Influence of herbal feed additives (*Emblica officinalis* and *Asparagus racemosus*) on ruminal fermentation and physiological parameters of Murrah buffaloes

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Abstract

The study was carried out to evaluate the effect of blend of *Emblica officinalis* fruit powder and *Asparagus racemosus* root powder feeding on rumen fermentation parameters in fistulated buffalo calves and haemato-biochemical parameters in lactating Murrah buffaloes. Feeding of plant blend to fistulated male buffalo calves was done for two months and rumen fermentation parameters were analyzed in rumen liquor samples at 0, 30 and 60 days. The results revealed a significant ($p \leq 0.05$) decrease in ruminal fluid pH at 30 days and at 60 days as weak acids, volatile fatty acids break down in the rumen releasing protons that lower the pH. Total volatile fatty acids concentration increased significantly at 30 days and at 60 days post feeding plant blend. For haemato-biochemical studies, twenty-four lactating Murrah buffaloes of early and mid-lactation stage were divided into control and treatment groups having six animals in each group. The animals in treatment groups were fed plant blend along with standard ration for three months and blood samples were taken at 0, 45 and 90 days for analysis of haemato-biochemical parameters. The results revealed a significant increase in hemoglobin concentration in treatment groups of early lactation stage at 45 and 90 days and in mid lactation stage at 45 days. Glucose concentration and total protein increased significantly ($p \leq 0.05$) in treatment group of early and mid-lactation at 45 day and 90 days. Feeding of *A. racemosus* and *E. officinalis* powder is likely to have positive impact on rumen fermentation parameters, having an antioxidant potential affecting the metabolism without any adverse effect on animal health.

Keywords: Amla; shatavari; rumen fermentation; lactation; Murrah buffaloes

Introduction

Ruminants are important in livestock to help the underprivileged since they can produce animal products from locally available feedstuffs. The microbial ecosystem in the rumen is a valuable resource with microbial richness and interaction complexity that changes based on the season, location and feeding schedule. Microorganisms are in charge of bioconverting ligno-cellulose feed into volatile fatty acids. A symbiotic relationship exists between ruminants and the microbes in their gut. These microbes in the rumen play a key role in fermenting carbohydrates to produce volatile fatty acids (VFAs), which account for over half of the total metabolizable energy available to ruminants. The current need for organic food, the prohibition on the use of some antibiotics, the unwanted side effects, and the affordability of animal feed have made the search for substitute feed additives imperative. It is well recognized that the herbal galactogogues positively impact milk production (Kachhawaha *et al*., 2025). Shatavari is a significant tropical and subtropical herbal medicine plant found in India. Its steroidal saponins, sapogenins, and phytochemicals provide it therapeutic value such as antibacterial, astringent, diuretic and galactogogue leading to increased animal products, particularly heightened milk yield and improved composition (Kumar, 2014, Patel *et al.*, 2016). *Emblica officinalis*, Indian Gooseberry or Amla, is a deciduous tree that is primarily found in tropical and subtropical regions of India. It is chemopreventive and possesses strong antioxidant, hepatoprotective and anti-inflammatory qualities (Variya *et al*., 2016). Amla fruit can increase milk production and bring value to the worldwide herbal market without negatively affecting feed intake or ruminal fermentation (Kumar *et al*., 2016). Regular feeding of feed additives help in balancing beneficial rumen microbe's thus optimizing volatile fatty acids production, improving nutrient utilization and preventing loss of milk yield. The present study was planned to see the effects of feeding Amla and Shatavari powder blend on rumen fermentation and biochemical profile in Murrah buffaloes.

Materials and Methods

The study was planned and carried in Department of Veterinary Physiology and Biochemistry in collaboration with Department of Livestock Production and Management and Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar from November 2023 to March 2024. In first phase of experiment, rumen fermentation studies were conducted in fistulated buffalo calves and in second phase, hemato biochemical parameters were studied in lactating Murrah buffaloes after feeding *E. officinalis* and *A. racemosus* powder after taking Institutional Animal Ethical Committee approval.

Rumen fermentation studies

The feeding trial was conducted on three male fistulated buffalo calves, kept in an open shed with appropriate set up for watering and feeding each animal individually. The animals were provided ration having 40% concentrate and 60% roughage as per ICAR (2013). The concentrate mixture containing 17.58% CP and 70% TDN comprised of maize 30%, groundnut cake 15%, mustard cake 20%, wheat bran 32%, mineral mixture 2% and common salt 1% containing + 10-12 kg green fodder + wheat straw *ad lib.* ***Asparagus racemosus***(Shatavari root) and ***Emblica officinalis*** dried fruit (Amla) were purchased form local market, dried in oven, ground and sieved. The ration was supplemented with plant blend of *E. officinalis* powder and *A. racemosus* powder (2:1) @ 2% of dry matter for a period of two months. Rumen liquor samples were collected at 0 hour at 0 day, 30 days and 60 days for two consecutive days, strained through four layered muslin cloth into a thermos flask which was previously flushed with carbon dioxide to maintain anaerobic conditions. Ruminal fluid temperature and pH were measured immediately. One fraction of 15 ml of rumen fluid was preserved with few drops of saturated mercuric chloride solution to determine ammonia nitrogen and total nitrogen concentration. Estimation of ammonia nitrogen (NH_3-N) was done by Conway (1962) disc method and total nitrogen was estimated by Micro Kjeldahl method (AOAC, 2005). The other fraction of 5 ml ruminal fluid was preserved with equal volume of 5% sulphuric acid for estimation of total volatile fatty acids by Markham (1942) steam distillation method.

Haemato-biochemical studies in lactating buffaloes

Twenty-four lactating Murrah buffaloes were selected and divided into two groups having twelve in early lactation and twelve in mid lactation stage from the herd of buffalo farm of the Department of Livestock Production Management, College of Veterinary Sciences, Lala Lajpat Rai University of Sciences, Hisar. Further animals were divided into subgroups (control and treatment) having six in each subgroup in both early and mid-lactation stage. The buffaloes in control group were fed ration composed of concentrate mixture and roughage individually as per ICAR (2013). The animals in treatment groups were fed standard ration concentrate along with plant blend of *E. officinalis* and *A. racemosus* (2:1) @ 2% of dry matter for a period of three months. Blood samples were collected at 0, 45 and 90thday of experiment aseptically during early morning hours before watering and feeding of buffaloes by jugular vein puncture. Sample collection was done in two sterile plastic tubes, one containing anticoagulant for haematological parameters and other without anticoagulant for serum separation. The clear sera were collected in separate clean, dry and labelled capped vials and stored under deep freezing (-20°C) for further analysis.

Hemato-biochemical parameters

The blood samples were analysed immediately for haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), lymphocytes, monocytes, granulocyte, mean corpuscular haemoglobin (MCH), and mean corpuscular volume (MCV) analysis by using fully automated haematology analyzer (MS4Se®).The sera samples were analyzed for estimation of blood biochemical parameters (glucose (mg/dl), total protein (g/dl), albumin (g/dl), blood urea nitrogen (BUN) (mg/dl), triglyceride (mg/dl), cholesterol (mg/dl), calcium(mg/dl), phosphorus (mg/dl), aspartate aminotransferase (AST) (IU/L) and alanine aminotransferase (ALT) (IU/L)) using Automated Random Access Clinical Chemistry Analyser (EM Destiny 200)^{TM}, Erba Diagnostics Mannheim GmbH).

Assay of lipid peroxidation

The malonaldehyde determination was carried out using the method described by Ohkawa*etal*. (1979). The technique relies on the reaction between malondialdehyde (MDA), a byproduct of lipid peroxidation and thiobarbituric acid (TBA), which produces a pink-colored complex in an acidic environment (pH = 3.5). The absorbance of this complex was measured at a wavelength of 532 nm.To a 0.1 ml sample, 0.2 ml of 8.1% SDS solution was added and mixed. Then, 1.5 ml of 20% acetic acid solution was incorporated, and the pH was adjusted to 3.5. Next, 1.5 ml of 0.8% aqueous thiobarbituric acid (TBA) solution was added. The final volume was adjusted to 4.0 ml with distilled water. The reaction mixture was heated in a water bath at 95°C for 60 minutes and then cooled immediately. Following this, 1.0 ml of distilled water and 5.0 ml of the n-butanol and pyridine mixture (15:1) were added. The test tubes were shaken vigorously and centrifuged at 4000 rpm for 10 minutes. The organic layer was separated and the absorbance of organic layer was taken at 532 nmat different concentrations of TMP.

Statistical Analysis

The data obtained was analyzed statistically with the SPSS (version 17) software using one way ANOVA followed by Duncan multiple range test. Significant differences were considered at p level ($p \leq 0.05$). The data obtained was analysed statistically using the Paired "t" test as described by Snedecor and Cochran (1994).

Results and discussion

Rumen fermentation parameters

The results of rumen fermentation parameters after supplementing *Emblica officinalis and Asparagus racemosus* powder in the diet of fistulated buffalo calves has been presented in Table 1. A significant ($p \leq 0.05$) decrease in ruminal fluid pH was observed at 30 days (6.54±0.03) and 60 days (6.44±0.13) after feeding plant blend. TVFA concentration increased significantly ($p \leq 0.05$) at 30 days (72.50± 5.77) and at 60 days (86.67±2.47) post feeding plant blend.Total nitrogen (mg/dl) concentration increased significantly ($p \leq 0.05$) at 30 days (56.41±0.50) and at 60 days (59.90±1.29) after feeding plant mixture.

Haematological parameters

The results (mean ± S.E.) of haematological parameters in two groups of Murrah buffaloes at different intervals have been presented in Table 2. The results revealed a significant ($p \leq 0.05$) increase in haemoglobin (g/dl) concentration at 45 days and 90 days after feeding the plant blend in treatment group in early lactation group and at 45 days in treatment group as compared to control group during the mid-lactation stage.

Biochemical parameters

The (mean ± S.E.) values of biochemical parameters have been presented in Table3. Glucose concentration(mg/dl) increased significantly ($p \leq 0.05$) at 90 days (62.80±3.48) in treatment group after feeding plant blend of early lactation stage as compared to control group (49.32±3.12). The results revealed a significant difference ($p \leq 0.05$) in glucose concentration at 45 days between treatment (53.70±1.21) and control group (46.93±2.73) and at 90 days 55.65±2.48 (treatment) and 46.83±2.45 (control) in mid lactation stage. Total protein concentration(g/dl) increased significantly ($p \leq 0.05$) in treatment group in both early and mid-lactation stages at 45 days and 90 days. The protein concentration observed in control (6.10±0.27) and in treatment (6.80±0.17) group at 45 days indicating a significant increase ($p \leq 0.05$) in treatment group in early lactation stage. The lipid peroxidation (MDA,nmol) values ranged from 320±15.04 (0 day) to 360±21.06 (90 day) in control group and from 348±23.05 (0day) to 241±24.03 (90 day) in treatment group in mid lactation animals. A significant decrease ($p \leq 0.05$) of lipid peroxidation was observed in treatment group of mid lactation stage at 90 days post feeding.

Table 1: Effect of *E. officinalis and A. racemosus*feeding on rumen fermentation parameters in buffalo calves

Parameter	Days			p-value
	0 day	30 days	60 days	
Temperature(ºC)	37.85±0.55	38.33±0.08	37.51±0.70	0.06
pH	6.79^{a}±0.20	6.54^{b}±0.03	6.41^{c}±0.13	0.01
Ammonia nitrogen (mg/dl)	4.90±0.31	4.66±0.29	4.78±1.12	0.34
Total – nitrogen (mg/dl)	52.65^{a}±2.69	56.41^{b}±0.50	59.90^{c}±1.29	0.03
Total VFA (meq/L)	68.50^{a}±5.77	72.50^{b}±5.77	86.67^{c}±2.47	0.02

^{a,b,c}Mean values bearing different superscripts in a row varies significantly ($p \leq 0.05$)

Table 2: Effect of *E. officinalis and A. racemosus* feeding on haematological parameters of Murrah buffaloes under different lactation stages

Lactation Stage		Early			Mid		
Parameter	**Days**	**Control**	**Treatment**	**p-value**	**Control**	**Treatment**	**p-value**
Haemoglobin (g/dl)	0	10.25±0.31	10.40±0.40	0.78	10.79±0.55	11.22±0.28	0.58
	45	10.48^{a}±0.27	11.29^{b}±0.23	0.04	11.02^{a}±0.20	11.84^{b}±0.20	0.02
	90	10.79^{a}±0.32	11.80^{b}±0.31	0.05	11.10±0.23	11.71±0.34	0.16
TEC (million/mm^3)	**0**	7.35±0.21	6.95±0.31	0.31	6.48±0.12	6.87±0.24	0.19
	45	6.50±0.15	6.73±0.34	0.55	6.42±0.20	6.75±0.16	0.23
	90	7.19±0.20	7.63±0.12	0.51	6.80±0.16	7.68±0.21	0.30
MCH (pg)	0	17.77±0.96	16.33±0.76	0.27	16.17±0.54	15.33±1.09	0.51
	45	15.83±1.14	16.67±0.71	0.55	17.08±0.70	16.33±0.71	0.17
	90	17.33±1.05	16.67±0.61	0.60	16.92±1.12	17.33±1.20	0.62
MCV (fl)	0	40.47±1.85	42.33±1.61	0.46	42.72±2.32	41.77±1.04	0.72
	45	41.83±1.35	42.33±1.26	0.79	43.93±1.42	44.86±1.21	0.34
	90	42.50±2.19	41.67±0.99	0.50	43.38±1.32	42.22±1.66	0.30
TLC (10^3/mm^3)	0	8.78±0.33	9.61±0.37	0.13	10.70±0.70	10.89±0.32	0.81
	45	9.21±0.57	9.13±0.94	0.95	10.89±0.46	11.65±0.30	0.20
	90	9.40±0.35	10.89±0.74	0.10	11.28±0.53	11.34±0.58	0.94
Granulocytes (%)	0	54.88±3.50	56.98±5.21	0.74	60.07±2.55	58.22±8.62	0.84
	45	56.72±2.63	58.37±3.40	0.71	58.49±3.49	55.81±4.07	0.63
	90	53.93±2.92	55.37±2.81	0.73	56.95±2.75	58.53±5.99	0.82
Lymphocytes (%)	0	42.18±2.21	39.65±3.25	0.53	42.38±26.88	38.38±3.53	0.31
	45	35.87±2.34	39.20±2.32	0.39	36.65±3.09	38.28±1.81	0.66
	90	38.38±2.62	41.67±2.71	0.40	36.23±2.96	40.88±3.29	0.30
Monocytes (%)	0	2.23±0.30	2.18±0.14	0.88	2.42±0.23	2.32±0.34	0.82
	45	2.48±0.20	2.33±0.14	0.55	2.30±0.24	2.13±0.53	0.78
	90	2.25±0.28	2.02±0.23	0.53	2.55±0.56	1.83±0.23	0.26

^{a,b,c}Mean values bearing different superscripts in a row varies significantly p≤0.05

Table 3: Effect of *E. officinalis and A. racemosus* feeding on biochemical parameters of Murrah buffaloes under different lactation stages

	lactation	Early			Mid		
Parameter	**Days**	**Control**	**Treatment**	**p-value**	**Control**	**Treatment**	**p-value**
Glucose (mg/dl)	0	50.62±5.74	53.23±1.89	0.64	44.12±2.82	48.76±2.82	0.25
	45	50.78±2.50	59.53±3.28	0.06	46.93^{a}±2.73	53.70^{b}±1.21	0.05
	90	49.32^{a}±3.12	62.80^{b}±3.48	0.01	46.83^{a}±2.45	55.65^{b}±2.48	0.05
Triglyceride (mg/dl)	0	15.83±1.40	12.57±0.87	0.07	15.33±1.71	14.16±2.24	0.69
	45	18.83±2.36	13.76±1.18	0.08	13.23±1.24	13.50±0.96	0.87
	90	16.16±2.46	15.99±2.14	0.95	14.50±1.09	13.83±1.64	0.47
Cholesterol (mg/dl)	0	121.83^{a}±2.87	135.66^{b}±3.63	0.01	110.50±6.18	103.16±13.43	0.63
	45	126.67±4.22	107.5±16.79	0.29	101.83±10.10	108.83±10.70	0.64
	90	110.17±9.99	108.5±10.67	0.91	108.17±9.19	101.33±12.12	0.66
Total protein (g/dl)	0	6.13±0.28	6.30±0.20	0.64	6.58±0.36	7.10±1.26	0.70
	45	6.10^{a}±0.27	6.80^{b}±0.17	0.05	6.55±0.22	7.57±1.21	0.43
	90	6.12±0.43	6.98±0.18	0.09	6.48±0.64	7.76±0.65	0.19
Albumin (g/dl)	0	2.76±0.27	2.32±0.06	0.14	2.12^{a}±0.07	2.32^{b}±0.06	0.03
	45	2.41±0.14	2.19±0.25	0.47	2.41±0.14	2.19±0.25	0.47
	90	2.54±0.37	2.30±0.11	0.71	2.54±0.37	2.39±0.11	0.71
Blood Urea (mg/dl)	0	24.55±1.78	28.13±1.77	0.18	26.29±2.74	27.8±3.02	0.72
	45	28.63±1.74	29.8±1.69	0.64	26.48±2.04	26.58±1.70	0.97
	90	28.87±1.80	24.33±0.51	0.13	27.28±2.13	29.90±2.23	0.42
Calcium (mg/dl)	0	11.65±0.69	11.28±0.40	0.65	11.06±0.29	11.32±0.34	0.59
	45	11.30±0.47	11.60±0.42	0.64	11.24±0.30	11.48±0.18	0.50
	90	11.47±0.46	11.33±0.31	0.81	11.17±033	11.23±0.40	0.92
Phosphorus (mg/dl)	0	5.88±0.20	5.78±0.20	0.75	6.13±0.20	6.27±0.10	0.56
	45	5.93±0.22	5.85±0.22	0.62	5.51±0.31	5.71±0.44	0.71
	90	5.79±0.53	5.86±0.26	0.81	5.92±0.55	5.21±0.43	0.34
ALT (IU/L)	0	35.10±5.14	31.10±0.5	0.52	22.83±3.53	33.45±4.28	0.08
	45	34.00±4.93	36.9±3.87	0.65	26.3±2.92	30.83±4.22	0.40
	90	31.93±3.73	28.16±3.50	0.42	31.16±2.65	27.07±4.91	0.47
AST (IU/L)	0	120.67±4.56	108.36±3.66	0.06	112.2±3.28	117.8±3.49	0.27
	45	119.02±4.45	113.11±6.39	0.11	113.15±4.92	110.71±5.22	0.74
	90	117.57±4.50	106.28±3.74	0.15	114.33±3.36	111.46±3.21	0.13
Lipid peroxidation (nmol)	0	360±11.06	335±21.03	0.61	320±15.04	348±23.05	0.73
	90	340±10.55	280±22.05	0.58	360^{a}±21.06	241^{b}±24.03	0.05

$^{a, b, c}$Mean values bearing different superscripts in a row varies significantly p≤0.05

The biochemical findings are in accordance with Razo Ortiz et al. (2020) who reported significant increase in hemoglobin (g/dL) after feeding with herbal supplements in lambs, may be associated with vitamin C present in amla fruit able to promote iron absorption in lower tract facilitating hemoglobin synthesis. The inclusion of *E. officinalis* powder impacted nutrient metabolism and led to a significant rise in total erythrocyte count (TEC), hemoglobin (Hb) concentration, total protein and albumin levels ($p \leq 0.05$) in the treatment group (Sarthak et al. 2025). A significant ($p \leq 0.05$) increase in glucose concentration in treatment groups might be due to more gluconeogenesis as mentioned by Tilahun et al. (2022) who also reported that supplementation with amla fruit led to an increase in serum glucose levels at the rate 15.4%, 18.3%, and 6.3% for daily doses of 200, 400, and 600 g amla respectively in dairy cows as compared to the control group. The results are in accordance with the Galbat et al. (2014) and Saini et al. (2018) in Sahiwal cows supplemented with Shatavari root powder. A significant ($p \leq 0.05$) increase in total protein levels (g/dL) following Shatavari supplementation in lactating goats was also reported by Galbat et al. (2014). The significant increase in albumin, total protein, and globulin levels at the 400 g/d (FAF) dose of amla fruit supplementation in dairy cows affects protein utilization efficiency and enhances liver function (Tilahun et al. 2022). Madan *et al*. (2015) evaluated the effect of *Emblica oficinalis* and *Mentha piperata* supplementation on biochemical parameters in 36 growing beetal kids (3-4 months age) and concluded that feeding of *E. officinalis* has shown significant hypolipedemic effects in kids as compared to *M. piperata*.

Conclusion

Considering all together, feeding of *A. racemosus and E. officinalis* may alter the ruminal microbial environment leading to improvement in fermentation and volatile fatty acids productions beneficial for productive purpose without any adverse effect on haemato-biochemical parameters and health of animals.

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