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Effect of Chicory Root Powder supplementation on faecal microbiology on Murrah buffalo

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Abstract
Twenty eight Murrah buffalo calves (7-10 d old and 31±2kg) were randomly selected and divided into four groups. All the four groups were fed as per ICAR (2013) feeding schedule except that these were additionally supplemented with 0, 8, 16 and 24 g/d chicory root powder (in the four respective groups i. e. T_0, T_1, T_2, T_3) for 90 days. Results showed that the faecal *Lactobacillus* and *Bifidobacterium* count was significantly ($P<0.05$) higher in all the supplemented groups with concomitant reduction in faecal *Coliform* count as compared to control. Faecal score was significantly ($P<0.01$) decreased in treatment group. There is no significant difference observe in the clostridium count in treatment and control. Thus, it may be concluded that the supplementation of chicory root powder (8, 16 and 24g) may be useful for enhancing health status and performance of calves.

Keywords: Prebiotic, calf, chicory root powder, performance, faecal microbiology

Introduction
For a profitable dairy industry, calves, being future replacement stock of the herd, are an important asset and key determinants of the economic future of dairy farm. Hence, healthy young stock is indispensable for a successful and profitable dairy enterprise. But, calf health is a very critical factor affecting the welfare and economics of young stock, dairy and rearing enterprises. The maintenance of health and growth rate of calves is very important especially during first 2 to 3 months of age. It also decides the economics of replacement stock rearing and has immense bearing on early maturity and production of the animals (Ghosh and Mehla, 2012) [3]. Calf diarrhoea, in particular, is a significant health issue in dairy rearing enterprises, with 38% of producers reporting it to be a significant problem (Morrison *et al*., 2010) [9]. Diarrhoea has been related to an increase of Coliform bacteria counts in the intestines and a decrease in Lactobacilli and Bifidobacteria counts (Ouwehand *et al.*, 2002) [11]. The increase of Coliform bacteria in the intestines may produce putrefactive substances and harm the host (Fujisawa *et al*., 2010) [22]. As a result, gut microbiota are important to the health maintenance and development of the host (Ng *et al*., 2009; Rowland *et al*., 2010) [10, 15].
The development of antimicrobial resistance and transference of antibiotic resistance genes from animal to human microbiota (Salyers *et al*., 2004) [16] and ban on the use of antibiotics as growth promoters in the European Union since January 1, 2006 (EC, 2001) [1] urged the scientist to find a suitable alternatives to antibiotics. To overcome these problems and to replace the use of antibiotics, prebiotics came up as a good adjuvant to promote the health (Heinrich *et al*., 2003) [4]. Prebiotic supplementation has gained interest in recent years as a method to improve gastrointestinal health in livestock. It has been provided that prebiotic supplementation may be most effective in times of stress or increased pathogen exposure throughout the calf's lifetime (Quirk *et al*., 2010) [22]. Inulin is one of the fructans, naturally occurring in many plants, mostly extracted from chicory root (Cichoriumintybus) or Jerusalem artichoke (Helianthus tuberosus). It is composed of oligo and polysaccharides, which give inulin its unique prebiotic properties (Samanta *et al*., 2013) [17]. Keeping all these facts in consideration, the objective of present study was effect of chicory root powder supplementation as a prebiotic on faecal microbiology and faecal score.

Material and methods
Animal housing environment and dietary treatment
Twenty eight Murrah buffalo calves (7-10 d old and 31 ± 2.0 kg of body weight), were

randomly assigned into four groups with seven animals in each group. All the calves were fed a similar basal diet (ICAR 2013) [5]. with group 1 (T_0) without any supplementation served as control while animals in Group II (T1), Group III (T2) and Group IV (T3) were supplemented with 8, 16, 24 g chicory root powder per calf/day respectively. The total duration of experimental period was of 120 days in (October 2016 to April 2017).

Housing and environment

The study was conducted in the individual calf sheds of ICAR - National Dairy Research Institute Karnal, India. The calves were housed individually in well-ventilated pens.

Feeding management

The diet comprised of concentrate mixture (maize, bajra, GNC, SBM, MOC, wheat bran, rice polish and mineral mixture. The animals were offered green fodder containing maize and jowar. All the calves had 24 hr access to ad libitum clean water. The feeding of milk was carried out twice a day. Whole milk fed to the calves at 1/10th of actual BW up to 2 weeks, 1/15th of actual BW in the third and fourth week, 1/20th of actual BW in the fifth and sixth week, and 1/25th in the seventh and eighth week of study. Calf starter was offered from the second week onwards. All the calves were fed ad libitum concentrate mixture and green fodder (Ramaswami *et al.,* 2005) [13].

Faecal collection and procedure

Rectal fecal samples were collected at 0, 15, 30, 45, 60 and 90 days to enumerate the faecal microbial populations. Sterile gloves were used to obtain 5 to 6 g of feces following perianal cleansing with dilute Betadine solution. Faeces were enumerated by two sets of serial ten-fold dilutions (10-1 to 10-12) with the total volume of 10 mL including one gram homogenized faeces and 9 mL normal saline (0.9% NaCl) and plated in duplicate onto selective media: MRS agar for Lactobacilli (Himedia), EMB Agar, Levine (Himedia) for coliforms, Clostridial agar (Himedia) for clostridia, and bifidobacteria agar for bifidobacteria (Himedia). A cfu is defined as a distinct colony measuring at least one mm in diameter (Swanson *et al*., 2002) [19]. Fecal consistency score in calves was recorded by adopting standard protocol (Larson *et al.,* 1977) [7].

Statistical analysis

The experimental data generated were analysed by ANOVA using the statistical software program SPSS (SPSS Inc., Chicago, Illinois, USA. Data for parameters involving periodic collections were analysed adopting repeated measures procedure using GLM of SPSS; the analysis included between-subjects main effect of treatment, within-subjects main effect of period of sampling and interaction between the periods of sampling × treatment. The effects were considered to be significant at $p < .05$.

Result and discussion

Faecal microbiology

The data of faecal microbial count are preseted in Table 1. There is significant difference observed in faecal *Lactobacillus* count between treatments then control it was further notice that no significant difference exists among the three treatment group. If we consider the period they will increase significantly ($P<0.01$) in the period from day one to day 120th of experiment. Same pattern was observed in *Bifidobacterium*. Similar to our finding, Mendoza *et al.* (2011) [18] also reported Lactobacilli count in feces was higher (P=0.05) and *Bifidobacteria* tended to be higher in calves fed Incompatible to present findings, (Kara *et al*., 2015) [6] reported fecal concentration of Lactobacillus was lower ($P<0.05$) in experimental group (4g MO.) compared with control.

Coliform and *Clostridium* counts due to supplementation of different levels of chicory root powder are given in Table 1. There is significant difference in *Coliform* count due to treatment as well period. *Coliform* counts decreased significantly ($P<0.01$) with increasing period of supplementation of chicory root powder. T2 and T3 groups were statistically similar but different from T1 which had comparatively higher count but significantly less than control group. There is no significant (P>0.05) difference observed in clostridia count between treatment and control. The possible reasons for lower fecal *Coliform* counts in treatment groups may be due to competitive inhibition and competition for binding sites in intestine with the pathogenic microorganisms. Our results are in agreement with the findings of (Roodposhti and Dabiri, 2012) [14], the workers reported that fecal E. coli count reduced significantly ($P<0.05$) on supplementation of prebiotic than control. (Ghosh and Mehla, 2012) [3] also observed decreased number of *coliform* count in MOS supplementation group Our results are also supported by (Takagi *et al.,* 2011) [20] who used difructose anhydride III as prebiotic in calves and found that its use significantly decreased fecal coliform at each sampling. Apposite to our finding Spring *et al.* (2000) [18] reported no significant difference between prebiotic (MOS) and control group in faecal *coliform* count of salmonella challenged broilers.

Faecal score

The data of faecal score are presented in Fig 1. There is significant difference observe in Fecal score. Prebiotic feeding significantly ($P<0.01$) decreased the average fecal score in the three chicory root fed groups. Concordant findings were reported by (Heinrichs *et al.,* 2003) [4] and (Ghosh and Mehla, 2012) [3] who observed decrease in fecal score in MOS supplemented in calves as compared to control and they postulated that the declined fecal score might be due to the reduction in pathogenic bacteria which are responsible for toxin production leading to intestinal secretion However, (Kara *et al*., 2015) [6] reported no effect on fecal score as a result of prebiotic supplementation.

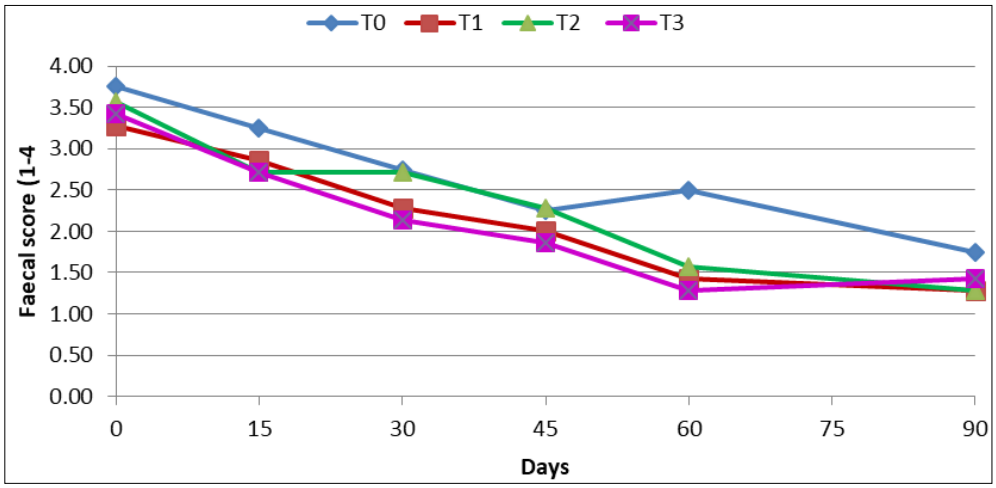

Fig 1: Temporal changes in faecal score of Murrah buffalo calves on supplementation with chicory root powder 8g (T_1), 16 g (T_2)) and 24g (T_3) as compared to the control (T_0) (significance: T =< 0.001, P =< 0.001 andT*P = 0.342]

Table 1: Effect of dietary supplementation of chicory root powder on faecal microbiology of Murrah buffalo calves

Attributes	Dietary group				Period mean	Significance		
	T_0	T_1(8g)	T_2 (16g)	T_3(24g)		T	P	T*P
Health positive bacteria (log_{10}cfu/g of fresh faeces) *Lactobacillus*								
0d	8.44±0.01	8.38±0.06	8.25±0.012	8.41±0.05	8.37^{p}± 0.03	<0.001	<0.001	<0.001
15d	8.46±0.01	8.48±0.03	8.56±0.01	8.45±0.06	8.49^{q}±0.02			
30d	8.53±0.02	8.45±0.07	8.63±0.01	8.45±0.04	8.52^{q} ±0.02			
45d	8.47±0.04	8.57±0.01	8.56±0.01	8.51±0.04	8.52^{q} ±0.02			
60d	8.40±0.02	8.61±0.01	8.54±0.03	8.57±0.03	8.53^{q} ±0.02			
90d	8.06±0.04	8.71±0.02	8.75±0.02	8.60±0.03	8.53^{q} ±0.06			
Average	8.39^{a}±0.03	8.53^{b}±0.02	8.75^{b}±0.02	8.50^{b}±0.02				
Bifidobacterium (log_{10}cfu/g of fresh faeces)								
0d	7.63±0.06	7.68±0.06	7.77±0.06	7.77±0.06	7.71^{p}±0.03	<0.001	<0.001	0.85
15d	7.62±0.08	7.73±0.05	7.82±0.05	7.78±0.05	7.74^{pq} ±0.03			
30d	7.66±0.07	7.77±0.08	7.84±0.05	7.81±0.05	7.77^{pqr}±0.03			
45d	7.71±0.05	7.91±0.10	7.89±0.07	7.86±0.05	7.84^{qrs} ±0.04			
60d	7.72±0.04	8.00±0.08	7.89±0.07	7.89±0.04	7.87^{rs} ±0.03			
90d	7.75±0.05	8.03±0.08	8.05±0.08	7.91±0.04	7.93^{s}±0.04			
average	7.68^{a}±0.02	7.85^{b}±0.04	7.88^{b} ±0.03	7.84^{b} 0.02				
Health positive bacteria *Coliform* (log_{10}cfu/g of fresh faeces)								
0d	7.80±0.04	7.86±0.04	8.01±0.05	7.84±0.04	7.88^{p}±0.03	<0.001	0.003	<0.001
15d	7.86±0.03	7.82±0.08	7.57±0.07	7.61±0.02	7.71^{q}±0.04			
30d	7.96±0.03	7.78±0.07	7.54±0.01	7.55±0.02	7.68^{q} ±0.03			
45d	8.02±0.06	7.79±0.06	7.39±0.05	7.56±0.14	7.69^{q} ±0.06			
60d	8.17±0.01	7.24±0.08	7.14±0.06	7.27±0.03	7.46^{r}±0.08			
90d	8.22±0.02	7.21±0.06	7.12±0.06	7.22±0.04	7.44^{r} ±0.09			
Average	7.99^{c}±0.03	7.62^{b} ±0.05	7.46^{a} ±0.06	7.51^{a} ±0.04				
Clostridia (log_{10}cfu/g of fresh faeces)								
0d	7.38±0.06	7.38±0.08	7.38±0.06	7.38±0.10	7.38±0.04	0.564	0.64	1
15d	7.35±0.06	7.34±0.12	7.38±0.05	7.36±0.09	7.36±0.04			
30d	7.34±0.04	7.32±0.07	7.36±0.05	7.33±0.07	7.34±0.03			
45d	7.34±0.04	7.26±0.08	7.35±0.05	7.33±0.07	7.32±0.03			
60d	7.37±0.05	7.28±0.05	7.29±0.05	7.35±0.06	7.32±0.03			
90d	7.38±0.05	7.26±0.06	7.28±0.04	7.32±0.06	7.31±0.03			
Average	7.36±0.02	7.31±0.03	7.34±0.02	7.34±0.03				

Basal diet with no supplementation (T_0,) or supplemented with chicory root powder 8g (T_1), 16 g (T_2)) and 24g (T_3)

$^{a,b/pqrs}$Means bearing different superscripts in a row (a,b) or column (P,q,r,s) differ significantly (P<0.01)

$^{\$}$Significant effects of dietary treatment (T), period (P) or their interaction (T*P)

Conclusion

Chicory root powder has potential for improving gut health fecal microbiota. So it could be conclude that 8g/d chicory root powder supplementation can reasonably be recommended for the calves for the overall health.

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