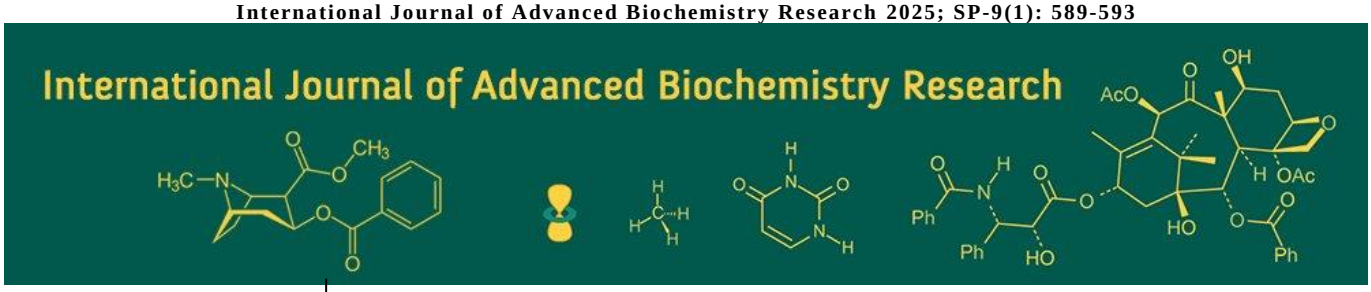

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Effect of feeding *E. officinalis* on rumen fermentation and biochemical parameters in growing buffalo calves

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Abstract
This study sought to evaluate the potential of amla dried fruit powder as a feed supplement for rumen fermentation, growth and hematobiochemical parameters in male murrah buffalo calves. *E. officinalis* powder was supplemented at 30 grams per day per animal in the diet of three fistulated buffalo calves for a period of three months. Rumen fermentation parameters were analyzed in the rumen samples collected at 0, 30, 60 and 90 days of the trial. In the second phase, six growing male buffalo calves aged 12-14 months were fed *E. officinalis* fruit powder along with standard ration for three months, and another group of six buffalo calves were fed standard ration (control group). Growth parameters and blood samples were taken at 0, 45 and 90 days of the trial period. The results indicated an improvement in microbial fermentation, as indicated by an increase in the total volatile fatty acid, propionate and butyrate concentrations in the ruminal fluid at 60 and 90 days post feeding, increasing the amount of glucogenic energy available for productive purposes. Feeding of *E. officinalis* did not significantly affect growth parameters. The hematological parameters indicated an increase in hemoglobin and total erythrocyte count without any significant changes in other parameters. The plasma glucose, cholesterol, total protein and albumin concentrations indicated a normal physiological status of the treatment group animals. This study provides a comprehensive conclusion that the inclusion of *E. officinalis* in the diet of buffalo calves improved rumen fermentation and metabolism, maintaining the physiological health status of the animals.

Keywords: Buffalo, rumen, *Emblica officinalis*, metabolites, plasma

Introduction
Ruminants are considered to be best at converting low-quality feed to useful products, which also provides an additional advantage of being non competitive in the human food chain. Tropical ruminant livestock are fed mainly poor-quality forages or crop residues, and the microbial enteric fermentation pattern in the rumen represents a waste of a considerable portion of gross energy depending on the feed composition and geographical location (Wang *et al*. 2011) [22], which can otherwise be used for productive purposes. To find an alternative to energy loss, several approaches, such as chemical treatments of feed, antibiotics and ionophores have been used to decrease proteolysis and enhance fiber degradation in the rumen by enzymatic inhibition in the rumen (Kamra *et al*. 2012) [14], but residual effects and antibiotic resistance have decreased consumer acceptance for their use in animal feed. This has caused a shift in interest in the search for natural feed alternatives that are environmentally friendly and have better acceptance with regard to feed safety issues. Plant secondary metabolites (phenolic and tannin compounds), the main active components as rumen modifiers, are considered better than chemicals or antibiotic-based modifiers. More efficient feed conversion, improved animal growth and improved performance in terms of the use of alternative hydrogen by rumen microbes enhancing volatile fatty acid and reducing gas production. A significant reduction in methane emission has been observed without any adverse effects on feed utilization by using essential oils, tannins and saponins in *in vivo* trials at a rate of 1-2% of dry matter intake (Kamra *et al*. 2012) [14]. *Emblica officinalis*, commonly known as amla, which is grown throughout tropical parts of India and has medicinal properties such as antioxidant and chemopreventive properties. Regular intake of amla in the diet promotes nutrient absorption and counteracts the effects of environmental stress by lowering free radicals (Gujman *et al*. 2020) [10]. Studies related to rumen fermentation, growth and metabolism in buffalo calves supplemented with locally available

herbs are scarce. The present study investigated the effects of feeding locally available amla dried fruit on the fermentation pattern, growth and hematobiochemical profile of buffalo calves.

Materials and Methods

The study was planned and carried out in the Department of Veterinary Physiology and Biochemistry, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, after receiving prior approval from the Institutional Animal Ethical Committee (VCC/IAEC/2022/624-57). In the first phase of the study, rumen fermentation parameters were analyzed in fistulated buffalo calves, and in the second phase, growth and hematobiochemical parameters were analyzed in growing male buffalo calves after feeding *E. officinalis* powder to the calves.

Rumen fermentation parameters

The study was conducted on three rumen-fistulatedmurrah buffalo calves housed in individual open sheds with free access to feed and water. The rations were provided to individual animals according to their body weight (ICAR, 2013) [13]. The concentrate mixture comprised 30% maize, 15% groundnut cake, 32% wheat bran, 20% mustard cake, 2% mineral mixture and 1% common salt and contained 17.58% crude protein and 70% total digestible nutrients plus 10 kg of green fodder plus wheat straw *ad libitum.* Dried *E. officinalis* fruit was purchased from local market, ground and sieved. The feed was supplemented with 30 gram of *E. officinalis* powder per day per animal for three months. Rumen fluid was collected at 0, 30, 60 and 90 days of the trial, strained through four-layered muslin cloth and divided into different fractions for further analysis. The rumen fluid pH was measured immediately by a pH meter (Hanna Instruments). One 15 ml fraction of rumen fluid was preserved with a few drops of saturated mercuric chloride solution to determine the ammonia nitrogen (Conway 1962) [5] and total nitrogen concentrations as per AOAC (2005). The second fraction of 5 ml of ruminal fluid was preserved with an equal volume of 5% sulfuric acid for estimation of total volatile fatty acidsby Markham (1942) [17]. One 10 ml fraction of ruminal fluid was processed with 2 ml of metaphosphoric acid (25% w/v), incubated overnight at room temperature and centrifuged at 5000 rpm for 10 minutes to obtain a clear supernatant. For the estimation of individual volatile fatty acids by gas liquid chromatography, the sample supernatant rumen liquor or standard was injected with the help of a 10 µL Hamilton microsyringe, which is capable of delivering 0.1 µL into a gas chromatograph equipped with a flame ionization detector (FID) and a glass column packed with chromosorb-101 according to Erwin (1961) [7].

Growth and hematobiochemical parameters

Twelve male buffalo calves aged 12-14 months were divided into two groups (control and treatment) of six animals in each group. *E. officinalis* powder (30 g/day/animal) was supplemented in the diet of the treatment group for a period of three months. The body growth parameters of each animal were recorded in the morning before providing feed and water for two consecutive days at 0, 45 and 90 days of the experiment.

Blood samples were collected at 0, 45 and 90 days of the trial via jugular puncture in two separate vials with heparin as anticoagulant in the morning before feeding and mixed well by rotating tubes between the palms. One set of the samples was centrifuged at 3000 rpm for 15 minutes to separate the plasma and stored at -20 °C for further analysis of biochemical parameters. The second set of blood samples was used for analysis of hematological parameters (total erythrocyte count (TEC), hemoglobin (Hb), total leucocyte count (TLC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total granulocytes, lymphocytes and monocytes) by an automated hematology analyzer (MS4Se®). The analysis of plasma biochemical parameters (glucose (mg/dl), total protein (g/dl), albumin (g/dl), blood urea nitrogen (BUN, mg/dl), triglyceride (mg/dl), cholesterol (mg/dl), phosphorus (mg/dl), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), high-density lipoprotein (HDL, mg/dl) and low-density lipoprotein (LDL, mg/dl)) was carried out using kits (M/s Transasia Biomedical Limited) with an automated clinical chemistry analyzer (EM 200^{TM}Erba Mannheim - Germany).

Statistical analysis

The data obtained were analyzed statistically with SPSS (version 17) software using one-way ANOVA followed by Dunken's post hoc test. The data are expressed as the mean ± SD with a significance level of $p \leq 0.05$.

Results and Discussion

Rumen fermentation parameters

The data (Table 1) revealed a significant decrease in ruminal fluid pH and a significant increase in VFA concentration (meq/l) at 60 and 90 days after feeding *E. officinalis* powder. Hosoda *et al.* (2006) [11, 12] reported a decrease in ruminal pH after the consumption of an herbal mixture. A significant increase in VFA, propionate and butyrate concentrations was observed at 60 and 90 days ($p \leq 0.05$) post feeding, indicating improved ruminal fermentation, also reported earlier by Wang *et al.* (2022) [23] after supplementing spearmint in the diet of calves. Acetate was not strongly affected during the feeding trial except for a decrease at 30 days. The ammonia nitrogen concentration increased significantly ($p \leq 0.05$) at 60 and 90 days, possibly because the rate of ammonia nitrogen formation exceeds that of cellulose fermentation and bacterial nitrogen use (Ghizzi *et al.* 2020) [8]. The hydrolysable tannins found in amla fruit may be responsible for the degradation of proteins to increase ammonia nitrogen levels in the rumen (Avila *et al.* 2020) [3]. Our results are in consistent with those of Tilahun M *et al.* (2022) [21], who reported an increased ruminal ammonia nitrogen concentration and molar proportions of propionate and butyrate after feeding fresh amla fruit at 600 g/day,while acetate proportion increased compromising butyrate at 400 g/day amla inclusion in the diet of lactating cattle without affecting nutrient digestibility. Aboagye *et al.* (2019) [1] suggested that the hydrolysable tannin metabolite pyrogallol present in amla fruit subjected to microbial degradation in the rumen is converted to acetate and butyrate. A significant increased total nitrogen concentration and increasing trend of VFA concentration was observed in rumen fluid after supplementation with *E. officinalis* plant extract in an *in vitro* trial (Sarthak *et al.*, 2023) [19].

Table 1: Effect of feeding *Emblica officinalis* on rumen fermentation parameters in buffalo calves (Mean ± S.D.)

Parameter	Days				P-value
	0 day	30 day	60 day	90 day	
pH	6.5^{a}±0.03	6.83^{b}±0.1	6.31^{c}±0.12	6.27^{c}±0.08	0.03
Ammonia –N (mg/dl)	9.15^{a}±0.17	8.74 ^{a}±0.2	11.16 ^{c}±0.33	11.16^{c}±0.17	0.12
Total – N (mg/dl)	69.69^{ab}±1.91	73.37^{b}±2.86	64.45^{a}±2.18	68.6^{ab}±1.09	0.03
TVFA (mEq/L)	103.04^{a}±2.98	99.6^{a}±3.72	109.66^{b}±2.3	111.05^{b}±2.58	0.02
Acetate (mEq/L)	67.11±1.03	63.87±2.85	67.23±1.65	66.47±0.69	0.07
Propionate (mEq/L	17.22^{c}±0.53	21.67^{b}±0.59	24.03^{b}±1.49	22.15^{b}±0.47	0.02
Butyrate (mEq/L)	8.78^{b}±0.33	8.44^{b}±0.46	12.88^{a}±0.31	13.02^{a}±0.29	0.05

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

Growth parameters

Body growth parameters of buffalo calves of two groups were recorded at the beginning and at the end of the trial (Table 2), and no significant differences were detected between the groups during the trial period. The findings of Wang *et al*. (2022) [23] support our results showing no noticeable effect on the body weight of crossbred cattle supplemented with Chinese herbal medicine, whereas the feed efficiency and average body weight of newborn crossbred (Holstein cross) calves improved significantly after supplementation with *Allium sativum* extract at 250 mg/kg body weight per day for two months (Ghosh *et al*. 2010) [9].

Table 2: Effect of feeding *Emblica officinalis* on growth parameters in buffalo calves (Mean ± S.D.)

Parameter	Days	Group		P-value
		Control	Treatment	
Body weight (kg)	0 day	156.83±9.78	159.5±4.36	.808
	90 day	176.17±12.71	172.83±8.69	.833
Body height (mts.)	0 day	1.03±0.03	1.04±0.01	.868
	90 day	1.14±0.03	1.14±0.02	.877
Heart girth (mts.)	0 day	1.32±0.02	1.37±0.03	.245
	90 day	0.97±0.06	1.39±0.06	.424
Body length (mts.)	0 day	1.11±0.04	0.92±0.04	.478
	90 day	1.29±0.04	1.07±0.04	.557
Abdominal girth (mts.)	0 day	1.68±0.09	1.33±0.03	.705
	90 day	1.56±0.07	1.57±0.02	.173

Hemato-biochemical parameters

The results of hematological parameters (Table 3) revealed a significant (p≤0.05) increase in TEC, hemoglobin and PCV at 45 and 90 days in the treatment group. The TLC concentration decreased significantly (p≤0.05) at 45 days and 90 days in the *E. officinalis* fed group. Hemoglobin and hematocrit values are based on whole blood and dependent on plasma volume. Increased packed cell volume is associated with an increase in RBC count or decreased volume (Kaneko 2008) [15]. The increased TEC and hemoglobin without compromising MCV in the supplemented group may be associated with vitamin C present in amla fruit being able to promote iron absorption in the lower tract, facilitating hemoglobin synthesis (Cook and Reddy 2001) [6]. Our results are in agreement with those of Razo Ortiz *et al*. (2022) [18], who also reported a similar pattern of hematological parameters in lambs fed a polyherbal mix containing *E. officinalis*.

A significant (p≤0.05) difference in the ALT concentration was observed between the treatment group and the control group at 90 days (Table 4). Triglyceride concentration (mg/dl) was greater (p≤0.05) in the control group at 45 and 90 days, while it was lower (19.33±2.16) at 90 days in the treatment group than at 0 days (32.66±7.65). The glucose concentration increased significantly with time in the treatment group as compared to control group, which may be attributed to polyphenolic compounds present in amla being responsible for improving ruminal microbial fermentation and enhancing propionate, the major precursor of glucose used for growth and maintenance, as documented by Bhatt *et al*. (2009) [4]. A significant variation was observed in the low-density lipoprotein (LDL) concentration (p≤0.05) at 45 and 90 days in both control and supplemented groups. The normal range of cholesterol and consistent HDL concentration with decreasing triglyceride levels in the present treatment group indicate that normal and sustained lipid metabolism might be a sequela of antioxidants present in amla. It is worth mentioning that in earlier studies conducted by Madan *et al*. (2015) [16], amla feeding had a hypolipedemic effect in beetal goat kids. Seifzadeh *et al*. (2016) [20] observed a significant effect on daily weight gain without affecting blood glucose, cholesterol and albumin after feeding a medical plant mix at a rate of 1.5% in suckling buffalo calves. The total protein concentration was within the normal range in both groups. The albumin concentration varied from 1.53±0.05 to 2.43±0.04 and showed an increasing trend in both groups, whereas significant increase in BUN in the control group may be due to increased protein degradation and absorption of ammonia from the rumen. A significant increase in ruminal ammonia reflecting a concomitant increase in plasma urea nitrogen in steers observed after feeding peppermint and lemongrass indicated ammonia absorption from rumen and conversion to urea in the liver (Hosoda *et al*. 2006a) [11, 12].

Table 3: Effect of feeding *Emblica officinalis* on haematological parameters in buffalo calves (Mean ± S.D.)

Parameters	Group	Days		
		0 day	45 day	90 day
TEC (million/mm^3)	Treatment	$4.80^{A,a}$±0.60	6.70^{B}±0.10	7.21^{C}±0.18
	Control	7.87^{b}±0.48	6.87±0.38	7.81±0.49
Haemoglobin (g/dl)	Treatment	$7.80^{A,a}$±1.03	10.84^{B}±0.29	11.68^{B}±0.31
	Control	12.97^{b}±0.90	11.71±0.64	11.37±0.66
TLC (million/mm$^{3)}$	Treatment	$11.57^{B,b}$±0.79	9.08^{A}±0.31	8.43^{A}±0.57
	Control	8.00^{a}±1.09	8.79±0.54	8.37±0.76
PCV (%)	Treatment	$21.37^{A,a}$±2.50	$32.1^{B,a}$±0.65	30.70^{Ba}±1.33
	Control	36.83^{b}±1.68	36.01^{b}±1.55	39.00^{b}±2.12
MCV (fl)	Treatment	44.98^{a}±1.08	48.01±1.34	46.75±0.92
	Control	48.35^{b}±0.95	53.27±3.79	50.22±2.13
MCH (pg)	Treatment	16.17±0.20	16.2±0.42	16.17±0.36
	Control	15.85±0.17	17.39±1.48	15.85±0.28
MCHC (g/dl)	Treatment	36.12^{b}±1.02	33.88±1.37	34.75±0.89
	Control	32.83^{a}±0.42	32.54±1.12	31.32±1.42
Lymphocytes (%)	Treatment	67.68±3.38	64.43±2.6	63.65±2.74
	Control	58.40±5.47	59.03±3.94	59.62±3.87
Monocytes (%)	Treatment	3.25^{B}±0.32	2.93^{AB}±0.23	2.57^{A}±0.26
	Control	2.95±0.31	2.87±0.22	2.82±0.15
Granulocytes (%)	Treatment	29.07±3.51	32.64±2.64	33.78±2.72
	Control	38.65±5.72	38.1±4.02	37.57±3.97

$^{a, b}$Mean values bearing different superscripts between groups and $^{A, B, C}$ between days varies significantly (p≤0.05)

Table 4: Effect of feeding *Emblica officinalis* on biochemical parameters in buffalo calves (Mean ± S.D.)

Parameters	Group	Days		
		0 day	45 day	90 day
Alanine aminotransferase(U/L)	Treatment	51.78±11.09	48.88±13.77	63.08^{b}±5.09
	Control	45.5±5.76	65.02±13.35	48.73^{a}±3.7
Aspartate aminotransferase(U/L)	Treatment	131.32±8.02	119.75±12.15	130.95±4.24
	Control	122.27±11.96	147.13±31.32	126.35±8.86
Phosphorus(mg/dl)	Treatment	6.1^{A}±0.35	6.68^{B}±0.54	6.67^{B}±0.63
	Control	6.14^{A}±0.44	6.56^{AB}±0.54	7.19^{B}±0.99
BUN(mg/dl)	Treatment	29.03^{b}±4.15	36.53±5.25	29.35±1.14
	Control	15.28^{aA}±4.6	35.72^{B}±6.26	30.18^{B}±3.1
Glucose(mg/dl)	Treatment	54.5^{A}±5.93	74.9^{B}±6.35	79.3^{B}±2.3
	Control	65.58^{A}±3.98	68.52^{A}±5.46	80.5^{B}±1.3
Triglyceride(mg/dl)	Treatment	32.67^{bA}±7.65	23.00±2.91	19.33^{B}±2.16
	Control	$14.67^{A,a}$±2.81	26.33^{B}±2.69	25.33^{B}±2.4
Cholesterol(mg/dl)	Treatment	62.5^{A}±4.6	77.67^{B}±4.18	78.17^{B}±1.74
	Control	53.17^{A}±3.61	71.50^{B}±4.85	75.33^{B}±2.03
HDL(mg/dl)	Treatment	43.15^{a}±2.9	44.15±3.37	46.78^{a}±3.26
	Control	34.33^{Ab}±3.82	43.83^{B}±4.11	37.75^{Ab}±2.65
LDL(mg/dl)	Treatment	13.17^{A}±2.31	22.94^{B}±4.55	21.81^{B}±2.46
	Control	11.42^{A}±1.87	22.57^{B}±1.5	19.3^{B}±0.89
Albumin(g/dl)	Treatment	1.53^{A}±0.05	1.87^{B}±0.08	2.43^{C}±0.04
	Control	1.59^{A}±0.08	1.91^{B}±0.06	2.41^{C}±0.12
Total protein(g/dl)	Treatment	$6.72^{A,b}$±0.38	7.19^{B}±0.38	$7.17^{B,a}$±0.72
	Control	$7.38^{A,a}$±0.53	6.99^{B}±0.42	$5.65^{C,b}$±0.35

$^{a, b}$ Mean values bearing different superscripts between groups and ^{A,B,C} between days varies significantly (p≤0.05)

Conclusions

The promising effects of feeding *E. officinalis* on ruminal fermentation have been reflected by increased levels of TVFAs and propionic acid in rumen fluid, which can be utilized more efficiently for productive purposes. Furthermore, these studies revealed that *E. officinalis* powder feeding had no adverse effect on metabolism, as no such significant alteration in plasma metabolites was observed. An increase in the TEC and hemoglobin levels in the treatment group indicated improved nutrient absorption and metabolism. More studies are requisited to strengthen the current evidence and can be extrapolated for further studies.

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