Effect of *Enterococcus faecium* SF68 on growth performance and *in vivo* digestibility in buffalo calves

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ABSTRACT: The effect of dietary supplementation with *Enterococcus faecium* strain SF68 on growth performance, faecal consistency and in vivo digestibility in buffalo (*Bubalus bubalis)* calves was evaluated. Forty calves were randomly assigned at 10 d of age to one of four treatments: (A) milk replacer with no additive, (B) milk replacer supplemented with 0.17 g/l of viable (2 x 10^9 cfu/g) *E. faecium* bacteria daily for 3 days with an interval of 7 days throughout 11 weeks, (C) milk replacer supplemented with *E. faecium* daily for 4 weeks, (D) milk replacer supplemented with *E. faecium* daily for 11 weeks. A total mixed ration was offered *ad libitum* from 5th week of the experimental period. Faecal score was significantly better in *E. faecium*-treated calves than control ones. The use of *E. faecium* had no effect on average daily gain at any stage, total body weight (BW) gain, dry matter intake or total tract digestibility. Therefore, *E. faecium* supplementation may be able to act favourably on the health of the gastrointestinal tract.

Key words: Enterococcus faecium SF68, Calf, Growth, Digestibility.

INTRODUCTION - In most buffalo (*Bubalus bubalis*) farms calf mortality from birth to weaning averages 10-20%, but if animals are reared under sub-optimal conditions this percentage can double. Neonatal diarrhoea is one of the main causes of buffalo calf death. *Enterococcus faecium* SF 68 is a well-studied Lactobacillus (LAB) strain which has been used for more than a decade as a probiotic in animal nutrition as well as in the prevention and treatment of diarrhoea in humans (Vanbelle *et al.*, 1990). The aim of this research was to evaluate the effect of *E. faecium* strain SF68 (EF68), offered with different frequency, on faecal score, growth performance and in vivo digestibility in buffalo calves during the weaning period.

MATERIAL AND METHODS - The study took place at a dairy buffalo farm. The experimental period was 11 weeks. Starting at 10 d of age, forty buffalo calves were placed in individual cages and allotted to one of four treatment groups (10 animals per group).Treatment A was the control diet and received a milk replacer without additive. For treatment B, a commercial culture (Primalac NF m) of viable (2 x 10^9 cfu/g) EF68 was added to milk replacer (0.17 g/l) for 3 days with an interval of 7 days throughout the experimental period. For treatment C, EF68 was added to milk replacer daily during the first 4 weeks of the experimental period. For treatment D, EF68 was administered to the animals daily for 11 weeks. Commercial milk replacer (22.9% crude protein, 25.0% fat, 6.9% ash, 0.3% crude fibre) was fed at a rate of 10% of initial body weight (BW) for the first two weeks (on average 4 l/d) and thereafter according to this scheme: 6 l/d at week 3, 7 l/d at week 4, 8 l/d from week 5 until week 8, 7 l/d at week 9, 6 l/d at week 10 and 4 l/d at week 11. Animals were maintained in individual calf pens for 5 weeks and then they were sorted according to the BW into eight pens (5 calves per pen, 2 pens per treatment). Calves were fed twice daily, in addition to milk replacer and on a free-choice basis, the same total mixed ration (TMR) fed lactating buffalo cows with additional (30% wt) flaked barley (on average on a DM basis 14.8% crude protein, 32.1% NDF, and 7.21 MJ ENL/kg DM). Each calf was weighed individually when assigned to treatment (average BW 40.3±5.7, 39.0±6.2, 38.7±5.5 and 40.5 ± 5.8 kg for groups A, B, C, and D, respectively) and thereafter every two weeks. Faeces of each

calf were observed twice weekly, and consistency was scored using the following scale: (0) severe scours, (1) scours, (2) soft, (3) normal, (4) firm. TMR dry matter intake (DMI) was measured per pen weekly. At the end of the 11-weeks feeding period, total tract apparent digestibility was measured by using acid-insoluble ash (AIA) as indigestible internal marker. The experimental protocol included a 5 d preliminary period followed by 3 a d faeces collection period, replicated 3 times, during which calves received 4 l/d of milk replacer and TMR on a free choice basis. Daily, feed consumption was measured along with the collection of samples of faeces which were combined at the end of the collection period to provide one sample per pen. Feed and faecal samples were analyzed according to Martillotti *et al.* (1987) and Van Keulen and Young (1977). Data were analysed using the Statistical Analysis System package (SAS, 1990). Faecal score and average daily gain (ADG) underwent analysis of variance for repeated measures with treatment (A, B, C, D) as non-repeated factors, and week of observation and week of observation x treatment as repeated factors, while the calf was the experimental unit. DMI over the 3-d faeces collection period and the apparent digestibility coefficients were analysed by analysis of variance for repeated measures with the pen used as the experimental unit. Total BW gain and final BW were analysed by one-way analysis of variance with treatment as factor; the pen was the experimental unit.

RESULTS AND CONCLUSIONS - Two control group calves died (26 and 32 days of age) and were not replaced. Because necropsies of dead calves were not performed, causes of death were not determined. The calves that died, however, showed in the days just before death severe diarrhoea symptoms. The interaction week of observation x treatment was not significant (P>0.4) for all variables tested except faecal score (P<0.017). Control calves had a significantly lower faecal score (P<0.05) than the animals treated with EF68 (Table 1). Although calves fed treatment D showed the highest score, no significant differences were found when the three groups receiving EF68 were compared. An effect of the observation week was found (P<0.001), which may be ascribed to the fact that incidence of diarrhoea progressively declined as the trial progressed (Table 1). A positive effect of viable culture of LAB on faecal consistency has been widely reported, regardless of the type of microorganism used (Abe *et al.*, 1995; Abu-Tarboush *et al,* 1996; Donovan *et al.*, 2002; Khuntia and Chaudhary, 2002). Milk replacer was entirely consumed by the calves. The milk allowance for the first 2 weeks was determined by initial BW and thereafter was the same for all calves. It could not therefore be affected by microbial supplementation. TMR - DMI increased throughout the experimental period. This accounts for the effect of the observation week being always significant (P<0.001).

Table 1. Total mixed ration dry matter intake (TMR DMI), average daily gain (ADG), final BW and total BW gain of calves on different treatments.

	Treatment				SE	Effect (P)	
	A	B	C	D		Week	Treatment
Faecal score	1.1[a]	1.6[b]	1.7[b]	1.8[b]	0.7	0.0001	0.04
TMR DMI (g/d) 5-11 wk	406	417	410	432	10.7	0.0001	0.89
Final BW (kg)	89.9	95.4	97.6	97.3	3.96		0.46
Total BW gain (kg)	50.4	56.4	58.9	56.8	3.10		0.23
ADG, (g/d) 1-11 wk	640	708	718	749	122	0.0001	0.20

[a,b] P<0.01.

Table 2. Dry matter intake (DMI) over the faeces collection period and digestibility coefficients of calves on different treatments.

	Treatment				SE	Effect (P)	
	A	B	C	D		Replicate	Treatment
DMI (g/d)	1665	1590	1450	1725	0.015	0.3592	0.19
Organic matter digestibility	0.76	0.80	0.81	0.80	0.015	0.89	0.37
Crude protein digestibility	0.65	0.70	0.74	0.71	0.022	0.80	0.19
NDF digestibility	0.52	0.53	0.59	0.55	0.032	0.65	0.63

EF68 administration unaffected intake of TMR (Table 1). Calf weight increased linearly and no weight loss was observed. The effect of week of observation on ADG was highly significant (P<0.001). The data on final BW and ADG varied among the four groups. Although final BW, total BW gain and ADG tended to be greater in EF68 treated groups than in control calves, the differences did not reach statistical significance. Extensive research (Jenny *et al.*, 1991; Higginbotham and Bath, 1993; Abu-Tarboush *et al.*, 1996; Cruywagen *et al.*, 1996) failed to find improvement in growth from LAB. DMI over the 4-d faeces collection periods and the apparent digestibility coefficients are presented in Table 2. The replicate x treatment interactions were not significant (P>0.5). DMI was not affected by treatment. No significant differences in digestibility coefficients were found as the effect of EF68 administration. A similar finding was noted by Abu-Tarboush *et al.* (1996) on dairy calves fed LAB. It may be concluded that the addition of *E. faecium* to buffalo calf diets does not contribute to higher BW at weaning in these experimental conditions, but may be able to reduce diarrhoea and may indirectly affect the mortality rate. Daily supplementation for 3 consecutive days every 7 days up to 3 months of age allows this effect to be achieved at lower cost.

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