Effect of Herbal Formulation AV/DAC-16 Supplementation on Rumen Profiles in Buffalo Calves (*Bubalus bubalis*)

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Abstract: 12 healthy buffalo calves with BW range 100-150 kg were fistulated and divided into two groups of 6 animals each. Control group animals were fed on conventional diet comprising of wheat straw (2 kg), green fodder (8 kg), concentrate (1.0 kg) and mineral mixture (0.050kg). The animals of the treatment group were kept on diet similar to the control group along with feeding of herbally formulated drug AV/DAC-16@ 15 gm/day for 21 days. Each animal was sampled for three consecutive days at 0 hr i.e., immediately before feeding and subsequent samples were taken at 2, 4 and 6 h intervals after feeding. There was a significant fall in pH at 2 and 4 h post-prandial and in MBRT during the entire observation period. TVFA concentration increased significantly in the treatment group. Though oral administration of AV/DAC-16 did not have any prominent effect on the protozoal count, the bacterial count increased significantly in comparison to control group. Total nitrogen concentrations fell significantly while a significant increase was observed in the ammonia nitrogen content in the supplemented group at 6 hours after feeding. The animals of supplemented group showed a significant increase in body weights.

Keywords: Digestibility, wheat straw.

INTRODUCTION

Ruminants have long lived in close symbiosis with humans. They contribute greatly to human food supplies by transforming products of little or no value into nutritious human food. Animal products provide one sixth of human food energy and one third of the protein on global basis [1]. Though ruminants have evolved a digestive system capable of utilizing wide range of low-grade roughages, the mechanisms involved are rather inefficient in terms of energy conversion [2]. Therefore to increase the energy conversion and nitrogen utilization, great stress has been laid on the use of certain feed additives including antibiotics and antibacterial drugs, which help in improving the health of dairy animals. Improvement of ruminant digestion results in better nutrient utilization and early attainment of puberty.

MATERIALS AND METHODS

Twelve apparently healthy male buffalo calves within the age group of 12-14 months and weighing 100-150 kg were procured from the local market of Ludhiana (Punjab) and housed separately under standard managemental conditions.

The animals were divided into two groups comprising six animals each. Control group animals were fed on conventional diet comprising of wheat straw (2 kg), green fodder (8 kg), concentrate (1.0 kg) and mineral mixture (0.05 kg). Treatment group animals were kept on diet similar to the control group along with feeding of herbally formulated drugAV/DAC-16 @ 15 gm/day in two doses of 7.5gms, one dose each in the morning as well as in the evening. Feeding was done twice daily for a period of 21 days for microbial adaptation. Diet was computated as per recommendations by Banerjee [3]. Fresh and clean drinking water was provided *ad libitum* immediately after feeding. Permanent rumen fistula was fitted to each animal following the technique adopted by Roychoudhary [4]. Animals were operated four weeks prior to the commencement of experiment.

Rumen liquor samples were collected through the rumen fistula to obtain a representative sample with the help of suction pump. Each animal was sampled for three consecutive days before feeding, i.e., at 0 hr and subsequently after 2, 4 and 6 hr intervals of feeding. Collected samples were strained through double layer of muslin cloth to remove solid particles and designated as strained rumen liquor (SRL).

pH of rumen liquor was determined immediately after collection of sample by electronic pH meter. Sedimentation Activity Time (SAT) and Methylene blue reduction time (MBRT) were noticed according to the method adopted by Dirksen [5]. Total volatile fatty acids (TVFAs) were estimated using equal volumes of SRL and Scaribrick buffer in Merkham's microkjeldahl distillation apparatus and titrating against standard 0.01

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N NaOH solution. Conway microdiffusion technique was used to estimate ammonia nitrogen in SRL. The total nitrogen in the SRL was determined by the method used by Singh [6]. Rumen protozoal count was done as per the method of Naga and El-Shazly [7] using formalinized sample and Lugol's iodine. Total Bacterial Count was made using nigrosine slide technique. The data thus collected was analyzed using computer programme 'SPSS 12.0'.The mean values of treatment group were compared with those of control group using paired 't' test.

RESULTS AND DISCUSSION

Ruminal pH

The pH of the rumen liquor in control and treatment groups varied between 6.80 ± 0.05 to 7.09 ± 0.30 and 6.66 ±0.04 to 7.05 ± 0.04, respectively (Table **1**). These values are similar to 6.9-7.1 [8], 6.56 ± 0.04 [9], 6.88 ± 0.03 [6] and 6.87 ± 0.03 [10] as reported in buffalo calves. Early studies reported that pH of the rumen liquor varied from 5.0 to 7.5, reaching the lowest level at 2-6 hr after feeding. Ruminal pH declined from 0-6 hr post-feeding in control as well as treatment groups. These observations are in accordance to the findings of Iqbal [11], Gill [9], Singh *et al.* [12], Singh [6], Singh [13] and Sharma [10]. Post-prandial decline in ruminal pH may be attributed to increased ruminal fermentation and accumulation of organic acids in the rumen. Singh [14] observed that pH changes were closely related to the production of volatile fatty acids in the rumen liquor. Rogers *et al.* [15] reported an inverse relationship between the pH of the rumen liquor and the total volatile fatty acid production in cattle and buffaloes. Administration of AV/DAC-16 caused a significant ($P<0.01$, 0.05) decrease in pH at 2nd and 4th hour after feeding, thereby, indicating that the drug resulted in increased fermentation and hence, better digestion of nutrients in the rumen as is suggested by a significant increase in TVFA in AV/DAC-16 supplemented group.

Methylene Blue Reduction Time (MBRT)

Methylene blue reduction time (MBRT) at different time intervals in control and treatment group ranged between 4.00 ± 0.28 to 7.33 ± 0.89 min and 1.83 ± 0.19 to 2.83 ± 0.23 min (Table **1**). The values are lower than 4.58 ± 0.37 [6] and 6.22 ± 0.31 min [10]. Garry [16] stated that MBRT of normal ruminal fluid ranged from 3-6 min. A progressive decrease in MBRT was observed till 6 hr after feeding. This may be due to high anaerobic fermentative metabolism of bacterial population [5]. Sharma [10] stated that MBRT reflects the anaerobic fermentation mechanism of bacterial population. A rapid discolouration of methylene blue

Table 1: Changes in Various Physiological Parameters of Rumen Liquor During Different Time Intervals After Oral Administration of Herbal Formulation AV/DAC-16 in Buffalo Calves

Parameter		0 hr	2 hr	4 hr	6 hr
pH	Control	7.09 ± 0.30	6.91 ± 0.06	6.80 ± 0.05	6.98 ± 0.19
	Treatment	7.05 ± 0.04	6.78 ± 0.02*	6.69 ± 0.02**	6.66 ± 0.04
MBRT (min)	Control	7.33 ± 0.89	4.00 ± 0.28	5.39 ± 0.51	4.28 ± 0.43
	Treatment	2.83±0.23**	2.22±0.19**	1.83 ± 0.19**	2.61±0.28**
SAT (min)	Control	8.50 ± 0.47	6.06 ± 0.24	6.61 ± 0.53	6.28 ± 0.30
	Treatment	8.89 ± 0.46	5.83 ± 0.27	6.89 ± 0.39	7.28 ± 0.48
TVFA (mEq/L)	Control	53.72 ± 1.28	61.83 ± 1.69	66.28 ± 1.40	77.28 ± 2.29
	Treatment	84.28±2.77**	83.61 ± 2.49	82.44±2.35**	86.22±2.52**
NH_3-N_2 (mg%)	Control	7.33 ± 0.23	8.67 ± 0.31	13.28 ± 0.58	7.00 ± 0.29
	Treatment	7.67±0.37	8.67±0.46	11.56±1.06	9.56 ± 0.73**
N_2 (mg%)	Control	76.41 ± 3.63	81.17 ± 5.02	84.40 ± 5.68	87.43 ± 6.08
	Treatment	68.80 ± 4.28	67.21±5.81**	63.33 ± 8.28*	54.51±5.21**
Protozoal Count (x 10^5/ml)	Control	2.32 ± 0.22	3.79 ± 0.25	3.49 ± 0.20	3.89 ± 0.26
	Treatment	1.83 ± 0.07*	2.18 ± 0.12**	3.28 ± 0.25	3.44 ± 0.33
Bacterial Count (x 10^9/ml)	Control	4.00 ± 0.35	5.71 ± 0.33	7.65 ± 0.34	9.08 ± 0.22
	Treatment	7.52 ± 0.56**	9.31 ± 0.61**	12.69±1.23**	11.74±0.80**

Each figure is a mean of 18 observations representing triplicate samples from 6 experimental animals.
*Significant at 5% level, **Significant at 1% level.

indicated a very active microflora whereas a delayed reaction indicated less activity.

Sedimentation Activity Time

The data of sedimentation activity time (SAT) after administration of AV/DAC-16 has been shown in Table **1**. The SAT values ranged between 6.06 ± 0.24 to 8.50 ± 0.47 min in control group and 5.83 ± 0.27 to 8.89 ± 0.46 min in treatment group. Early works have reported SAT range to be 4-8 min [5] and 3-9 min [17] in animals just fed. SAT was highest before feeding and there was a sharp decrease 2 hr after feeding in control as well as treatment group. Similar results have been reported by other workers [6, 10, 13]. Average SAT values of the treatment group were significantly ((P<0.01) higher than the control values after 4 and 6 hr of feeding. Settling of particulate matter rapidly in comparison to control group is thereby suggesting that AV/DAC-16 improves rumen metabolism.

Total Volatile Fatty Acids

The TVFA concentration in rumen liquor in control and experimental group varied between 53.72 ± 1.28 to 77.28 ± 2.29 mEq/L and 82.44 ± 2.35 to 86.22 ± 2.52 mEq/L, respectively. The results of the control group are closer to the earlier reported TVFA level of 83.35 ± 1.00 mEq/L [10]. There was a progressive increase in the levels of TVFA from 0-6 hr post-feeding Treatment group showed a significant (P<0.01) increase in TVFA concentration during all periods of observation. This may be attributed to higher fermentation rate [18] due to increased availability of nutrients [19]. Kumar *et al.* [20] noticed that an increase in microbial population was generally accompanied by an increase in the levels of rumen metabolites. A progressive increase in TVFA concentration was seen till 6 hr after feeding in control group. This pattern of change in TVFA concentration between 4-6 hr post-feeding has been reported by Devi [21], Singh *et al.* [22] and Singh [12]. A peak in TVFA concentration 4-6 hours post-feeding has been reported in buffaloes [23] and goats [24]. This may be due to fermentation of carbohydrates [18] and catabolism of amino acids leading to the formation of organic acids. However in the experimental group, there was an increase in TVFA concentration even at 6^{th} hour. This may be due to improvement in fermentation upon administration of AV/DAC-16.

Ammonia Nitrogen

The ammonia nitrogen (NH_3-N_2) concentration in rumen liquor during different time intervals in the control and treatment groups ranged between 7.00 ± 0.29 to 13.28 ± 0.58 mg/100 ml and 7.67 ± 0.37 to 11.56 ± 1.06 mg/100 ml, respectively (Table **1**). These values are similar to 8.95 ± 0.33 [13] but lower than 14.6 ± 1.56 [6] and 21.85 ± 2.25 [9]. Results indicate that there was a progressive increase in NH_3-N_2 till 4 hr after feeding. Sinha *et al.* [25] noticed peak NH_3-N_2 levels at 2 and 4 hr post-feeding in cattle. Iqbal [10] recorded highest level of NH3-N_2 at 1 hr post-prandial with exclusive feeding of wheat straw. Sinha *et al.* [25] and Singh [12] reported peak NH_3-N_2 level 2 hr after feeding under different dietary regimes. Singh [6] observed peak levels of NH_3-N_2 3 hr after feeding. Initial post-prandial increase in NH_3-N_2 level could be attributed to increased availability of substrate, which on proteolysis and deamination leads to the formation of ammonia in the rumen [22]. After 6 hr, the level of NH_3-N_2 decreased in control group. This may either be due to direct absorption of nitrogen in the form of ammonia or the onward passage of nitrogen along with digesta from rumen or incorporation of nitrogen in the synthesis of microbial proteins [22]. In the treatment group, the NH_3-N_2 concentration was significantly (P<0.01) higher, thereby, indicating that AV/DAC-16 improved digestion in rumen.

Total Nitrogen

The total nitrogen (N_2) concentration in rumen liquor in control and treatment groups ranged between 76.41 ± 3.63 to 87.43 ± 6.08 mg/100 ml and 54.51 ± 5.21 to 68.80 ± 4.28 mg/100 ml, respectively (Table **1**). These values were slightly less than 90.50 ± 3.43 mg/100ml [6] but similar to 72.05 ± 1.09 mg/100ml [10]. There was an increase in total nitrogen content after 2 hr feeding in the control group which could be attributed to increased substrate availability. Peak N_2 levels in rumen liquor 2-4 hr post-feeding have earlier been reported [9, 12, 22, 26]. Sinha *et al.* [25] noticed peak levels of total nitrogen at 3 hr post-feeding with conventional diet. A progressive decrease in the levels of total nitrogen was observed after feeding. This may be due to utilization of nitrogen by microbes which show a progressive increase in concentration in the rumen liquor after administration of AV/DAC-16 (Table **1**). Miller [27] stated that the most important function of rumen microbes is utilization of compounds like non-protein nitrogen which are otherwise almost unusable.

Total Protozoal Count

The total protozoal count in rumen liquor at different time intervals in control and treatment groups varied

Table 2: Change in Body Weights of Buffalo Calves with Administration of Herbal Formulation AV/DAC-16

Week	Control (Mean±SE)	Treatment (Mean±SE)
0	143.00 ± 10.25	149.83 ± 8.48*
1	143.50 ± 10.06	155.00 ± 9.48*
2	144.50 ± 9.78	160.00 ± 10.08**
3	149.67 ± 8.68	164.33 ± 10.10**
Total Weight Gain/Loss (kg)	6.67	14.50
Weight gain/day (kg)	0.32	0.69

*Significant at 5% level, **Significant at 1% level.

between 2.32 ± 0.22 to 3.89 ± 0.26 (x 10^5/ml) and 1.83 ± 0.07 to 3.44 ± 0.33 (x 10^5/ml), repectively. The values are close to the earlier reported values of 2.60-3.60 (x 10^5/ml) [7], 2.80 ± 0.16 (x 10^5/ml) [9] and 3.27 ± 0.11 (x 10^5/ml) [6] in buffalo calves. A progressive increase in the protozoal count was observed till till the end of the observation period in treatment group and throughout in the control group. *Singh et al.* [22] and Singh [6] recorded peak levels of protozoal count at 2-4 hr post-feeding. Sinha *et al.* [25] reported highest protozoal population at 2-4 hr post feeding on maintenance and sub-maintenance ration, respectively in buffalo calves. A significant post-prandial increase in total protozoal count may be attributed to increased availability of substrate required for microbial growth and dislodging of microbes along with a significant increase in protozoal count before feeding (Table **1**), which may be attributed to the fact that AV/DAC-16 allowed better utilization of ration and made available higher amount of soluble carbohydrates, vitamins and nitrogen content and this promoted microbial growth. Post-prandial increase in protozoal count may also be due to increase in ammonia nitrogen concentrations after feeding. Sharma [10] was of the view that greater protozoal concentrations were associated with higher concentrations of ammonia in the rumen liquor as is also evident from the present investigation.

Total Bacterial Count

The total bacterial count in the rumen liquor at different time intervals in control and treatment groups varied between 4.00 ± 0.35 to 9.08 ± 0.22 (x 10^9/ml) and 7.52 ± 0.56 to 12.69 ± 1.23 (x 10^9/ml), respectively (Table **1**). The values of the control group are closer to 10.47 ± 0.22 (x 10^9/ml) [10]. There was a progressive increase in the concentration of bacteria after feeding and this value reached its peak at 6 hr post-feeding in the treatment group (Table **1**). These findings are supported by the observations of Gill [9] and Singh [12] who recorded peak bacterial counts 4 hr after feeding. Iqbal *et al.* [28] and Singh *et al.* [22] reported peak bacterial counts 2 hr after feeding. The total bacterial count after oral administration of AV/DAC-16 was significantly ($P<0.01$) higher than the control group at all periods of observation. This indicates that AV/DAC-16 causes better utilization of nutrients, as also observed by an increase in the levels of rumen metabolites. According to Sharma [10], the higher amounts of soluble carbohydrates and nitrogen intends better utilization of ration and makes available the nutrients in their soluble form. Significant progressive increase in the microbial population post feeding indicates beneficial effect of AV/DAC-16 which leads to better utilization of nutrients.

The data for weekly body weights (BW) of animals are given in Table **2**. The BW of animals in control and treatment groups ranged between 143.00 ± 10.25 to 149.67 ± 8.68 kg and 149.83 ± 8.48 to 164.33 ± 10.10 kg, respectively. Animals showed a significant increase in BW during 1st, 2nd and 3rd ($P<0.01$, 0.05) weeks of observation. Animals supplemented with AV/DAC-16 gained 690g of BW per day in comparison to the control group which gained 320g per day, suggesting the beneficial effect of the drug.

The results of the this study makes us conclude that the herbal formulation AV/DAC-16 supplementation improves the digestibility of the diet as well as improves the growth of the animals as is evident from better weight gain.

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REFERENCES

[1] Bradford GE. Contribution of animal. Livestock Prod Sci 1999; 59: 95-112. http://dx.doi.org/10.1016/S0301-6226(99)00019-6

[2] Leek BF. Clinical diseases. Vet Rec 1983; 113: 10-4. http://dx.doi.org/10.1136/vr.113.1.10

[3] Banerjee GC. Balanced ration, its characteristics and computation for cattle and buffaloes. Feeds and principals of animal nutrition. Oxford and IBH publishing Co. Pvt. Ltd. 1999; pp. 149-66.

[4] Roychoudhary RK. Clinicotherapeutic studies in hepatic dysfunction associated with acid indigestion in bovines. Ph.D. dissertation, Punjab Agricultural university, ludhiana-India 1981.

[5] Dirksen G. Digestive System. In: Rozenberger G, Ed. Clinical examination of cattle. Verlag Paul, Parey, Berlin and Hamburg, D-1000 Berlin-61, Germany 1979; pp. 208.

[6] Singh G. Effect of HB Strong on rumen profile in experimentally induced rumen dysfunction in buffaloes. M.V.Sc. thesis, Punjab Agricultural University, Ludhiana, India 2002.

[7] Naga MA, El-Shazly K. Activities of rumen. J Dairy Res 1969; 36: 1-10.

[8] Joshi BP, Misra SS. Epizootiological studies. Indian J Anim Sci 1974; 14: 742-6.

[9] Gill SS. Profile of rumen liquor under different dietary regimes in buffalo calves (Bubalus bubalis). M.V.Sc. thesis, Punjab Agricultural University, Ludhiana, India 1993.

[10] Sharma M. Studies on rumen profile during exclusive feeding of rice straw in buffalo calves. M.V.Sc. thesis, Punjab Agricultural University, Ludhiana, India 2004.

[11] Iqbal S. Studies on changes in the population of rumen microflora and fauna and some of the metabolites of rumen liquor under different dietary regimes in buffalo calves (Bubalus bubalis). M.V.Sc. thesis, Punjab Agricultural University, Ludhiana, India 1989.

[12] Singh R, Iqbal S, Ahuja AK and Setia MS. Profile of rumen. Buffalo J 1995; 11.

[13] Singh N. Study on the effect of nonensin feeding on rumen ecosystem in buffalo (Bubalus bubalis). M.V.Sc. thesis, Punjab Agricultural University, Ludhiana, India 2002.

[14] Singh G. Studies on effect of feed additives on rumen profiles in buffaloes. Ph.D. dissertation. Punjab Agricultural University, Ludhiana- India 2005.

[15] Rogers JA, Muller LD, Synder TJ, Maddox T. Milk production, nutrient digestion and rate of digesta passage in dairy cows fed long or chopped alfalfa hay supplemented with sodium bicarbonate Journal of Dairy Science 1985; **58:** 868-80.

[16] Garry F. Symposium on bovine. Veterinary Medicine 1990; 85: 660-70.

[17] Radostits OM, Gary CC, Blood DC, Hinchcliff KW. Special examination of the alimentary tract and abdomen of cattle. Veterinary Medicine: A textbook of the diseases of cattle, sheep, pigs, goats and horses. 9th edition. W B Saunders Company Ltd. 2000; pp. 273.

[18] Amos HE, Akin DE. Rumen protozoal. Appl Envirol Micro 1978; 36: 513-22.

[19] Kapoor PD, Nangia OP, Gupta M. Studies on some. Indian Vet J 1987; 64: 669-73.

[20] Kumar CK, Gupta BN, Mohini M. Protozoal status. Indian J Anim Sci 1988; 58: 112-5.

[21] Devi N. Rumen functions in relation to feeding frequency in buffaloes. M.V.Sc. thesis, Haryana Agricultural University, Hisar, India 1987.

[22] Singh R, Iqbal S, Singh DV, Setia MS. Effect of exclusive feeding. Buffalo J 1996; 12: 1-19.

[23] Bhatia SK, Pradhan K, Singh R. Rumen metabolic profile. Indian J Anim Sci 1980; 50: 16-20.

[24] Barbind RP, Kambale VJ. Volatile fatty acid. Indian Jl Anim Res 2002; 36: 52-4.

[25] Sinha RN Sharma DD, Nambudripad VKN, Ranganathan B. Preliminary observations. Indian J Anim Sci 1974; 44: 18-21.

[26] Gupta M, Kapoor P D and Nangia O P (1988) Effect of urea. Indian J Animal Sci 58: 507-10.

[27] Miller WJ. Introduction and utilization of nutrients by dairy cattle. Dairy cattle feeding and Nutrition, academic Press, New York 1979; pp. 13.

[28] Iqbal S, Singh R, Setia MS. Influence of diets. Buffalo J 1993; 9: 87-91.

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