0.06	6.97^{b}±0.06	6.90^{b}±0.04	6.71^{a}±0.08	0.003
Lactate (µmol/g) of fresh feces	2.98^{a}±0.11	3.42^{b}±0.14	3.46^{b}±0.10	3.76^{c}±0.15	0.001
Ammonia (µmol/g) of fresh feces	5.86^{b}±0.18	5.26^{ab}±0.18	5.32^{ab}±0.20	4.90^{a}±0.18	0.001
Volatile fatty acid (µmol/g) of fresh feces					
Acetate	13.0^{a}±0.63	14.1^{ab}±0.63	14.9^{ab}±0.54	15.5^{b}±0.78	0.001
Propionate	6.41^{a}±0.26	7.30^{ab}±0.25	7.20^{ab}±0.26	7.65^{b}±0.31	0.02
Butyrate	3.16^{a}±0.22	3.95^{ab}±0.31	4.01^{ab}±0.39	4.27^{b}±0.40	0.05
A:P	2.29^{b}±0.15	1.89^{a}±0.07	2.09^{ab}±0.07	2.05^{ab}±0.09	0.05

[a,b,c]Means bearing different superscripts in a row differ significantly (P<0.05)

butyrate producing bacteria I(Dueenas *et al.,* 2015), hence helpful in maintaining integrity and development of intestinal epithelium I (Mentschel *et al.,* 2001). On the other hand, it suppresses the growth of pathogenic microorganism like *IE. Coli* and *Clostridium perfringens*. Thus, it is evident that there is a noticeable beneficial effect of synbiotic supplementation on fecal microbial count. This could be linked to increased concentration of IVFA which have the bacteriostatic and bactericidal effect on health negative bacteria. Inulin is neither digested nor absorbed in the small intestine but it is selectively and quickly fermented by bacteria in large intestine that help in proliferation of lactobacillus and *Bifidobacterium*, which could be probable reason for higherI(P<0.05) count of both *Lactobacillus* and *Bifidobacteria* in synbiotics supplemented group. In the present study, pH was reduced Ion the provision of different combinations of synbiotics. In the similar line, Sharma *et al.* (2018) observed reduced fecal pH on supplementation of synbiotic and probiotic in calves. *Lactobacillus* ferment lactose present in the milk Ito lactic acid. Because of this reason lactic acid concentration was increased in synbiotic supplemented groups leading to reduced pH. Similarly, Bayatkoushar *et al.,* (2013) supplemented lactic acid bacteria to Holstein calves and found that faecal pH was reduced (P<0.05) in supplemented group as compared to control. Zhang *et al.* (2019) fed *Lactobacillus rhamnosus* GG to Holstein calves during pre-weaning stage and found that supplementation reduced (P<0.05) the pH of rumen. Supplementation of synbiotics also improved acetate, propionate and butyrate level indicating better fermentation in the supplemented groups (P<0.05).

Data pertaining to immunity and antioxidant status are presented in Table 4. Serum concentration of neither nor IgA was affected (P>0.05) by supplementation of different symbiotic formulations. Zhang *et al.* (2019) also did not find any effect I(P>0.05) of *Lactobacillus rhamanosus* IGG supplementation Ion serum IgG and IgA concentrations. Contrary Ito the present study ileal IgA concentrations of dogs increased due to supplemented of both mannan oligosaccharides and fructo-oligosaccharides (Swanson *et al.,* 2002). IgG concentrations were improved by 32% and 23% compared to controls in two trials involving mannan oligosaccharide-supplementation in pigletsI(Lazarevic *et al.,* 2010). Levels SOD and catalase were higher (P<0.05) in synbiotic supplemented groups as compared control. Between SYN 1 and SYN2, there was no difference in catalase level, while SOD level was similar in SYN 1, SYN2 and SYN3 groups. I Probiotics have the ability Ito enhance antioxidant activity because of their own antioxidant system, consisting of potent enzymes like ISOD and ICAT which stimulate the

Table 4. Effect of dietary supplementation of synbiotic formulations on immunoglobulin, antioxidant activity, and humoral and cell mediated immunity of pre-ruminant buffalo calves

Parameters	Dietary groups†				P-values
CON	SYN1	SYN2	SYN3		
IgG (mg/mL)	7.23±0.25	7.74±0.38	8.26±0.31	7.77±0.32	0.12
IgA (mg/mL)	0.30±0.01	0.31±0.01	0.29±0.01	0.33±0.01	0.15
Superoxide dismutase (U/mg Hb)	50.3^{a}±0.95	54.5^{b}±1.24	55.3^{b}±1.01	56.8^{b}±0.92	0.001
CAT (umole of H_2O_2 consumed /min/g Hb)	93.6^{a}±0.96	98.5^{b}±2.07	99.1^{b}±1.52	103.2^{c}±2.07	0.001
Absolute skin thickness (mm)*	8.53^{a}±0.28	8.89^{ab}±0.35	9.27^{bc}±0.29	9.63^{c}±0.35	0.001
Faecal score (1-4 scale)	2.94^{b}±0.08	2.78^{ab}±0.11	2.82^{ab}±0.10	2.64^{a}±0.11	0.03

[a,b,c]Means bearing different superscripts in a row differ significantly (P<0.05); *With response to post injection PHA-P (delayed type hypersensitivity reaction)

antioxidant signaling pathway (Wang *et al.,* 2017). Besides, probiotics have the restorative ability Ito enhance the antioxidant system of the host and raise its activity efficiently. Wang *et al.* (2009) studied the effect of *Lactobacillus fermentum* supplementation in pig and found increased serum level of ISOD and GPx.

There was a positive response of various formulations of synbiotic on fecal score of pre-ruminant buffalo calves (Table 4). The fecal score was lowest ($P<0.03$) in SYN3 group Most of health negative microorganism produces endotoxins which bind with specific receptors on intestinal epithelium and induce losses of water and electrolytes. In the present study supplementation of synbiotic resulted in reduced count of health negative bacteria (coliform and clostridium) which might have contributed to reduced fecal score. Prebiotic not only prevent from adhesion of pathogens to intestinal mucosal, but also stimulate growth and activity of beneficial bacteria and depress proliferation of pathogens in digestive tract by agglutination of gram-negative bacteria. However, Sharma *et al.* (2018) did not find any effect of supplementation of synbiotics on fecal score. Seifzadesh *et al.* (2016) also did not find any effect synbiotic addition on fecal score in suckling Holestein calves. The observed discrepancy could be due variation in source of probiotic and prebiotic along with climatic variables. While there was no effect on cough and nasal discharge, the eye discharge reduced in supplemented group which may be due to the improvement of mucosal immunity.

CONCLUSION

It was concluded that symbiotic combination of 9 g inulin and 50 g *Lactobacillus plantraum* CRD-7 having 10^8 CFU/ml to pre-ruminant calves improved immunity, antioxidant status, gut health and growth performance of pre-ruminant Murrah buffalo calves.

ACKNOWLEDGEMENT

The authors are thankful to the Director, NDRI, Karnal for providing required fund, infrastructure and facilities to carry out the work.

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Received on 01-10-2-2020 and accepted on 16-10-2020