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 (0.050 kg). 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 managerial 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 drug AV/DAC-16@ 15 gm/day for 21 days. Each dose was given 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 computed 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 measured 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
Observations are in accordance with the findings of these authors. 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 et al. [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 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 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 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.

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**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 et al. [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 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.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 hr</th>
<th>2 hr</th>
<th>4 hr</th>
<th>6 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Control</td>
<td>7.09 ± 0.30</td>
<td>6.91 ± 0.06</td>
<td>6.80 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>7.05 ± 0.04</td>
<td>6.78 ± 0.02*</td>
<td>6.69 ± 0.02**</td>
</tr>
<tr>
<td>MBRT (min)</td>
<td>Control</td>
<td>7.33 ± 0.89</td>
<td>4.00 ± 0.28</td>
<td>5.39 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>2.83±0.23**</td>
<td>2.22±0.19**</td>
<td>1.83 ± 0.19**</td>
</tr>
<tr>
<td>SAT (min)</td>
<td>Control</td>
<td>8.50 ± 0.47</td>
<td>6.06 ± 0.24</td>
<td>6.61 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>8.89 ± 0.46</td>
<td>5.83 ± 0.27</td>
<td>6.89 ± 0.39</td>
</tr>
<tr>
<td>TVFA (mEq/L)</td>
<td>Control</td>
<td>53.72 ± 1.28</td>
<td>61.83 ± 1.69</td>
<td>66.28 ± 1.40</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>84.28±2.77**</td>
<td>83.61 ± 2.49</td>
<td>82.44±2.35**</td>
</tr>
<tr>
<td>NH₃-N₂ (mg%)</td>
<td>Control</td>
<td>7.33 ± 0.23</td>
<td>8.67 ± 0.31</td>
<td>13.28 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>7.67±0.37</td>
<td>8.67±0.46</td>
<td>11.56±1.06</td>
</tr>
<tr>
<td>N₂ (mg%)</td>
<td>Control</td>
<td>76.41 ± 3.63</td>
<td>81.17 ± 5.02</td>
<td>84.40 ± 5.68</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>66.80 ± 4.28</td>
<td>67.21±5.81**</td>
<td>63.33 ± 8.28*</td>
</tr>
<tr>
<td>Protozoal Count</td>
<td>Control</td>
<td>2.32 ± 0.22</td>
<td>3.79 ± 0.25</td>
<td>3.49 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>1.83 ± 0.07**</td>
<td>2.18 ± 0.12**</td>
<td>3.28 ± 0.25</td>
</tr>
<tr>
<td>Bacterial Count</td>
<td>Control</td>
<td>4.00 ± 0.35</td>
<td>5.71 ± 0.33</td>
<td>7.65 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>7.52 ± 0.56**</td>
<td>9.31 ± 0.61**</td>
<td>12.69±1.23**</td>
</tr>
</tbody>
</table>

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 6th hour. This may be due to improvement in fermentation upon administration of AV/DAC-16.

**Ammonia Nitrogen**

The ammonia nitrogen (NH₃-N₂) 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 NH₃-N₂ till 4 hr after feeding. Sinha et al. [25] noticed peak NH₃-N₂ levels at 2 and 4 hr post-feeding in cattle. Iqbal [10] recorded highest level of NH₃-N₂ at 1 hr post-prandial with exclusive feeding of wheat straw. Sinha et al. [25] and Singh [12] reported peak NH₃-N₂ level 2 hr after feeding under different dietary regimes. Singh [6] observed peak levels of NH₃-N₂ 3 hr after feeding. Initial post-prandial increase in NH₃-N₂ level could be attributed to increased availability 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 NH₃-N₂ concentration was significantly (P<0.01) higher, thereby, indicating that AV/DAC-16 improved digestion in rumen.

**Total Nitrogen**

The total nitrogen (N₂) 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 N₂ 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
between 2.32 ± 0.22 to 3.89 ± 0.26 (x 10⁵/ml) and 1.83 ± 0.07 to 3.44 ± 0.33 (x 10⁵/ml), respectively. The values are close to the earlier reported values of 2.60-3.60 (x 10⁵/ml) [7], 2.80 ± 0.16 (x 10⁵/ml) [9] and 3.27 ± 0.11 (x 10⁵/ml) [6] in buffalo calves. A progressive increase in the protozoal count was observed 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 10⁹/ml) and 7.52 ± 0.56 to 12.69 ± 1.23 (x 10⁹/ml), respectively (Table 1). The values of the control group are closer to 10.47 ± 0.22 (x 10⁹/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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