



Effect of feeding fermentable synbiotics (*Lactobacillus rhamnosus* NCDC 298 and fructo-oligosaccharide) to Jersey crossbred calves up to 3 months of age

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ABSTRACT

The synbiotic combination of *L. rhamnosus* NCDC 298 and fructo-oligosaccharide (FOS) is known to be effective against diarrhoea by preventing the adhesion of pathogens to the gut, hereby, preventing infection and pathology. Such synergistic effect of the combination has emerged as a favourable alternative to antibiotic use against diseases and growth promoters in the livestock industry. The present study assessed the health and growth performance of the calves supplemented fermentable synbiotic fortified milk from 4 to 90 days and observed its performance till 120th day of age. Body temperature and passive immunity (IgG) were significantly better in calves fed synbiotics with notable increase in body weight and heart girth post synbiotic feeding period. These calves had increased dry matter intake per animal/day when compared to the control animals. The populations of *Lactobacillus* sp. elevated with decreased pathogenic bacteria (*Coliform*) in faeces on feeding synbiotics and it continued even after 90 days of age indicating colonization of beneficial bacteria. Therefore, feeding of fermentable synbiotics had observable beneficial effects on health, immunity and growth of the Jersey crossbred calves. The findings encourage further verification of fermentable synbiotics fortified milk as a potential growth promoter to young dairy calves.

Keywords: Body growth, Calf health, Fructo-oligosaccharides, *Lactobacillus* bacteria, Synbiotic supplement

The healthy calf is essential for a successful and profitable dairy venture but common use of antibiotics against diseases and growth promoters have come to rigorous public scrutiny (Langford *et al.* 2003). To address the problem there is a need to look for viable alternative strategies to enhance calf performance and reduce the massive use of antibiotics in farms. One method is the synergistic combination of probiotics and prebiotics known as synbiotics which favourably modulated the gut microbiota by preventing the colonization of pathogenic bacteria, produce metabolic substrates (e.g. vitamins and short-chain fatty acids) and enhance feed efficiency and weight gain in calves (Soberon *et al.* 2012, Zang *et al.* 2016). Besides, they are also known to stimulate the immune system and reduced the incidence of neonatal diarrhea caused by the enterotoxigenic *E. coli* (de Vaux *et al.* 2002) thereby, maintaining the overall calf health (Tuohy *et al.* 2005). Probiotics commonly used in livestock and poultry industry are *Lactobacillus*, *Enterococcus*, *Bacillus* and *Saccharomyces* while prebiotics used are mainly Fructo oligosaccharide (FOS), Galacto oligosaccharide (GOS), Mannan oligosaccharide (MOS) (Gaggia *et al.* 2010).

The study, for the first time, investigated the effect of feeding fermentable synbiotic (*Lactobacillus rhamnosus* NCDC 298 and fructo-oligosaccharide) fortified whole milk

to the Jersey crossbred calves on their health and growth performance. Our hypothesis states that synbiotic combination of *Lactobacillus rhamnosus* NCDC 298 and fructo-oligosaccharide (FOS) may have beneficial effect on account of its *in vitro* antibacterial property against *E. coli* toxin (Anand *et al.* 2017).

MATERIALS AND METHODS

The present study was carried out at National Dairy Research Institute (NDRI), Eastern Regional Station, Kalyani, India. Kalyani is located at the lower Gangetic basin of West Bengal in Nadia district. The climatic condition is hot and humid with average annual maximum and minimum temperatures of 39°C and 12°C, respectively. The average annual rainfall is around 1,250 mm with a maximum relative humidity of 90%. The duration of the experiment was 120 days.

Housing and feeding of the experimental calves: All the calves born in November–December 2018 and January 2019 were taken into study. Jersey crossbred calves (16) were randomly assigned to 2 groups (8 group), treatment (T) and control (C). Each of the calves was separated immediately after birth from their dams and housed in well-ventilated calf sheds. Colostrum and milk was fed as per the standard farm practices till the calves reached 90 days of age. *Ad lib.* supply of concentrates and green fodder as the basal diet was offered as early as one week of age to the

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Table 1. Chemical composition of the feed and fodder offered to the calves during the experimental period

Chemical composition (%)	Concentrate	Maize fodder
DM	93.11	18.62
CP	22.61	8.50
CF	7.08	25.15
Total ash	7.83	8.30

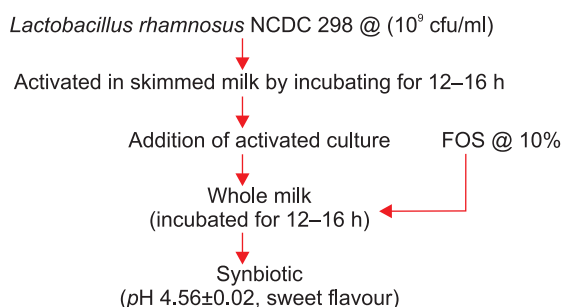


Fig. 1. Flow diagram of fermentable synbiotic preparation

calves in both the groups but the treatment calves received an additional supply of fermented synbiotics (*Lactobacillus rhamnosus* NCDC 298 and fructo-oligosaccharide) fortified with whole milk till 3 months of age. Calves were gradually weaned off the whole milk and observation was taken till 120 days of age. The nutrient profile of the concentrates and green fodder are shown in Table 1.

Preparation of fermentable Synbiotics: Synbiotic was prepared by combining *Lactobacillus rhamnosus* NCDC 298 @ 3.4×10^9 CFU/ml and fructo oligosaccharides (FOS) @ 10%, in skimmed milk (Fig. 1).

Health monitoring and sample collection: The health of the calves was monitored daily by examining temperature, respiration, scours, medical treatments or presence of any abnormalities. Blood samples were collected in evacuated

glass tubes containing EDTA at 1 day and weekly intervals for analysis of serum total protein (Refractrometer) (Buczinski *et al.* 2016) and total leukocyte count (Haemocytometer). Plasma IgG was estimated at 7 day and 28th day of age using ELISA (Bovine IgG, BlueGene Biotech^R) (Beam *et al.* 2009). Bodyweight, body measurements (length, withers height and heart girth) and dry matter intake were measured at weekly intervals (Siddiqui *et al.* 2015). Faecal score (1, normal; 2, slight liquid consistency; 3, moderate diarrhoea and 4, severe diarrhoea (Morrison *et al.* 2010) and faecal dry matter (AOAC 2005) was estimated on weekly basis. The faecal samples collected from the rectum at 1 day and the weekly interval was enumerated for *Lactobacillus* and *Coliform* bacteria (Hasunuma *et al.* 2011).

Statistical analysis: The serum cortisol level, body weight, body measurements, DMI, faecal score, faecal dry matter, faecal *Lactobacillus/Coliform* count in treatment and control calves were compared by analysis of variance (ANOVA) with repeated measures using SPSS Ver. 20 (SPSS Inc., USA). Faecal bacteria counts were transformed (\log_{10}) before statistical analysis. Treatment effects were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

No mortality occurred during the experimental period. The difference in body temperature, respiratory rate, serum total protein and plasma IgG was also detected in calves supplemented fermentable synbiotics. The increase in body temperature of the calves in the control group may be associated with severe dehydration which generally interferes with the temperature homeostasis (Naylor 1989). Most recently, prebiotics has shown to enhance immunity, growth and feed efficiency in calves (Henrichs *et al.* 2003) while in early-weaned piglets (Hong *et al.* 2005) and broilers it increased the insulin-like growth factor-1 (Huang *et al.* 2007). Though 7/8 calves in both the groups suffered from

Table 2. General physiology, disease incidence, medical treatment in control and synbiotic supplemented calves

Parameter	Control	Synbiotic fed	Sig.
Body temperature (°F)	101.27±0.07	100.94±0.08	P<0.01
Respiration rate/min	34.66±0.58	33.19±0.49	P<0.05
Serum total protein (g/dl)	7.27±0.70	7.10±0.73	P<0.05
Faecal score	1.47±0.08	1.42±0.07	NS
Faecal dry matter (%)	20.46±0.53	21.14±0.49	NS
IgG concentration at one week of age (mg/ml)	22.40±1.39	20.55±2.54	NS
Overall IgG concentration at 28 th day of age (mg/ml)	20.83±4.42	29.38±4.80	P<0.05
Total leukocyte count at one month of age ($10^3/\mu\text{l}$)	4.66.63±0.80	4.83.50±0.94	NS
Days suffered due to diarrhea (range)	9.07±4.06 (5–28)	4.84±2.17 (5–16)	–
No of calves suffered due to respiratory problems	2	0	–
No of calves suffered due to joint ill (days suffered)	1 (84 days)	0	–
Total number of drugs used (names of drugs)	5 (Sulphanomaide, Oxytetracycline, Tinidazole+ Norfloxacin, Ceftriaxone+ Tazobactam, Amikacin)	3 (Sulphanomaide, Oxytetracycline, Ceftriaxone+ Tazobactam)	–

Statistical significance (NS=not significant, **P<0.01, *P<0.05).

Table 3. Performance of the calves fed synbiotic supplement

Parameter/indicator	Control	Treatment	Sig.
<i>Body weight/DMI</i>			
Initial body weight (kg)	23.43±1.30	22.00±1.17	NS
Body weight during synbiotic feeding period (4–90 days)	37.15±1.17	39.64±1.28	NS
Body weight post synbiotic feeding period (91–120 days) (kg)	62.35±1.84	66.78±1.79	P<0.05
Total DMI (kg/animal/day)	1.13±0.05	1.84±0.24	P<0.05
<i>Body length</i>			
Initial body length (cm)	58.62±1.97	59.50±1.03	NS
Body length during synbiotic feeding period (4–90 days)	70.95±0.78	72.55±0.71	NS
Body length post synbiotic feeding period (91–120 days)	86.80±1.08	87.40±0.86	NS
<i>Heart girth</i>			
Initial heart girth (cm)	68.00±1.07	68.37±1.58	NS
Heart girth during synbiotic feeding period (4–90 days)	80.37±0.84	82.30±0.88	NS
Heart girth post synbiotic feeding period (91–120 days)	95.56±1.07	99.28±1.17	P<0.05
<i>Body height</i>			
Initial body height (cm)	66.00±1.21	66.75±1.34	NS
Body height during synbiotic feeding period (4–90 days)	75.94±0.61	77.50±0.70	NS
Body height post synbiotic feeding period (91–120 days)	88.60±0.90	89.36±1.05	NS
<i>Faecal coliform load count (log cfu/g of faeces)</i>			
At one day age of the calves (log cfu/g of faeces)	4.58±1.73	4.57±1.83	NS
Synbiotic feeding period (4–90 days)	5.92±0.41	4.48±0.31	P<0.01
Post synbiotic feeding period (91–120 days)	5.04±0.64	4.42±0.60	NS
<i>Faecal Lactobacillus load count (log cfu/g of faeces)</i>			
At one day age of the calves (log cfu/g of faeces)	2.42±1.59	2.64±1.73	NS
Synbiotic feeding period (4–90 days)	5.91±0.35	7.70±0.32	P<0.01
Post synbiotic feeding period (91–120 days)	5.97±0.53	6.86±0.46	NS

Statistical significance (NS, not significant, **P<0.01, *P<0.05).

various illnesses, control calves exhibited a higher incidence of diarrhoea, problems of respiration and joint ill with an enhanced antibiotic use. To maintain the health of the calves in treatment, 3 drugs (Sulphanomide, Oxytetracycline and Ceftriaxone + Tazobactam) had to be used against diarrhoea while in control calves 5 drugs (Tinidazole + Norfloxacin, Tazobactam, Sulphanomide, Oxytetracycline and Ceftriaxone + Tazobactam) were used for respective health problems. One of the control calves suffered due to joint ill for a long period and at the end of the experiment, there was lameness associated with slow growth. Beneficial bacterial species are known to support the animal's defense system by stimulating the gastrointestinal immune system with increased surveillance of the leukocytes thus, elimination the disease-causing pathogens (Hughes and Heritage 2002). Similarly, Donovan *et al.* (2005) have also found a reduction in duration and occurrence of diarrhea in calves offered probiotics (*L. acidophilus*). No statistical differences were detected in scour scores and faecal dry matter however, the control group had a higher scour score than the treatment calves.

Though the calves in the study were fewer in number, the true potential of synbiotic supplements to calves cannot be concluded but our observation on body weight, growth measurements, fecal *Coliforms* and *Lactobacillus* load count is presented in Table 3. Body weight and heart girth showed

significant results at the end of the experimental period of 91–120 days with enhanced DMI in the treatment group. Though, Mudgal and Baghel (2010) believed that feeding *Lactobacillus* sp. at early age promoted weight gain and rumen development our study revealed that feeding fermented synbiotics up to the milk feeding period of 90 days maintained higher weight gain, good health where beneficial bacteria sustained even after the synbiotic feeding period of 91–120 days. Therefore, there is a possibility that feeding of fermentable synbiotics during the milk feeding period is suitable for the young calves whose rumen are still in the developmental phase. *Lactobacillus* is one of the most beneficial organisms in the monogastric animals, the forestomach of the pre ruminant calves also resembles one and hence its supplementation during that time enhanced digestibility and ultimate growth of the calves (Hill *et al.* 2009). Also, calves fed fermentable synbiotics had significant faecal shedding of *Coliforms* and increased the population of *Lactobacillus* sp. Dar *et al.* (2017) also found similar results on calves. It is identified that lactic acid bacteria inhibit the growth of pathogenic bacteria by decreasing the pH in the large intestine and compete with the pathogenic organism for attachment (Riddell *et al.* 2010). We observed this trend even after the synbiotic feeding period of 91–120 days. It may be due to the colonization and development of the *Lactobacillus* sp. in

the intestine with intestinal microbial balance (Fuller, 1989) that might facilitate favourable bacterial community.

Feeding of fermentable synbiotics in the first 90 days had a detectable health and growth benefits to the calves. Health and growth performance was enhanced with comparatively lesser antibiotic use; the population of *Lactobacillus* maintained even after the synbiotic feeding period suggesting further validation of the duration and synergism of synbiotics as growth promoters in young Jersey crossbred calves.

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