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Effect of prebiotic supplementation on faecal characteristic on buffalo calves

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Abstract
Ban on the antibiotic as a growth promoter European Union since January 1, 2006 prebiotic come in existence. Twenty eight Murrah buffalo calves 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. CON, T1, T2 T3) for 90 days. Results showed that there is significant ($p<0.05$) decrease in Fecal, ammonia and faecal pH on supplementation. Faecal lactate is significantly increase in treatment group. 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. As there is no significant difference observe in the treatment group in 8g and 16 g faecal score, faecal pH, so we can conclude that 8gm of chicory root powder beneficial for calves.

Keywords: prebiotic, calf, faecal pH, faecal lactate

Introduction
Major portion of India's economy is based on agriculture and animal husbandry in which milk production plays a vital role. India ranks first in milk production in the world. Milk production in India was around 155 million tonnes in 2015-16 according to NDDB and buffaloes contribute 56% the nation's milk production (FAO, 2004) [20]. The rural people are mostly dependent on agriculture for their livelihood and livestock provides additional support to them. For a lucrative 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) [6]. 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) [12]. 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) [14]. The increase of Coliform bacteria in the intestines may produce putrefactive substances and harm the host (Fujisawa *et al*., 2010) [5]. As a result, gut microbiota are important to the health maintenance and development of the host (Ng *et al*. 2009; Rowland *et al*. 2010) [17]. The development of antimicrobial resistance and transference of antibiotic resistance genes from animal to human microbiota (Salyers *et al*. 2004) [18] and ban on the use of antibiotics as growth promoters in the European Union since January 1, 2006 (EC, 2001) 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) [8]. 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) [15]. Inulin is one of the fructans, naturally occurring in many plants, mostly extracted from chicory root (*Cichorium Intybus*) or Jerusalem artichoke (Helianthus tuberosus). It is composed of oligo and polysaccharides, which give inulin its unique prebiotic properties (Samanta *et al*. 2013) [19].

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) [9]. 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.

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) [16].

Faecal collection and procedure

Rectal fecal samples were collected at 0, 15, 30, 45, 60 and 90 days to enumerate the faecal pH, ammonia and faecal microbial populations. Sterile gloves were used to obtain 10 to 12 g of feces following perianal cleansing with dilute Betadine solution. The pH of the samples before aliquoting of the faeces was determined directly with digital pH meter (pH Spear, Eutech Instruments, Klang Selangor, DE, Malaysia; pH range −1.00–14.00, resolution 0.01 pH, accuracy ±0.01 pH) specially designed for direct pH measurement of semi-solid samples (Kore *et al.* 2009) [11]. Additional three aliquots were collected for determination of fermentative end products. About 2g of fresh faeces was diluted with 6 mL of 6 N HCl, centrifuged and was stored at –20oC for analysis of ammonia. The ammonia estimation was done by modified method of (Chaney and Marbach, 1962) [2]. Another aliquot of about 2g was diluted with an equal volume of distilled water and centrifuged at 10,000 rpm and the supernatant was stored at -20oC for analysis of lactate. Lactic acid was estimated as per method of (Baker and Summerson, 1941) [1].

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 analyzed 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 Charecterstic

The data of faecal pH were presented in table 1. It is evident from the pH values showed a declining trend in all the groups including control, however, decline was more pronounced in chicory root powder supplemented group. Gibson and Roberfroid. (1995) [7] observed that the pH reduced due to Bifidogenic mechanism of action which is based on selective fermentation of fructans by *Bifidobacteria* through synthesis of beta-fructosidases enzyme decomposing beta-2, 1 glycosidic bonds in inulin and oligofructoses. As a result of bifidogenic effect change of bacterial microflora in intestine, decrease in the number of harmful bacteria is observed. Their proliferation is inhibited by *Bifidobacteria* that produces short-chain fatty acids (SCFA) and lowers the pH of intestinal chyme, with concomitant adverse conditions for pathogens. This may also be the case in our study and plausible reason for reduction in faecal pH. Similar results were found by Chen *et al*. (2005) [3] who studied the effect of oligofructose and inulin and observed a reduction in the fecal pH in fresh feces during the first four weeks of production in diet containing oligofructose in broilers. Little or no effect on fecal pH was observed for the inulin treatment. However, Fujisawa *et al*. (2010) [5] observed that the fecal pH did not differ from those of the control group, when calves were fed prebiotic which is opposite to our findings.

Faecal Metabolite

Data of faecal lactate and faecal ammnia is presented in Table 1. There is significant ($P<0.01$) increase in faecal lactate concentration in treatment group as compare to control. Lactate concentration is highest in T_2 then control (T_0). Fecal ammonia concentration is also significant and similar trend was observed as lactate. Increased lactate concentration with lowering of ammonia concentration. So that minimum value is observed in T2 (4.30±0.10) then control (4.97±0.09) Increased lactate concentration with lowering of ammonia concentration result in lowering of pH, thus supporting the data obtained for pH too. Similar results were found in the study of (Fujisawa *et al*., 2010) [5] indicated that fecal ammonia ($P<0.05$) was significantly lower in the prebiotic supplemented group as compared to the control group. Our studies are also in agreement with the findings of (Chen *et al.* 2005) [3] who studied the effect of oligofructose and inulin and observed reduction in volatile ammonia concentration and faecl pH in fresh feces during the first four weeks of age in diet containing oligofructose. However, (Fujisawa *et al*. 2010) [5] observed that the fecal pH did not differ from those of the control group, when calves were fed prebiotic which is opposite to our findings.

Table 2: Effect of dietary supplementation of chicory root powder on faecal characteristics 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
Fecal pH								
0d	7.64±0.15	7.58±0.14	7.50±0.18	7.53±0.18	7.56^{s}±0.08	0.011	<0.001	1
15d	7.46±0.05	7.28±0.28	7.18±0.16	7.21±0.13	7.28^{rs} ±0.09			
30d	7.58±0.16	7.25±0.41	7.26±0.32	7.29±0.23	7.34^{rs}±0.14			

45d	7.38±0.15	6.81±0.25	6.84±0.33	6.87±0.17	6.98^{qr}±0.12			
60d	7.17±0.10	6.66±0.23	6.69±0.28	6.70±0.12	6.80^{q}±0.11			
90d	6.52±0.11	6.12±0.31	6.20±0.26	6.30±0.23	6.29^{p}±0.12			
Average	7.29 ^{b}±0.08	6.95^{a}±0.13	6.94 ^{a} ±0.12	6.98 ^{a} ±0.11				
Lactate (µmol/g) of fresh feces								
0d	3.11±0.21	3.05±0.17	3.13±0.13	3.12±0.23	3.10^{p}±0.18	<0.01	<0.01	<0.01
15d	3.07±0.13	3.23±0.09	3.20±0.19	3.29±0.17	3.20 ^{pq}±0.16			
30d	3.07±0.22	3.40±0.13	3.41±0.19	3.41±0.15	3.32^{r}±0.22			
45d	3.21±0.14	3.56±0.18	3.67±0.12	3.61±0.22	3.51^{s}±0.24			
60d	3.23±0.20	3.83±0.19	3.89±0.16	4.05±0.23	3.75^{t}±0.37			
90d	3.14±0.14	4.04±0.09	4.64±0.41	4.06±0.32	3.97^{u}±0.60			
average	3.14^{a}±0.18	3.52^{b}±0.37	3.66^{c}±0.56	3.59^{bc}±0.42				
Ammonia (µmol/g) of fresh feces								
0d	5.67±0.02	5.42±0.06	5.08±0.17	5.04±0.22	5.30 ^{s}±0.08	<0.01	<0.01	.121
15d	5.22±0.10	5.03±0.06	4.90±0.14	5.12±0.21	5.07^{s}±0.07			
30d	4.98±0.19	4.69±0.14	4.53±0.11	4.55±0.20	4.69^{r}±0.08			
45d	4.60±0.27	4.49±0.15	4.11±0.07	4.57±0.24	4.44^{qr}±0.10			
60d	4.78±0.19	4.16±0.15	3.78±0.06	4.11±0.10	4.21^{q}±0.09			
90d	4.60±0.08	3.77±0.15	3.39±0.06	3.72±0.10	3.87^{p}±0.10			
average	4.97^{c}±0.09	4.59^{b}±0.10	4.30^{a}±0.10	4.52^{b}±0.11				

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 pH and fecal metabolites of calves. 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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