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Full Length Research Paper

Manipulation of ruminal protozoa of crossbred calves by herbal rumenotoric drugs

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An experiment was conducted to investigate the efficacy of market drug (*Rumizyme*) and self formulated rumenotoric drug on the rumen protozoa such as *Dasytricha, Isotricha, Entodinium* and *Diplodinium* and effects on rumen pH of crossbred calves. The trial was conducted with twenty seven crossbred calves and divided randomly into three similar groups (3 calves in each) for three different trials (1st, 2nd & 3rd Trials) and each trial was conducted for 60 days excluding pre experimental period. Group T_2 and T_3 were supplemented with market drug (one bolus/day) and self formulated rumenotoric drug (40 gm/day) respectively for each animal while group T_1 (control) was fed without any drug. During each trial at 15, 30, 45 and 60 days of interval the *Dasytricha, Isotricha, Entodinium Diplodinium,* and rumen pH were measured. Results revealed that the administration phytogenic feed additives significantly ($P<0.05$) increased the population of *Dasytricha, Isotricha, Entodinium* and *Diplodinium* in T_3 followed by T_2 and minimum in T_1 group in each trial at different periods. The rumen pH significantly ($P<0.05$) decreased in T_3 group followed by T_2 and T_1 at different time intervals (15, 30, 45 and 60 days) during each trial.

Key words: Herbal, rumenotoric, rumen protozoa, crossbred.

INTRODUCTION

The ban on nutritive antibiotic use in Europe and the increased awareness of the consumers triggered a need for natural and safe feed additives to achieve better production results from farm animals. Plant extracts are used in animal nutrition as appetizer and digestive stimulants, stimulants of physiological functions, for prevention and treatment of certain pathological conditions, as colorants and antioxidants. Rumen ecology plays an important role in the fermentation process in ruminant digestion. Role of rumen microflora in digestion of feed is vital for nutrient utilization in ruminants. Rumen function modulator optimizes population and activity of ruminal microflora. This also results in efficient cellulose digestion and also, it facilitates maintenance of normal rumino-reticular and intestinal movement for proper maceration as well as the mixing and passage of ingesta and normal expulsions of gases. Consequently, phytogenic feed additives help to increase the resistance of the animals exposed to different stress situations and increase the absorption of essential nutrients, thus improving the growth of the animals (Windisch et al., 2008). There are numerous studies showing beneficial effects of phytogenic feed additives on feed intake, immune functions and health, rumen fermentation and productivity of calves, dairy cows, heifers and beef cattle (Wawrzynczak et al., 2000; Kraszewski et al., 2002; Greathead, 2003; Cardozo et al., 2006). Characteristics of ruminal protozoa such as *Dasytricha, Isotricha, Entodinium* and *Diplodinium* as well as ruminal pH differed significantly between control and treatments with herbal formulations (El-Kholy and Salama, 1995; Randhawa et al., 1995; Shukla et al.,1999, Mishra et al., 2004; Singh and Singh, 2006; Phengvilaysouk et al., 2008; Bhatt et al., 2009; Yadav et al., 2009; Santra et al.,2012). Furthermore, Dolezal et al., (2011) found that addition of yeast culture significantly increased ruminal pH and numbers of protozoa in the rumen of dairy cows. With the above views the current study was designed to evaluate the efficacy of natural formulation like *Apium graveolens, Amomum subulatum, Tinospora cordifolia, Andrographis*

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Table 1. Composition of marketed herbal drug *(Rumizyme)*.

Sl. No.	Ingredients	**Properties** (Garter, 1948)	Each 100gm Contains
1	*Pueraria tuberose*	Antioxidant	10 gm.
2	*Hemidesmus indica*	-do-	5 gm.
3	*Phyllanthus niruri*	Stomachic and diuretic	10 gm.
4	*Terminalia chebula*	Antipyretic and purgative	5 gm.
5	*Andrographis paniculata*	Laxative and rejuvenative	15 gm.
6	*Trachyspermum ammi*	stimulant and carminative	4 gm.
7	*Pimpinella anisum*	antimycotic and antimicrobial	4 gm.
8	*Zingiber officinale*	stimulant and carminative	2 gm.
9	*Leptadenia reticulate*	Antibacterial and diuretic	5 gm.
10	*Boerhavia diffusa*	Antibacterial and Antinociceptive	4 gm.
11	*Eclipta alba*	Antibacterial and antihaemorrhagic	5 gm.
12	*Sindhav salt*	laxative and digestive	14 gm.
13	*Beed Lavan*	-	5 gm.
14	*Excipients*	-	12 gm.

paniculata, Phylanthus nuriry, Trachyspermum ammi, Terminalia chebula, Cuminum cyminum, Piper nigrum, Zingiber officinale, Piper longum, Plumbago rosea, Pmbelia ribes, yeast, Ammonium chloride Black Salt and market rumenotoric drug (*Rumizyme*) as feed additives on rumen protozoal population and pH in three sets of trial.

MATERIALS AND METHODS

The experiment was conducted in the Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005 (India) to evaluate the effect of marketed drug (*Rumizyme*) and self formulated rumenotoric drug on the rumen protozoa such as *Dasytricha, Isotricha, Entodinium* and *Diplodinium* of crossbred calves. In this study twenty seven crossbred calves with an average body weight 77 to 80 Kg and 6 to 12 months of age were used. During the investigation, three experimental trials were conducted; the first trial was held from 01.07.2008 to 30.09.2008; the second trial took place from 01.11.2008 to 30.01.2009 and the third trial was from 01.03.2009 – 02.06.2009. The experimental calves were divided randomly into three groups (3 calves in each) for each trial. **Table 1** shows the composition of marketed rumenotoric drug; while in case of self formulated rumenotoric drug, some modification were brought in the composition of market rumenotoric drug (*Rumizyme* that is, *Pueraria tuberose, Hemidesmus indica, Pimpinella anisum, Eclipta alba, Sindhav salt, Boerhavia diffusa, Pimpinella anisum* and *Leptadenia reticulate* were replaced by *Apium graveolens, Amomum subulatum, Tinospora cordifolia, Cuminum cyminum, Piper nigrum, Piper longum, Plumbago rosea, Pmbelia ribes, yeast,* Ammonium chloride and Black Salt. Calves of the T_1 group (control) were reared on normal feeding (As per recommendation of NRC-1989), T_2 and T_3 groups received same ration with marketed drug (one bolus/day) and self formulated rumenotoric drug (40gm/day) respectively for each calf. The animals of various experimental groups were fed with standard farm ration comprising of green fodder (Bajara, Maize and Berseem etc. depending on seasonal availability) and wheat straw as the dry roughage along with a balanced concentrate mixture and mineral to meet their nutritional requirements. During the entire period possible scientific care was exercised to maintain hygienic conditions and to avoid infectious diseases in the experimental animals. These animals were dewormed using bolus of *Bandy Kind Plus* before initiating the experiments. During each trial at 15, 30, 45 and 60 days interval, meanwhile the rumen protozoa such as *Dasytricha, Isotricha, Entodinium and Diplodinium* and ruminal pH of crossbred calves were measured.

Collection and Processing of Rumen Liquor

Rumen liquor from non-fistulated animals was collected with the help of a stomach tube at 0 hr (immediately before feeding) by using aspiration bottle and collection was done once in a day of the last three days of each feeding trail. On the days of collection of rumen liquor; water was offered to the animals at 8.00 A.M. Immediately after collection, about 100 ml of representative sample of rumen liquor was brought to the laboratory in a flask closed with a rubber stopper to

Table 2. Rumen pH and protozoal concentration (x10^5/ml) in different groups of crossbred calves at different intervals during first trial.

Group	Parameters	1st Trial			
		15 Days	30 Days	45 Days	60 Days
Control Group (T_1)	*Isotricha*	0.31^{e}±0.00	0.35^{e}±0.01	0.36^{e}±0.01	0.39^{de}±0.01
	Dasytricha	0.09^{d}±0.03	0.13^{cd}±0.04	0.18^{bcd}±0.04	0.21^{bcd}±0.01
	Entodinium	2.45^{cd}±0.05	2.49^{cd}±0.06	2.53^{bcd}±0.06	2.54^{bc}±0.04
	Diplodinium	0.21^{g}±o.00	0.24^{g}±0.00	0.28^{fg}±0.00	0.34^{ef}±0.01
	Rumen pH	7.38^{ab}±0.15	7.22^{abcd}±0.05	7.43^{a}±0.07	7.34^{abc}±0.05
Market herbal drug (T_2)	*Isotricha*	0.33^{e}±0.01	0.45^{cd}±0.03	0.52^{c}±0.05	0.63^{b}±0.05
	Dasytricha	0.11^{d}±0.02	0.14^{bcd}±0.03	0.17^{bcd}±0.02	0.25^{abc}±0.01
	Entodinium	2.27^{e}±0.06	2.36^{de}±0.07	2.48^{cd}±0.05	2.54^{bc}±0.07
	Diplodinium	0.37^{def}±0.03	0.44^{cde}±0.03	0.50^{bc}±0.04	0.58^{ab}±0.04
	Rumen pH	7.14^{cd}±0.01	7.24^{abcd}±0.00	7.25^{abcd}±0.04	7.16^{bcd}±0.01
Self Compounded herbal drug (T_3)	*Isotricha*	**0.34^{e}±0.01**	**0.48^{c}±0.01**	**0.61^{b}±0.00**	**0.75^{a}±0.01**
	Dasytricha	**0.11^{d}±0.05**	**0.22^{abcd}±0.06**	**0.26^{ab}±0.05**	**0.34^{a}±0.03**
	Entodinium	**2.58^{bc}±0.03**	**2.69^{ab}±0.03**	**2.77^{a}±0.03**	**2.85^{a}±0.01**
	Diplodinium	**0.37^{ef}±0.02**	**0.47^{cd}±0.02**	**0.52^{abc}±0.05**	**0.62^{a}±0.04**
	Rumen pH	**7.11^{cd}±0.02**	**7.15^{bcd}±0.03**	**7.80^{de}±0.02**	**6.87^{e}±0.14**

Means with different letters differ significantly (p<0.05).

Table 3. Rumen pH and Protozoal concentration (x10^5/ml) in different groups of crossbred calves at different intervals during second trial.

Group	Parameters	2nd Trial			
		15 Days	30 Days	45 Days	60 Days
Control Group (T_1)	*Isotricha*	0.34^{bc}±0.03	0.29^{cde}±0.04	0.30^{cd}±0.02	0.34^{bc}±0.01
	Dasytricha	0.76^{bcd}±0.02	0.82^{bc}±0.02	0.87^{b}±0.02	0.82^{bc}±0.01
	Entodinium	2.89^{cd}±0.03	3.02^{cd}±0.11	3.22^{c}±0.02	3.08^{cd}±0.05
	Diplodinium	0.64^{abc}±0.05	0.63^{abc}±0.02	0.71^{a}±0.06	0.69^{ab}±0.03
	Rumen pH	7.11^{ab}±0.04	7.20^{ab}±0.03	7.10^{ab}±0.01	7.12^{ab}±0.04
Market herbal drug (T_2)	*Isotricha*	0.21^{e}±0.02	0.22^{e}±0.03	0.30^{cd}±0.04	0.31^{bcd}±0.02
	Dasytricha	0.42^{f}±0.03	0.57^{ef}±0.10	0.59^{def}±0.06	0.65^{cde}±0.07
	Entodinium	2.58^{d}±0.05	2.99^{cd}±0.23	3.43^{bc}±0.29	3.06^{cd}±0.24
	Diplodinium	0.34^{e}±0.01	0.47^{d}±0.04	0.58^{bcd}±0.06	0.55^{cd}±0.06
	Rumen pH	7.34^{a}±0.06	7.16^{ab}±0.12	6.80^{bc}±0.28	6.62^{cd}±0.10
Self Compounded herbal drug (T_3)	*Isotricha*	**0.25^{de}±0.01**	**0.34^{bc}±0.02**	**0.39^{ab}±0.01**	**0.44^{a}±0.01**
	Dasytricha	**0.67^{cde}±0.06**	**0.81^{bc}±0.07**	**0.92^{b}±0.03**	**1.18^{a}±0.10**
	Entodinium	**2.59^{d}±0.06**	**3.79^{b}±0.04**	**4.60^{a}±0.31**	**4.71^{a}±0.08**
	Diplodinium	**0.60^{abc}±0.03**	**0.67^{abc}±0.05**	**0.68^{ab}±0.00**	**0.73^{a}±0.01**
	Rumen pH	**7.17^{ab}±0.01**	**6.65^{cd}±0.23**	**6.30^{de}±0.13**	**6.13^{e}±0.03**

Means with different letters differ significantly (p<0.05).

maintain anaerobic condition during transport. The rumen liquor was strained with two layers of muslin cloth to remove debris. About 50 ml of strained Rumen liquor (SRL) was poured into a clean plastic bottle containing few drops of 10% mercuric chloride and thoroughly mixed. For protozoal count, 50 ml volume of SRL was preserved by diluting with an equal volume of 50% formalin solution (10% formaldehyde) (Franzolin and Dehority 1996).

Identification and Counting of Rumen Protozoa

The rumen protozoa were identified by making temporary preparations using acidified methylene blue (0.5 gm Methylene blue, 2 ml acetic acid and distilled water up to 100 ml) as a nuclear stain and Lugal's iodine (1 gm Iodine, 2.0 gm Potassium Iodide and distilled water up to 30 ml) for skeletal plats (Dehority 1993). Sketches by Ogimoto and Imai (1981) and

Table 4. Rumen Protozoal concentration (x105/ml) in different groups of crossbred calves at different intervals during third trial.

Group	Parameters	3rd Trial			
		15 Days	**30 Days**	**45 Days**	**60 Days**
Control Group (T_1)	*Isotricha*	1.26^{a}±0.05	1.19^{ab}±0.04	1.21^{ab}±0.06	1.24^{ab}±0.02
	Dasytricha	0.44^{ab}±0.01	0.42^{bc}±0.01	0.45^{ab}±0.01	0.44^{ab}±0.01
	Entodinium	4.60^{a}±0.31	4.38^{ab}±0.32	4.28^{ab}±0.31	4.39^{ab}±0.29
	Diplodinium	0.68^{bc}±0.00	0.73^{abc}±0.01	0.77^{ab}±0.05	0.75^{abc}±0.02
	Rumen pH	6.15^{b}±0.03	6.58^{b}±0.35	6.51^{b}±0.20	6.35^{b}±0.11
Market herbal drug (T_2)	*Isotricha*	0.89^{d}±0.03	1.11^{bc}±0.03	1.19^{ab}±0.04	1.19^{ab}±0.03
	Dasytricha	0.34^{de}±0.01	0.40^{bcd}±0.01	0.41^{bc}±0.04	0.42^{bc}±0.02
	Entodinium	3.22^{cd}±0.02	3.08^{d}±0.05	3.32^{cd}±0.05	3.40^{cd}±0.03
	Diplodinium	0.71^{bc}±0.05	0.73^{abc}±0.04	0.78^{ab}±0.04	0.83^{ab}±0.03
	Rumen pH	7.09^{a}±0.03	7.13^{a}±0.08	6.58^{b}±0.03	6.56^{b}±0.10
Self Compounded herbal drug (T_3)	*Isotricha*	**0.76^{e}±0.05**	**1.03^{c}±0.08**	**1.19^{ab}±0.03**	**1.32^{a}±0.03**
	Dasytricha	**0.31^{e}±0.02**	**0.37^{cde}±0.02**	**0.43^{abc}±0.01**	**0.48^{a}±0.02**
	Entodinium	**3.43^{cd}±0.29**	**3.39^{cd}±0.17**	**3.78^{bc}±0.06**	**4.26^{ab}±0.01**
	Diplodinium	**0.59^{c}±0.08**	**0.78^{ab}±0.10**	**0.82^{ab}±0.03**	**0.89^{a}±0.03**
	Rumen pH	**6.61^{b}±0.08**	**6.30^{b}±0.02**	**6.32^{b}±0.15**	**6.26^{b}±0.10**

Means with different letters differ significantly (p<0.05).

williams and coleman (1992) formed the basis of identification. The rumen protozoa were counted with the help of a haemocytometer under l00x magnification. The pH of rumen fluid was determined with the help of digital pH meter or automatic pH meter in the laboratory immediately after straining; collected rumen liquor through muslin cloth. Finally the data were statistically analyzed using GLM procedure of SAS (1992). Duncan's test (1955) was applied to separate means that were significantly different.

RESULTS AND DISCUSSION

Although the presence of the market drug (*Rumizyme*) and self formulated rumenotoric drug had significant effect on the protozoa absolute numbers, they tended to increase with advancement of age in all the trials (**Table 2, 3 and 4**). Also, the concentration of *Dasytrichia* increased significantly ($P < 0.05$) higher in T_3 followed by T_2 and lowest in T_1 group in each trial. Arakaki et al., (2000) reported that the proportion of *Dasytrichia* increased due to application of yeast culture. Furthermore, the same trend was found in the case of *Isotricha* that is, significantly ($P < 0.05$) higher in T_3 followed by T_2 and lowest in T_1 group in all the trials at various intervals (15, 30, 45 and 60 Days). Bhatt et al., (2009) found that the percentage of *Isotricha* was increased due to administration of herbal formulation (Ruchamax @30 g per day). Likewise *Entodinium* was found to significantly ($P < 0.05$) differ amongst T_1, T_2 and T_3 during all three trials at different periods (15, 30, 45 and 60 Days). *Diplodinium* was observed to be significantly ($P < 0.05$) higher in T_3 followed by T_2 and lowest in T_1 group in all trials at various intervals (15, 30, 45 and 60 Days). Characteristics of ruminal protozoa such as *Dasytricha, Isotricha, Entodinium* and *Diplodinium* along with ruminal pH differed significantly between control and treatments with herbal formulation (El-Kholy and Salama, 1995; Randhawa et al., 1995; Shukla et al., 1999; Mishra et al., 2004; Singh and Singh, 2006; Phengvilaysouk et al., 2008; Yadav et al., 2009; Santra et al., 2012). The rumen pH was significantly higher ($P<0.05$) in T_1 and T_2 groups but lowest in T_3 group at various intervals during each trial therefore the above protozoa counts found increased vice versa amongst the same groups. The herbal rumenotoric drugs fed with the basic diet might have significantly modified the proportions of the different protozoa types, rumen pH and improved ruminal cellulolytic activity.

CONCLUSIONS

Administration of market drug (one bolus/day) and self formulated rumenotoric drug (40gm/day) to each animal as a feed additive with basic diets have significantly ($P<0.05$) increased the population of *Dasytricha, Isotricha, Entodinium* and *Diplodinium* and decreased the ruminal pH. Thus it can be concluded that the herbs used in the rumenotoric drugs not only improve the appetite and digestion process but also it stimulate gro-wth parameters and improves the rumen eco-system.

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