

Effect of Herbal Drug on Rumen Ecosystem

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Abstract

The rumen ecosystem is largely dependent on pH, VFA and microbial population. To determine the effect of herbal drug on pH, VFA and microbial population a mixture of herbal drug was given to two groups of calves along with one control group. The herbal drug was composed of some herbs like chirayita, kutki, cordimom, guruchi, kalmegh, bhumyamalki, neemchhal, annis seed, cumin or ajwain, black pepper, piplamul, ghee-kunwar, azmoda, yeast along with ammonium chloride and certain other minerals. Nine healthy cross-bred calves were selected ; divided into three groups with three calves in each group. The period of each trail was of three months including one month of pre-experimental feeding. The experimental trails were conducted in switch over design. Results showed positive effect of compounded herbal drug on pH, VFAs (acetic acid, propionic, butaric acid and other VFAs) and microbial population (protozoal number and types). The high level of drug ($D_3 = 80$ g) improved the rumen ecosystem (pH, VFA and protozoal population) compared to control group ($D_1 =$ without drug) and low dose of drug ($D_2 = 40$ g). This herbal drug had no adverse effect on animal body.

Key words : Herbal drug, Rumen ecosystem.

Rumen ecosystem is consisting of rumen flora and chemical status. The rumen flora is comprising 10^9 - 10^{10} bacteria, 10^5 - 10^6 Protozoa and 10^3 - 10^4 fungi per ml of rumen fluid. About 200 species of bacteria, 10 species of protozoan and eight species of fungi in the rumen have been reported by various researches. Chemical status is maintained by pH and quantity and types of VFA present in rumen. Recently various researches reported the change in rumen kinetic which have brought by using herbal mixture. Some herbs or their mixture are used to promote feed intake due to their flavoring and medicinal value, their essential oil content and other unidentified factors in chemical forms also play an important role in enhancing the animal performance. Besides, these are less expensive and safe than chemical additives and it may be a way of utilization of vast plant materials in nature. This is an example to sustain productivity of animal. A herbal mixture of 32 herbs have been added to compose a herbal product batisa, which is used to improve appetite of animals and helps in curing different types of indigestion by increasing the protozoal mass, bacterial population and absorption of nutrients. Wenk (1) concluded that herbs can

replace antibiotics for raising calves. Enhancement of acetate, butyrate and propionate, increased gas production, decreased methanogens were observed with some herbs containing high flavinoid. Present study was made to determine the effect of self compounded herbal drug on rumen flora and chemical status. Herbal mixture is compounded by using some herbs like chirayita, kutki, cordimom, guruchi, kalmegh, bhumyamalki, neemchhal, anis seed, ajwain, black pepper, piplamul, ghee-kunwar, azmoda, yeast along with ammonium chloride and certain other minerals.

Methods

Nine Healthy cross-bred calves were selected, divided into three groups D_1 , D_2 and D_3 . The average weight of calves in first group ranged from 80—82 kg while in second from 60—96 kg and in third group from 80—94 kg. The calves were randomly divided into three groups and three levels of herbal drug ($D_1 =$ without herbal drug, $D_2 = 40$ g herbal drug) and $D_3 = 80$ g herbal drug was offered to these calves. The period of each trail was of three months includ-

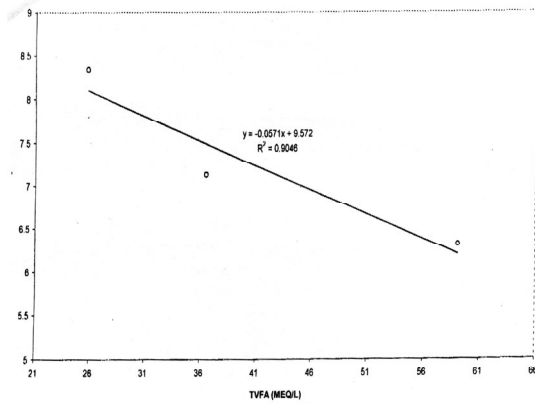


Figure 1. Effect of TVFA on rumen pH.

ing one month of pre-experimental feeding. The experimental trails were conducted in switch over design.

Rumen liquor was analyzed for pH, TVFA, Individual volatile fatty acids (acetic, propionic, butyric acid) and protozoan number. The protozoans present in the strained rumen liquor were identified and classified as total Protozoa, genera - *Isotricha*, *Dasytricha*, *Entodinium*, *Epidinium* and *Diplodinium*.

Results and Discussion

Results related to the effect of herbal drug on

rumen kinetics showed positive response.

Rumen pH

The average rumen pH was recorded as 8.34, 7.13 and 6.31 in D₁, D₂ and D₃, respectively (Table 1). The maximum average pH (8.34) was recorded in control group (D₁) followed by D₂ (7.13) and D₃ (6.31). The effect of herbal drug (D₁, D₂ and D₃) on rumen pH were found significant ($P < 0.05$).

Higher pH recorded in control group (D₁) as the animals were fed only coarse material (bhusa and straw). Coarse feed materials create an alkalinity condition and produce lesser amount of VFA in rumen.

TVFA

The effect of herbal drug (D₁, D₂ and D₃) on TVFA was significant ($P < 0.01$). The mean TVFA values were recorded to be 25.8, 36.5 and 59.1 meq/liter in D₁, D₂ and D₃. The maximum TVFA (59.1 meq/liter) was observed in D₃ as compared to D₁ (25.8 meq/liter) and D₂ (36.5 meq/liter) (Table 1).

Results indicated that relationship between rumen pH and TVFA was inverse. The average TVFA was recorded as 25.8, 36.5 and 59.1 meq/liter in D₁, D₂ and D₃ levels, respectively and correspondingly mean rumen pH recorded as 8.34, 7.13 and 6.31 (Table 1 and Fig. 1).

Table 1. Rumen pH, TVFA and protozoan population in rumen of cross bred calves fed different level of herbal drug. * Significant ($P < 0.05$), ** Significant ($P < 0.01$).

Parameter	Treatment group		
	D ₁ (Control)	D ₂	D ₃
Rumen pH*	8.34	7.13	6.31
TVFA** (meq/l)	25.8	36.5	59.1
Acetic acid	12.49 (48.64%)	19.67 (53.48%)	43.37 (74.27%)
Propionic acid	6.89 (26.76%)	8.64 (24.02%)	7.20 (12.42%)
Butyric acid	4.41 (16.19%)	5.73 (15.67%)	6.22 (10.46%)
Other VFAs	2.20 (7.47%)	2.33 (6.59%)	2.17 (2.74%)
Protozoan Population**	3.305×10^5	5.23×10^5	6.915×10^5
Holotricha*	0.838×10^5	1.246×10^5	1.63×10^5
Isotricha*	0.441×10^5	0.4546×10^5	0.970×10^5
Dasytricha*	0.398×10^5	0.598×10^5	0.76×10^5
Entodiniomorphids*	2.46×10^5	3.837×10^5	5.767×10^5
Entodinium**	2.30×10^5	3.267×10^5	5.226×10^5
Diplodinium	0.267×10^5	0.83×10^5	0.89×10^5
Epidinium	0.155×10^5	0.230×10^5	0.34×10^5

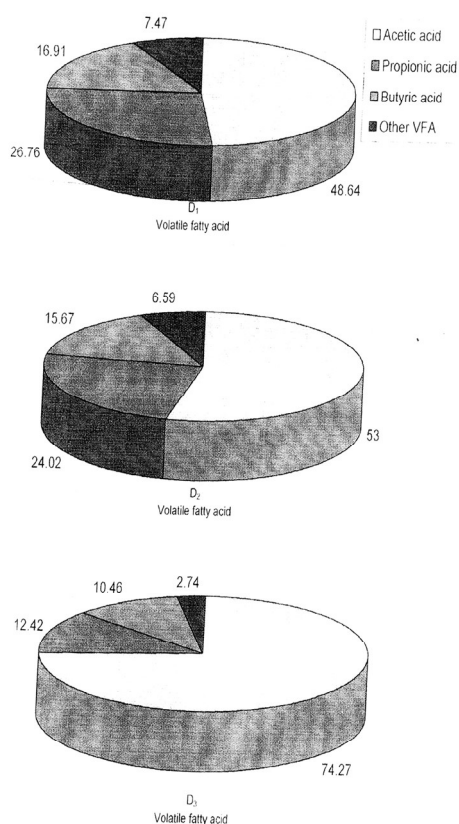


Figure 2. Average volatile fatty acid (per cent) in different treatments of compounded herbal drug.

Acetic Acid

Table 1 shows the effect of herbal drug on acetic acid. The average acetic acid was measured to be 12.49, 19.67 and 43.37 meq/liter in D₁, D₂ and D₃, respectively. These findings showed significant difference at 5% level of significance. The acetic acid at D₃ level (43.37 meq/liter) was found to be better than D₁ (12.49 meq/liter) and D₂ level (19.67 meq/liter) (Fig. 2).

Propionic Acid

The average propionic acid was observed to be 6.89, 8.64 and 7.20 meq/liter in D₁, D₂ and D₃ levels, respectively. There was no significant difference observed at different levels of herbal drug (D₁, D₂ and D₃).

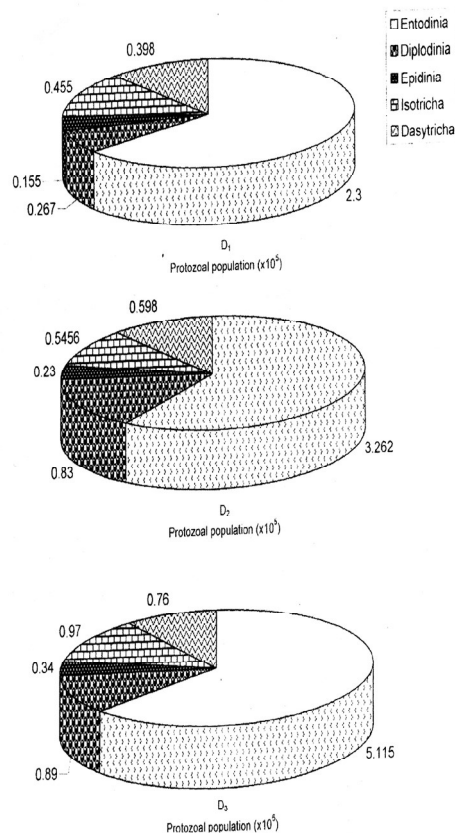


Figure 3. Average protozoan population ($\times 10^5$ / ml) in different groups.

Butyric Acid

The effect of herbal drug on butyric acid was recorded as 4.41, 5.73 and 6.22 meq/liter in D₁, D₂ and D₃ levels, respectively. The effect of herbal drug on butyric acid was found non-significant (Table 1, Fig. 2).

Other VFA

The data pertaining to the effect of herbal drug on other VFA of cross-bred calves showed no significant ($P < 0.05$) difference amongst different levels of drug (Table 1). The maximum other VFA (2.33 meq/liter) was recorded in D₂ followed in D₁ (2.2 meq/liter) and D₃ (2.17 meq/liter). (Fig. 2).

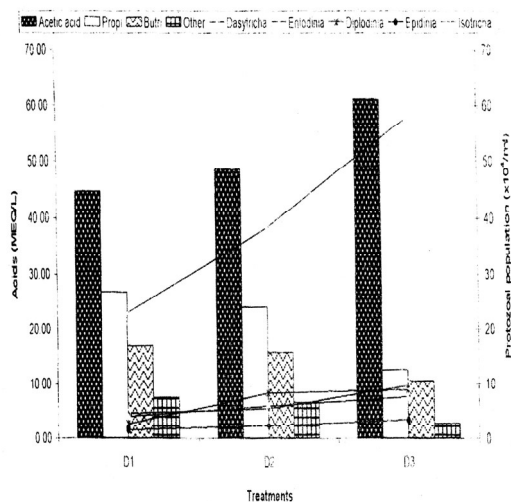


Figure 4. Effect of different protozoan species on volatile fatty acid in different treatments.

Shifts in VFA proportions may be of importance in two respects. Where the presence or absence of protozoan is associated with large changes in the ratio of acetate plus butyrate to propionate, there can be significant changes in methane (CH₄) formation and synthesis of milk fat. Whitelaw et al. (2) showed that where defaunation resulted in doubling the proportion of propionate the production of CH₄ was halved and metabolizability of the grass energy increased by 5%. Methanogenic ruminal bacteria have been observed to be attached to ciliate protozoans and the protozoans have been reported to be major CH₄ producing fraction in ruminal contents (3). Differences in the molar proportions of acetate, propionate and butyrate produced during formation affects the amount of hydrogen available for CH₄ production (4) as does the site of digestion (fermentation vs

Table 2. Effect of different protozoans on rumen pH. Multiple regression coefficient $r = 1^{**}$.

Treatments	Rumen pH	Protozoal population ($\times 10^5$)				
		Iso-tri-cha	Dasy-tri-cha	Ento-dinia	Diplo-dinia	Epi-dinia
D ₁	8.13	0.45	0.39	2.30	0.26	0.15
D ₂	7.13	0.55	0.59	3.26	0.83	0.23
D ₃	6.31	0.97	0.76	5.22	0.89	0.34

Table 3. Effect of different protozoans on acetic acid level in rumen. Multiple regression coefficient $r = 1^{**}$.

Treatments	Acetic acid (meq/l)	Protozoal population ($\times 10^5$)				
		Iso-tri-cha	Dasy-tri-cha	Ento-dinia	Dip-lodi-nia	Epi-dinia
D ₁	12.49	0.45	0.39	2.30	0.26	0.15
D ₂	19.69	0.55	0.59	3.26	0.83	0.23
D ₃	43.67	0.97	0.76	5.22	0.89	0.34

gastric) and the extent of digestion.

In present study yeast culture is incorporated with herbal drug and it may be positive response on VFA and NH₃ concentration in the rumen. Wiedmeir et al. (5) and Arambel and Tung (6) reported that acetate to propionate ratio in the rumen of animals receiving a yeast culture supplement increased while Harrison et al. (7) and Martin et al. (8) reported that acetate to propionate proportions decreased. Adams et al. (9) reported that yeast supplements did not influence the relative concentrations of VFA in the rumen. Yun-SangGi et al (10) have reported that concentrations of acetic acid decreased and those of propionic acid and valeric acid increased as the concentrate content of the diet increased. The proportions of VFAs are often changed by the presence or absence of protozoans in an inconsistent manner (11).

Effect on Rumen Protozoa

In the present study response of herbal drug supplementation on rumen protozoan population was observed to be positive. The average protozoan popu-

Table 4. Effect of different protozoans on propionic acid level in rumen. Multiple regression coefficient $r = 1^{**}$.

Treatments	Propionic acid (meq/l)	Protozoal population ($\times 10^5$)				
		Iso-tri-cha	Das-ytri-cha	Ent-odi-nia	Dip-lodi-nia	Epi-dinia
D ₁	6.86	0.45	0.39	2.30	0.26	0.15
D ₂	8.64	0.55	0.59	3.26	0.83	0.23
D ₃	7.20	0.97	0.76	5.22	0.89	0.34

Table 5. Effect of different protozoans on butyric acid level in rumen. Multiple regression coefficient $r = 1^{**}$.

Treat-ments	But- yric acid (meq/ l)	Protozoal population ($\times 10^5$)				
		Iso- tri- cha	Das- ytri- cha	En- todi- nia	Dip- lodi- nia	Epi- dina
D ₁	4.41	0.45	0.39	2.30	0.26	0.15
D ₂	5.73	0.55	0.59	3.26	0.83	0.23
D ₃	6.22	0.97	0.76	5.22	0.89	0.34

lation recorded in D₁, D₂ and D₃ were 3.3×10^5 , 5.23×10^5 and 6.915×10^5 /ml, respectively (Table 1). The maximum average number (6.915×10^5) was observed in D₃ followed by D₂ (5.23×10^5 /ml) and D₁ (3.305×10^5 /ml). Results indicated that effect of compounded herbal drug on protozoan population was significant ($P < 0.01$). Protozoan population is increased by herbal drug supplementation might be due to increase in the bacterial-protein-amino acid contents which have utilized by protozoans.

The concentration of Holotricha was observed as 0.838×10^5 , 1.246×10^5 and 1.63×10^5 /ml in D₁, D₂ and D₃ groups, respectively. Isotricha and Dasytricha population were more in both treated groups (D₂ and D₃) than control (D₁) which might be due to the large number of bacterial biomass were available in rumen which were utilized by Isotricha and Dasytricha. The microbes increase in number of Holotricha might be due to better digestibility which enhanced the large amount of soluble digesta in the rumen. (Table 1 and Fig. 2).

The concentration of Isotricha number was observed to be more than Dasytricha which might be due to better predation capability of bacterial cells by Isotricha than Dasytricha.

Entodiniomorphids

The concentration of Entodiniomorphids recorded in D₁, D₂ and D₃ were 2.46×10^5 , 3.837×10^5 and 5.767×10^5 /ml respectively. The maximum average Entodiniomorphids population (5.767×10^5) was observed in D₃ followed by D₂ (3.837×10^5 /ml) and D₁ (2.46×10^5 /ml). The data indicated significant effect ($P < 0.05$) of herbal drug on Entodiniomorphids protozoan population (Table 1 and Fig. 3).

Table 6. Effect of different protozoans on other VFA level in rumen. Multiple regression coefficient $r = 1^{**}$.

Treat-ments	Other VFA (meq/ l)	Protozoal population ($\times 10^5$)				
		Iso- tri- cha	Das- ytri- cha	Ent- odi- nia	Dip- lodi- nia	Epi- dina
D ₁	2.20	0.45	0.39	2.30	0.26	0.15
D ₂	2.33	0.55	0.59	3.26	0.83	0.23
D ₃	1.91	0.97	0.76	5.22	0.89	0.34

Entodinium

The average Entodinium population was recorded as 2.30×10^5 , 3.267×10^5 and 5.226×10^5 in D₁, D₂ and D₃ groups, respectively. The maximum average Entodinium number (5.226×10^5) was observed in D₃ followed by D₂ (3.26×10^5) and D₁ (2.30×10^5 /ml). Effect of compounded herbal drug on Entodinium population was found significant ($P < 0.01$). The experiment indicated that Entodinium population was more in both the treated groups (D₂ and D₃) than control (D₁) in rumen liquor, probable reason might be the large amount of roughage diet was (cellulogic feed) given to the animals.

Diplodinium

The average numbers of Diplodinium recorded in D₁, D₂ and D₃ were 0.287×10^5 , 0.83×10^5 and 0.89×10^5 per ml, respectively. The maximum number (0.89×10^5) was observed in D₃ followed by D₂ and D₁. The study indicated that the effect of herbal drug on Diplodinium population was non-significant, but the effect of D₃ level was observed better than D₂ level. (Table 1 and Fig. 3).

Epidinium

Average Epidinium population was recorded as 0.155×10^5 /ml, $.023 \times 10^5$ /ml and 0.34×10^5 /ml in D₁, D₂ and D₃ groups, respectively. Results indicated that the effect of herbal drug on Epidinium population was non-significant (Table 1 and Fig. 3).

Entodinium protozoan population was observed to be more amongst other genera (Diplodinium, Epidinium, Isotricha and Dasytricha). Therefore, the study indicated that the response of herbal drug was

more on Entodinium population than other genera (Fig. 3).

Rumen pH and Protozoan Number

The effect of different protozoan species (Isotricha, Dasytricha, Entodinium, Diplodinium and Epidinium) on rumen pH were observed to be significant ($P < 0.01$). Table 2 indicated the inverse relationship between large number of protozoan species and rumen pH. The maximum number of protozoan species (0.97×10^5 , 0.76×10^5 , 5.22×10^5 , 0.89×10^5 and 0.34×10^5) of Isotricha, Dasytricha, Entodinium, Diplodinium and Epidinium, respectively were found to be correlated to minimum rumen pH 6.31 in D_3 level. In the presence of large number of protozoan rumen pH decreased linearly (multiple regression coefficient $r = 1.0$) from 8.13 to 6.31 in D_1 to D_3 level. But protozoans regulate rumen pH to check the fall from normal acidic condition. The multiple regression equation calculated using pH as dependent variable and different protozoans as independent variables and equation is presented as below :

$$Y = 9.72 - 0.276 X_1 - 0.1989 X_2 - 1.523 X_3 - 0.350 X_4 - 0.1007 X_5$$

The regression equations between pH and various protozoan species were calculated separately and are given as :

$$Y = 0.27 X_1 + 2.6415 R^2 = 0.8373 \text{ (regression equation between pH (Y) and Isotricha (X}_1\text{)}$$

$$Y = -0.198 X_2 + 2.057 R^2 = 1.0 \text{ (regression equation between pH (Y) and Dasytricha (X}_2\text{)}$$

$$Y = -1.5255 X_3 + 14.527 R^2 = 0.9442 \text{ (regression equation between pH (Y) and Entodinium (X}_3\text{)}$$

$$Y = 0.3503 X_4 + 3.1808 R^2 = 0.863 \text{ (regression equation between pH (Y) and Diplodinium (X}_4\text{)}$$

$$Y = 0.1007 X_5 + 0.9656 R^2 = 0.2727 \text{ (regression equation between pH (Y) and Epidinium (X}_5\text{)}$$

These equations indicate that there was a linear relationship between rumen pH and different protozoan species. The equations indicated that Dasytricha and Entodinium played a major role for reduction of rumen pH. In the equations 2 and 3, R^2 values were 1.00 and 0.9442, respectively. In these equations con-

tribution of different protozoan species have shown as to how much pH was reduced by individual protozoan species.

Rumen pH and TVFA

Rumen pH decreased linearly ($r = 1.0$) with increasing TVFA. Rumen pH was recorded as 8.34, 7.13 and 6.31 in D_1 , D_2 and D_3 groups, respectively (Table 1 and Fig. 1) and TVFA recorded in D_1 , D_2 and D_3 were 25.8, 36.5 and 59.1 meq/liter, respectively. Results indicated that inverse linear relationship was found between rumen pH and TVFA. Shift proportions of volatile fatty acids were affected by rumen pH. Veira (12) reported that in animals receiving a corn silage : corn (1 : 1) diet, the post-feed variation in pH of the ciliated animals was in range of pH 6.3—6.6 while in the ciliate free animals the range of pH 5.95—6.85.

Purser and Moir (13) found a linear regression of mean daily protozoan population density and minimum daily pH. Protozoan stabilization of the environmental pH during the period of active ruminal fermentative activity may be significant as bacterial growth (14, 15) and enzymic activities (e.g. cellulolytic) (16) are inhibited at lower pH values.

A number of study supported the relationship between protozoan number and rumen pH (17—20). When the pH falls below 5.5 the protozoans start disintegrating and lyse completely. In defaunated and faunated animals the pH varies from 6.2—6.9 vs 6.5—7.1, respectively fed on wheat straw : concentrate diet (21). Some reports showed an increase in the proportion of VFAs while others reported a decrease (22) in defaunated animals. The molar proportion of the VFAs are variable but the molar proportion of propionic acid increase largely on defaunation at the expense of acetic acid and butyric acid. Most of the protozoans produce acetic acid and butyric acid on fermentation of sugars but no propionic acid is produced. Propionic acid has an inhibitory effect on the Holotrich protozoans and large Entodiniomorph (23). On the occasion due to high starch diet protozoans are eliminated which might be combining effect of lowering of pH and an increase in the molar proportion of propionate in the rumen liquor ; this is perhaps due to better efficiency of protozoans in lactic acid utilization than

bacteria conforms the role of protozoans in controlling lactic acidosis in ruminants (24).

Generally the molar proportion of acetate is less affected than either propionate or butyrate but has a tendency to be slightly lower in the rumen of defaunated animals (25, 26). The incorporation of single protozoan species (Polyplastron multivesiculatum or Entodinium) to meroxeme lamps resulted in an increase in the ruminal VFA concentration and changes in molar proportions of the principal acetate, propionate and butyrate.

Production of TVFA is drastically affected by number of protozoans and their activity. TVFA recorded in D₁, D₂ and D₃ were 25.8, 36.5 and 59.1 meq/liter, respectively. Similarly protozoan populations were recorded as 3.305×10^5 , 5.23×10^5 and 6.915×10^5 /ml in D₁, D₂ and D₃ groups, respectively (Table 1).

The effect of different of protozoan spp. (Isotricha, Dasytricha, Entodinium, Diplodinium, Epidinium) on acetic acid production was observed to be positive and acetic was measured at increasing rate (Table 3).

Volatile fatty acid increased linearly (multiple regression coefficient $r = 1$) with increasing protozoan population. Acetic was recorded as 12.49, 19.69 and 43.67 meq/liter in D₁, D₂ and D₃ groups, respectively. Similarly protozoan population (Isotricha, Dasytricha, Entodinia, Deplodinia and Epidenia) correspondingly increased in all groups (Table 3).

The data presented in the Tables 2 to 6 and figure 4 showed a significant ($P < 0.01$) effect of different protozoan species on various VFA's. This indicated positive effect of large number of different protozoan species to enhance propionic, butyric and other VFA level.

The multiple regression equations were calculated and are presented below :

$$Y = 28.51 + 0.0168X_1 + 0.0114X_2 + 0.087X_3 - 0.0421X_4 + 0.10056X_5$$

(Acetic acid as dependent variable and different protozoan species as independent variables).

$$Y = 29.71 - 0.0573X_1 + 0.6459X_2 + 0.001X_3 + 0.2105X_4 + 0.0071X_5$$

(Propionic acid as dependent variable and different protozoan species as independent variables).

$$Y = 18.09 + 0.2099X_1 + 0.1886X_2 + 1.252X_3 - 0.3528X_4 + 0.0848X_5$$

(Butyric acid as dependent variable and different protozoan species as independent variables).

$$Y = 9.39 - 1.1385X_1 - 0.53X_2 - 5.30X_3 - 0.4783X_4 - 0.3248X_5$$

(Other VFAs as dependent variable and different protozoan species as independent variables).

A number of studies supported the present finding (5—8, 27).

Conclusion

Thus this investigation indicated positive response of herbal drug on rumen pH, TVFA, individual VFAs (acetic, propionic, butyric acid and other VFA), and protozoan population. We conclude that by incorporation of herbal drug in the diet of dairy calves, the ecosystem of rumen of those calves can be improved.

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