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Ficus hookeri tree leaves as herbal feed additives to enhance ruminal fermentation and reduced protozoal population in growing crossbred cattle

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ABSTRACT

An experiment was conducted to evaluate the effect of dietary supplementation of *Ficus hookeri* leaf meal as herbal feed additive on rumen protozoal population, enzyme profile and fermentation characteristics in growing crossbred calves. Ten growing male crossbred (Jersey × Tharparker) calves with similar initial body weight of 91.8±1.37 kg, were randomly divided into two equal groups (T_1 and T_2) and fed individually under stall feeding for 140 days on a mixed ration containing rice straw and concentrate mixture in 50:50 ratio. Two types (C1 and C2) of iso-nitrogenous concentrate mixtures were prepared. Wheat bran in concentrate mixture (C2) of test group (T_2) was partially replaced (4 parts w/w) with *Ficus hookeri* leaf meal. Daily dry matter intake between two experimental groups was similar. Rumen *p*H, NH_3-N concentration and total rumen protozoal population decreased while ruminal TVFA and propionic acid production increased due to dietary supplementation of *Ficus hookeri* leaf meal as herbal feed additive. Activity of carboxymethyl cellulase, xylanase and β-glucosidase enzymes were significantly higher in the rumen liquor of calves fed *Ficus hookeri* leaf meal. It could be concluded that dietary supplementation of *Ficus hookeri* leaf meals as herbal feed additive have a potential for reducing rumen protozoal population and ammonia nitrogen concentration with improving ruminal TVFA and propionate production in growing crossbred calves.

Keywords: Calves, Herbal feed additive, Enzyme profile, Rumen fermentation, Rumen protozoa

The manipulation of the rumen microbial ecosystem is an important strategy for enhancing fibrous feed digestibility and reducing methane emission, rumen protozoal population and nitrogen excretion for ensuring efficient feed utilization, resulting in better performances in ruminants (Alexander *et al.* 2008, Choudhary *et al.* 2022). Synthetic/chemical feed additives have been proved very effective to manipulate rumen fermentation for reducing ruminal methanogenesis, dietary energy and protein losses. However, contemporary bio-security threats have restricted their use in the diet in many developed countries (Malik *et al.* 2019, e Silva *et al.* 2021) and even banned their use in ruminant diets in the European Union (Tilahun *et al.* 2022) because of the risk of their transmission into meat and milk as well as the antimicrobial resistance (e Silva *et al.* 2021, Swelum *et al.* 2021). Plant secondary metabolites in tree leaves are capable of manipulating rumen metabolism and this could have environmental and production merits (Terranova *et al.* 2018, Choudhary *et al.* 2022). The major factors influencing the effects of plant secondary metabolites in ruminant nutrition are the chemical nature of plant secondary metabolites, their concentration in diet and the diversity and abundance of rumen microbiota (Malik *et al.* 2017). Recently, plant herbs as well as tree leaves have been widely used as natural rumen manipulator to manipulate rumen ecology to improve production performances of ruminants *in vivo* (Cherdthong *et al.* 2019, Santra *et al.* 2020, Bhatt *et al.* 2021, Tilahun *et al.* 2022).

Based on previous *in vitro* studies on methane and protozoa population reduction potential (Choudhary 2022), *Ficus hookeri* tree leaves which have not yet been investigated, were selected in this *in vivo* study. To the best of our knowledge, no studies have been conducted to determine the effect of *Ficus hookeri* leaf powder as herbal feed additive on feed digestibility, rumen fermentation parameters, enzyme activities and microbial population in ruminants. Therefore, the aim of this study was to examine the effect of inclusion of *Ficus hookeri* leaf meal as herbal feed additive on ruminal fermentation, enzyme profile and protozoal diversity in growing cattle.

MATERIALS AND METHODS

Collection and processing of plant materials to use as herbal feed additive: Ficus hookeri tree leaves were collected from subtropical and temperate regions of Meghalaya, India, located between 24.58°N to 26.07°N latitudes and 89.48°E to 92.51°E longitudes. Samples consisted of a mixture of young and adult leaves collected

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during summer (May), monsoon (August), winter (December) and spring (March) season. Leaves were collected from ten different trees with a height of 6-12 ft, mixed properly and sun-dried for three days. Dried tree leaves were ground in a hammer mill, passed through 2 mm sieve and stored in air-tight container for experimental use.

Animals, feeding and management: Experiment was conducted on ten growing male cross-bred (Jersey × Tharparker) calves (age four months, body weight 91.8±1.37 kg). Two types (C1 and C2) of iso-nitrogenous concentrate mixtures were prepared (Table 1). Wheat bran, 4% (w/w) in concentrate mixture (C2) of test group (T_2) was replaced with *Ficus hookeri* leaf meal (sun-dried leaf powder). Roughage used for feeding to the experimental animals was paddy straw. All the experimental calves were fed individually under stall feeding on total mixed ration with roughage (paddy straw) to concentrate ratio of 50:50 for duration of 140 days. Feed was offered to all experimental animals once daily at 9:00 AM after discarding previous day's residue if any. Calves of test group (T_2) were fed *Ficus hookeri* leaf meal @ 2% of total diet as herbal feed additive. Record of daily feed intake was maintained throughout the experimental period. Proper health management and sanitation conditions were maintained throughout the experimental period. The animals were dewormed at the beginning of the experiment.

Collection of rumen liquor samples and counting of rumen protozoa: Rumen liquor was collected from all experimental calves on 01, 02, 04, 08, 15, 30, 45, 60, 75, 90, 105, 120 and 140 days of experimental feeding at 0 h post feeding by stomach tube for counting the rumen protozoal numbers. After collection of rumen liquor, the samples were processed immediately for counting of rumen protozoa as described earlier by Santra and Karim (2002).

Rumen liquor was also collected consecutively for three days from all experimental calves after 100 days of experimental feeding at 0, 2, 4, 6, 8 and 10 h post feeding by stomach tube. The collected rumen fluid was strained through two layer of muslin cloth having 100 µm pores to remove feed particles. After collection of the rumen liquor, pH was recorded immediately and a sub samples were preserved at -25°C for further chemical analysis.

Chemical analysis of biological samples: The feed offered and residues left were analyzed for DM by drying at 100°C for 24 h, OM by ashing at 550°C for 4 h and crude protein (CP) by Kjeldahl technique (AOAC 2012). NDF, ADF and ADL were estimated following the method of Van Soest (1991). Cellulose content was calculated by subtracting ADL from ADF. TVFA estimation of rumen liquor was carried out as described by Barnett and Reid (1957) and VFA fractionations were estimated in a gas chromatograph (Netel Ultima 2100) as described by Cottyn and Boueque (1968). Total and differential count of rumen protozoa were done as described by Kamra *et al.* (1991). Ammonia nitrogen concentration in the rumen liquor was estimated as per method of Weatherburn (1967). For estimation of enzyme activity, rumen liquor was processed as described by Patra et al. (2006) and enzyme activity was estimated by the dinitrosalicyclic acid method (Miller, 1959). The total phenols, total tannins (PVPP-tannins) and the condensed tannins in the feed samples were determined, as per the procedure given by Makkar (2003).

Statistical analysis: Data were analyzed by the methods described by Snedecor and Cochran (1994). The data were subjected to analysis of variance (one way ANOVA) by means of SPSS version 20.0 and significant treatment effect was determined by comparing the means with Duncan's multiple range test (Duncan 1955).

Table 1. Proportions of ingredients and chemical composition of concentrate mixtures, rice straw and *Ficus hookeri* leaf meal

Attribute	Concentrate mixtures		Rice straw	*Ficus hookeri* leaf meal
	C1	C2		
Ingredients, %				
Ficus hookeri leaf meal	00	4.0		
Maize grain	45.0	45.0		
Wheat bran	29.0	25.0		
Mustard cake	23.0	23.0		
Mineral mixture	2.0	2.0		
Common salt	1.0	1.0		
Vitablend® AD_3	@ 20 g/100 kg concentrate mixture			
Chemical composition, on % DM				
Organic matter (OM)	93.7	93.4	86.6	84.2
Crude protein (CP)	18.2	17.7	3.4	10.4
Ether extract (EE)	4.7	4.5	1.9	3.6
Total carbohydrate (TCHO)	70.8	71.2	81.3	70.2
Neutral detergent fibre (NDF)	34.8	35.7	71.5	55.4
Acid detergent fibre (ADF)	11.8	12.1	41.1	37.3
Cellulose	7.6	7.8	34.3	26.6
Acid detergent lignin (ADL)	3.9	4.1	6.1	11.5

RESULTS AND DISCUSSION

Diet composition: The concentrate mixture C1 and C2 contained 18.2 and 17.7% crude protein (CP), 7.6 and 7.8% cellulose and 3.9 and 4.1% acid detergent lignin (ADL), respectively (Table 1). The overall nutrient composition of concentrate mixture (C1) for feeding to the control group (G1) and concentrate mixture (C2) for feeding to the test group (G2) was similar. Rice straw was contained 3.4% CP, 34.3% cellulose and 6.1% ADL on DM basis. *Ficus hookeri* leaf meal consisted of 84.2% OM, 10.4% CP, 3.6% EE, 55.4% NDF and 26.6% cellulose. Moreover, *Ficus hookeri* leaf meal contained 5.1% condensed tannin, 0.1% hydrolysable tannin and 5.9% total phenolic compounds in the present study. The chemical composition of rice straw used in the present experiment was comparable to the values reported earlier by Santra *et al.* (2013). In the present experiment *Ficus hookeri* leaf powder contained 10.4% crude protein which was lower than earlier reported value for *Ficus sp.* tree leaves (Khan *et al.* 2014). Similar chemical composition of *Ficus hookeri* tree leave was earlier reported by Choudhary *et al.* (2022).

Nutrient intake: Feed intake is normally explained in relation to metabolic body size ($BW^{0.75}$) or more simply as a percentage of body weight. Dry matter intake through rice straw, concentrate mixture and total dry matter intake in terms of g/kg $BW^{0.75}$/d as well as total dry matter intake in terms of kg/100 kg body weight/d were similar between the two experimental groups (Table 2). The dry matter intake as % of body weight was 3.3 and 3.2% for the experimental calves of T_1 and T_2 groups, respectively. Dietary supplementation of *Ficus hokkeri* leaf meal as herbal feed additive had no effect on daily organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) intake. Similar dry matter intake in terms of per unit body weight due to dietary supplementation of herbal feed additive as observed in the present experiment was also reported by various workers earlier. It was earlier reported that supplementation of *Sapindus mukorossi* leaves @ 3% in diet as herbal feed additive had no effect on daily feed intake in Rathi calves (Meel *et al.* 2015). Olafadehan *et al.* (2016) observed that inclusion of tannin-containing *Ficus polita* foliage up to 700 g/kg of DM in a total mixed ration for goat did not compromise voluntary feed intake. Similarly, a non-significant effect of tropical tree leaves supplementation as herbal feed additive on dry matter intake (DMI, g/d) was reported in sheep between control and test group (Malik *et al.* 2017). On contrary, Totakul *et al.* (2022) reported that supplementation of 6% *Cnidoscolus aconitifolius* leaf in the diet of lactating cows, improved daily dry matter intake.

Rumen protozoal population: Ciliate protozoa present in the rumen liquor of experimental claves from both the experimental groups (T_1 and T_2), belonged to a B-type population due to presence of *Epidinium sp.* and the absence of *Polyplastron multivesiculatum* (Coleman 1980).

Table 2. Feed intake in growing calves fed *Ficus hookeri* leaf meal as herbal feed additive

Item	Level of *Ficus hookeri* leaf meal (%) / Experimental groups		SEM	Level of significance
	0 (T_1)	2 (T_2)		
DM intake				
Rice straw				
kg/d	2.2	2.2	0.03	NS
g/kg $BW^{0.75}$/d	53.9	52.9	1.83	NS
Concentrate				
kg/d	2.4	2.3	0.05	NS
g/kg $BW^{0.75}$/d	58.8	55.3	1.74	NS
Ficus hookeri leaf meal				
kg/d	00	0.09	0.01	-
g/kg $BW^{0.75}$/d	00	2.2	0.05	-
Total intake				
kg/d	4.6	4.6	0.07	NS
kg/100 kg BW/d	3.3	3.2	0.09	NS
g/kg $BW^{0.75}$/d	112.7	110.5	4.15	NS
Nutrient intake (kg/d)				
Dry matter (DM)	4.6	4.6	0.07	NS
Organic matter (OM)	4.2	4.1	0.05	NS
Crude protein (CP)	0.50	0.48	0.02	NS
Neutral detergent fibre (NDF)	2.4	2.3	0.07	NS
Acid detergent fibre (ADF)	1.1	1.2	0.04	NS

NS, Non-significant; SEM, Standard error of mean.

Rumen protozoal number decreased drastically up to 15 days of experimental feeding in the calves of T_2 group due to dietary supplementation of *Ficus hookeri* leaf meal and thereafter the number of rumen protozoa remains almost static during rest of the experimental period (Fig. 1).

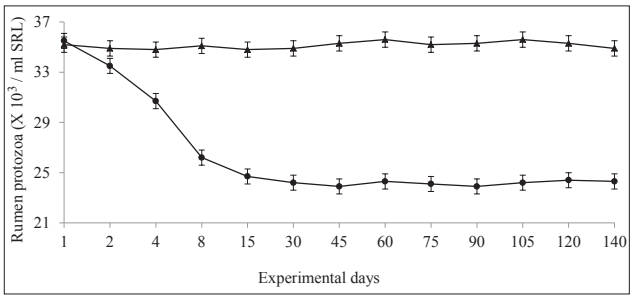

Fig. 1. Rumen total protozoal population in growing claves fed *Ficus hookeri* leaf meal (T_1: ▲ = un-supplemented: T_2: ● = supplemented with *Ficus hookri* leaf meal).

The total rumen ciliate protozoal number ranged from 26.5 to 40.8 ($\times 10^3$) per ml rumen liquor whereas spirotrich protozoa number ranged from 23.9 to 36.1 ($\times 10^3$) and holotrich protozoa ranged from 2.6 to 4.7 ($\times 10^3$) per ml of rumen liquor of experimental calves (Table 3). Number of total rumen protozoa was lower ($P<0.01$) in T_2 than T_1

Table 3. Rumen protozoal population (× 10^3 /ml)* in growing calves fed *Ficus hookeri* leaf meal as herbal feed additive

Attribute	Level of *Ficus hookeri* leaf meal (%) / Experimental groups		SEM	Level of significance
	0 (T_1)	2 (T_2)		
Large Holotrich	1.2	0.5	0.02	P<0.01
Small Holotrich	3.5	2.1	0.05	P<0.01
Total Holotrich	4.7	2.6	0.08	P<0.01
Large Spirotrich	11.5	7.2	0.13	P<0.01
Small Spirotrich	24.6	16.7	0.34	P<0.01
Total Spirotrich	36.1	23.9	0.47	P<0.01
Total rumen protozoa	40.8	26.5	0.83	P<0.01

SEM, Standard error of mean. *Each value is an average of sixteen separate observations and each observation is an average of the protozoa count in 30 microscopic fields (magnification 100 ×).

group. The holotrich protozoa had an average cell size of 103 × 54 µm (range 42 – 179 µm × 22 – 103 µm) while spirotrich protozoa had an average cell size of 87 × 49 µm (range 28 – 171 µm × 15 – 107 µm). Moreover, the ciliate protozoal population present in the rumen liquor of the calves from both the experimental groups in the present study was almost entirely spirotrich protozoa (88.5% of total protozoal population in T_1 group while, 90.2% of total protozoal population in T_2 group). Santra and Karim (2002) and Santra *et al.* (2012) also reported that spirotrich protozoa comprised more than 80% of total rumen protozoa population under Indian condition. The lowest number of rumen ciliate protozoal count was observed just before feeding with increased in total protozoal number at 6 h (peak value) followed by a gradual decrease at 8 to 10 h post-feeding in the calves of both the experimental groups (Fig. 2A). Feed offered was associated with an abrupt increase in the total protozoa population within 6 h of post feeding in the present experiment, which could be due to migration of ciliate protozoa from the reticulo-rumen wall from where they sequestered to the rumen medium in response to chemical stimuli originating from the diet (Santra and Karim 2002). The migration of ciliates from the reticulo-rumen wall into rumen liquor was due to chemotactic movement of protozoa towards the feed entering the rumen. After the feed was utilized, the protozoa gradually migrated back to the reticulo-ruminal wall resulting in the observed drop in their numbers in the rumen liquor at 8 to 10 h post-feeding in the experimental calves of T_1 and T_2 group as observed in the present experiment (Fig. 2A, B, and C). The total rumen protozoa number as well as holotrich and spirotrich protozoa number decreased (P<0.01) in the calves of T_2 group. It was earlier also reported by many workers that feeding of selected tropical/Indian tree leaves reduced rumen protozoa population in ruminant animals due to presence of plant secondary metabolites like tannin, saponin, essential oils and flavonoids (Meel *et al.* 2015, Santra *et al*. 2016, Malik *et al.* 2017, Cherdthong *et al.* 2019). Further, plants from the genus *Ficus* consist of a variety of phytochemicals including phenolics, polyphenols, flavonoids, tannins and saponins (Hilou 2012). The inhibitory effect of *Ficus hookeri* leaf meal as herbal feed additive on rumen protozoa in the present experiment could be due to saponin content. All types of saponins reduce the rumen protozoal population (Patra and Saxena 2009). The antiprotozoal effect of saponins is attributed to the binding of saponins to cholesterol in the protozoal cell membrane, causing cell lysis (Cheeke 2000) resulting in lower rumen protozoal number.

Rumen fermentation pattern and enzyme profile: Rumen *p*H was lower (P<0.05) and TVFA as well as propionic acid concentrations were higher (P<0.05) due to dietary supplementation of *Ficus hookeri* leaf meal as herbal feed additive in growing calves (Table 4). However, dietary supplementation of *Ficus hookeri* leaf meal as herbal feed additive had no effect on butyric acid, total nitrogen and TCA soluble nitrogen concentration in the rumen of calves. The *p*H decreased from 0 to 6 h post feeding in the serial rumen liquor sample, followed by an increase at 8 to 10 h post feeding in the calves of all experimental groups (Fig. 2B). TVFA production at similar time intervals exhibited reverse trend (Fig. 2C) to that of rumen *p*H. Lower rumen *p*H in *Ficus hookeri* leaf meal fed calves, was most probably due to the reduction in rumen

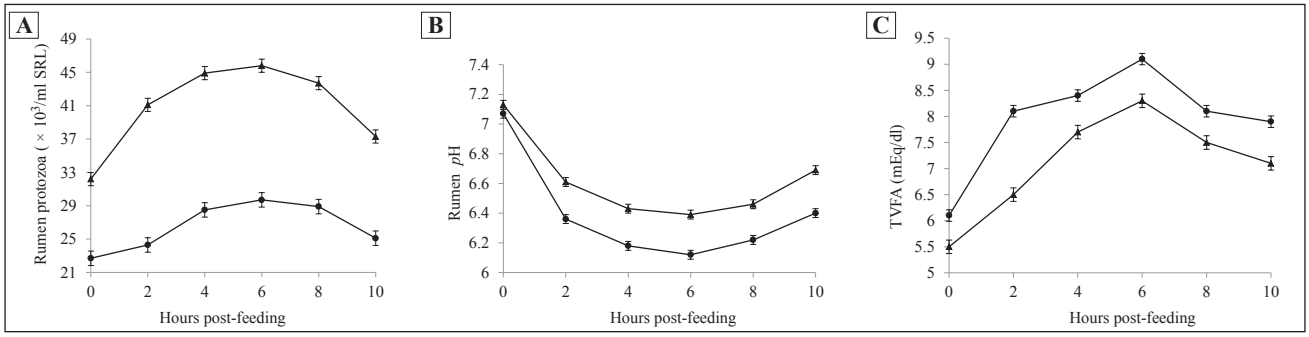

Fig. 2. A. Rumen total protozoal population in claves fed *Ficus hookeri* leaf meal (G1: ▲ = un-supplemented: G2: ● = supplemented with *Ficus hookri* leaf meal); B. Rumen *p*H in claves fed *Ficus hookeri* leaf meal (T_1: ▲ = un-supplemented; T_2: ● = supplemented with *Ficus hookri* leaf meal); C. Rumen TVFA concentration in claves fed *Ficus hookeri* leaf meal (T_1: ▲ = un-supplemented; T_2: ● = supplemented with *Ficus hookri* leaf meal).

Table 4. Rumen metabolites, volatile fatty acid production and enzyme profile in growing calves fed *Ficus hookeri* leaf meal as herbal feed additive

Attribute	Level of *Ficus hookeri* leaf meal (%) / Experimental groups		SEM	Level of significance
	0 (T_1)	2 (T_2)		
Rumen metabolites				
*p*H	6.62	6.39	0.019	P < 0.05
TVFA (meq/100 ml)	7.1	7.9	0.17	P < 0.05
Acetate (mol/100 mol)	68.5	67.4	0.24	P < 0.05
Propionate (mol/100 mol)	22.2	23.2	0.15	P < 0.05
Butyrate (mol/100 mol)	9.3	9.4	0.07	NS
Acetate: propionate ratio	3.1	2.9	0.01	P < 0.05
Total – N (mg/100 ml)	67.3	66.7	1.72	NS
TCA-soluble-N (mg/100 ml)	32.7	31.9	0.58	NS
NH_3-N (mg/100 ml)	8.4	7.6	0.13	P<0.01
Rumen enzyme activity (IU/100 ml)				
Carboxy methyl cellulase	95.6	110.7	2.83	P<0.01
Xylanase	126.5	139.5	3.02	P<0.01
β-glucosidase	13.9	16.8	0.27	P<0.05
Amylase	208.7	211.9	9.75	NS

NS, Non-significant; SEM, Standard error of mean.

protozoal population (Santra and Karim 2002). Rumen protozoa seem to have a *p*H stabilizing effect in the rumen due to engulfment of excess starch from the rumen (Hristov *et al.* 2001). Further, higher propionic acid production in the rumen of *Ficus hookeri* leaf meal fed calves was due to a shift in rumen fermentation towards propionate at the expense of acetate production (Shingfield *et al.* 2008). Lower (P<0.01) rumen ammonia nitrogen concentration was observed in the rumen liquor of calves fed *Ficus hookeri* leaf meal as herbal feed additive (T_2 group) in comparison to control group (T_1 group). The presence of protozoa in the rumen ecosystem is associated with increased recycling of microbial nitrogen in the rumen and therefore, decreased protozoal population in the rumen are usually associated with lowered ammonia concentrations, primarily as a result of a decrease in proteolysis of bacterial protein by ruminal protozoa (Hristov *et al.* 2005). Lower (P<0.01) rumen ammonia nitrogen concentration was observed in the rumen liquor of calves fed *Ficus hookeri* leaf meal as herbal feed additive (T_2 group) in comparison to control group (T_1group) in the present experiment might be due to lower number of rumen protozoa. The concentration of total nitrogen and ammonia nitrogen in the serial rumen liquor samples peaked at 6 h post feeding followed by a gradual decrease at 8 to 10 h post-feeding.

Activities of polysaccharide degrading enzymes, e.g. carboxymethyl cellulase, xylanase and β-glucosidase were significantly higher in the rumen of calves fed *Ficus hookeri* leaf meal as herbal feed additive (T_2 group). However, ruminal activity of amylase enzyme was not influenced by the dietary supplementation of *Ficus hookeri* leaf meal. Generally, presence of ciliate protozoa, inhibit the cellulolytic activity of rumen fungi (Widyastuti *et al.* 1995) as well as rumen bacteria (Lee *et al.* 1999). Decreased protozoal population in the rumen is usually associated with higher rumen bacterial and fungal population as evident from the defaunated animals (Chaudhary *et al.* 1995). Therefore, activity of fibre degrading enzymes, e.g. carboxymethyl cellulase, xylanase and β-glucosidase was increased by the dietary supplementation of *Ficus hookeri* leaf meal might be due to higher number of rumen bacteria and fungi due to lower rumen protozoal population.

From this study, it can be concluded that dietary supplementation of *Ficus hookeri* leaf meal as a herbal feed additive improved propionate concentration, whereas the number of protozoa and ammonia nitrogen concentration were decreased. Therefore, supplementation of *Ficus hookeri* leaf meal in ruminant feeding at 2% of total ration as herbal feed additive is recommended for manipulating rumen fermentation for improving the productive performance of the animals. However, future studies on the effect of *Ficus hookeri* leaf meal should be done to clarify this through production trial.

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