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RESEARCH ARTICLE

# Effect of dietary supplementation of *Emblica officinalis* fruit pomace on methane emission, ruminal fermentation, nutrient utilization, and milk production performance in buffaloes

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## Abstract

Effects of dietary supplementation of *Emblica officinalis* fruit (Indian gooseberry) pomace (EFP), a waste from fruit processing plants and rich in polyphenolic compounds, were investigated for ruminal fermentation, nutrient utilization, methane production, and milk production performance in buffaloes. An in vitro experiment was conducted using 0 to 50 g/kg of EFP (six treatments) to select an optimum dose for feeding of buffaloes. Organic matter (OM) degradability, total volatile fatty acid concentration, and acetate proportion decreased, but propionate proportion increased at the higher doses (> 30 g/kg). Methane production also decreased at the higher doses (≥ 20 g/kg). In the in vivo study, ten lactating buffaloes were randomly allotted into control and EFP groups ($n = 5$/group). The control group was fed a total mixed ration, whereas the EFP group was fed the control ration along with EFP at 20 g/kg of dry matter (DM) intake for 120 days. Feeding of EFP to buffaloes improved milk yield ($P < 0.01$) and milk production efficiency ($P < 0.01$). Concentration of milk protein tended ($P = 0.071$) to increase and that of solid not fat increased ($P = 0.032$) due to the EFP feeding. Yields (kg/day) of milk fat ($P = 0.026$), solid not fat ($P = 0.011$), and protein ($P = 0.002$) were greater in the EFP group than the control group. Somatic cell count in milk decreased ($P = 0.032$) due to EFP feeding. Digestibility of ether extract ($P < 0.001$) increased and OM ($P = 0.051$) tended to increase by EFP feeding. Methane production (g/d), yield (g/kg DM intake or g/kg digestible organic matter intake), and intensity (g/kg milk, g/kg milk fat, or g/kg milk protein), and methane conversion rate (percentage of gross energy intake) were lower ($P < 0.01$) in the EFP group than the control group. For milk fatty acid (FA) profiles, total saturated FA proportion tended to be greater ($P = 0.057$) in the EFP group than the control group, which was due to increased ($P = 0.045$) proportion of total short- and medium-chain FA (C4 to C14). Feed intake, digestibility of crude protein and fiber, and total n-6, n-3, mono-unsaturated FA, poly-unsaturated FA, and long-chain FA (C18 to C24) proportions were similar between the groups. This study suggests that feeding of EFP at 20 g/kg DM intake increases milk production and decreases methane production and intensity without impacting health of buffaloes and FA profiles of milk. This is a win–win situation for sustainable and cleaner buffalo production by improving milk production and decreasing environmental burdens of greenhouse gas emission and EFP residue disposal problems.

**Keywords** Digestibility · Feed intake · Methane intensity · Milk composition · Milk fatty acid profile

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## Introduction

Deterioration of planetary health such as degradation of land, climatic change, acidification of ocean, water scarcity, loss of biodiversity, deforestation, and overexploitation of fisheries over the past few decades is a great threat for the natural habitants including human civilization (Bell et al. 2019; IPCC 2013; Whitmee et al. 2015). Livestock farming has been considered as substantial divers of many of these ecological changes worldwide (Bouwman et al. 2013; Opio et al. 2013; Whitmee et al. 2015). Globally, livestock contributed 2771 Tg in $CO_2$-eq./year of GHG (i.e., methane and nitrous oxide) in 2010 (Patra 2014a) and 2720 Tg in $CO_2$-eq./year of methane in 2014 (Dangal et al. 2017) from enteric fermentation and manure management to the environment. Livestock shares approximately 10% of the anthropogenic GHG emissions produced globally without considering the contribution from the land use change (Smith et al. 2007) with 37% and 65% of the total anthropogenic methane and nitrous oxide emission, respectively (FAO 2006). Within the agricultural sectors, enteric methane emission contributes approximately 40% of total GHG, which has been estimated to increase by 11% from the period 2001 to 2011 (FAO 2014). Therefore, several efforts have been attempted to decrease ruminal methane production (Patra 2016; Patra et al. 2017).

A large amount of food wastes and residues of food and fruit processing plants is a global concern (Maina et al. 2017; Dahiya et al. 2018). The valorization of these wastes and residues are of paramount importance for transformation of linear economy to circular and sustainable bioeconomy-based processes (Maina et al. 2017; Dahiya et al. 2018; Singh et al. 2020). *Emblica officinalis* (Indian gooseberry or amla) fruit pomace (EFP), which is produced after extraction of juice from the fruits for human consumption, is required for downstream processing in the circular bioeconomy. An amount of over 1 million tons of gooseberries is produced annually in India (APEDA 2020). About 40% of raw fruits are wasted after juice extraction in juice processing industries, which is disposed of mainly to landfill sites. This EEP needs proper valorization including in animal feeding. Gooseberry contains high concentrations of plant bioactive principles such as polyphenols, tannins, flavonoids, vitamin C, and antioxidants (Wadhwa et al. 2015). Due to the presence of these bioactive compounds with several health benefits, *Emblica* fruit has been traditionally used in herbal medicines for longtime, and the trees are grown in many subtropical countries including Indian subcontinent, Southern China, Malaysia, and South East Asia (Variya et al. 2016; Yadav et al. 2017). *Emblica* fruit or its pomace improved growth (2.5 and 7.5 g/kg diet, Kumari et al. 2012; 4 and 8 g/kg diet, Patel et al. 2016; 2.5 to 10 g/kg diet, Dalal et al. 2018) and laying performance (2.5 g/kg diet; Aswal et al. 2017) in poultry. A few in vitro studies on the *Emblica* fruit or its extracts have shown beneficial effects on ruminal fermentation and methane production (Patra et al. 2006; Kumar et al. 2016; Singh et al. 2018).

However, there is no study investigating the effects of the EFP on ruminal fermentation, milk production performance, and methane production in vitro or in vivo in ruminants. Diets supplemented with plant bioactive compounds such as phenolics including flavonoid compounds has been suggested as a feeding strategy to improve ruminal fermentation, lower methane emission, and enhance the functional characteristics of milk and meat from ruminant origins (Vasta et al. 2008; Patra and Saxena 2010; Patra 2014b). It was hypothesized that EFP due to the presence of the plant bioactive compounds may impart positive responses to ruminal fermentation, methane emission, and milk production performance of buffaloes. Therefore, this study was conducted to investigate the effect of EFP on in vitro ruminal fermentation, in vivo nutrient utilization, methane production, milk production performance, and fatty acid (FA) profile in buffaloes.

## Material and methods

The study was carried out at the Directorate of Livestock Farm and Department of Animal Nutrition (305,452 N, 7,548,142 E, and 230 m above sea level), Guru Angad Dev Veterinary and Animal Science University, Ludhiana-1411004, Punjab (India).

### Procurement of *Emblica* fruit pomace

*Emblica* fruit pomace powder was procured from the Unati Cooperative Marketing-cum-Processing Society Ltd., Talwara-144216, Hoshiarpur, Punjab, and sampled in triplicates for analysis of total phenolics (Makkar et al. 1993), condensed tannins (Porter et al. 1986), flavonoids (Balbaa et al. 1974), 2, 2-diphenyl-1-picrylhydrazyl (DPPH; Kumaran and Karakumaran 2007), saponins (Baccou et al. 1977), and vitamin C (Jagota and Dani 1982).

### In vitro experiment

#### Substrate preparation and treatment

Control substrate used in the experiment was a total mixed ration prepared by using concentrate mixture, wheat straw, berseem hay, and oat hay with concentrate to forage ratio of 38:62 (Table 1). Berseem hay, oat hay, wheat straw, concentrate mixture, and EFP were dried at 60 °C for 72 h in a forced hot air oven (NSW-148B Tray drier, Narang Scientific Works, New Delhi, India). Dried samples were ground in a hammer mill, passed through 1-mm sieve and used for in vitro experiment. The in vitro experiment was conducted with six doses

**Table 1** Ingredient and chemical composition of total mixed ration (TMR) and *Emblica* fruit pomace (EFP) fed to lactating buffaloes

| Total mixed ration | | *Emblica* fruit pomace | |
|---|---|---|---|
| Item | Composition | Item | Composition |
| Ingredient composition, g/kg DM | | Chemical composition, g/kg DM | |
| Berseem hay | 390 | Total ash | 15.3 |
| Oat hay | 100 | Crude protein | 32.0 |
| Wheat straw | 130 | Ether extract | 13.3 |
| Concentrate[1] | 380 | Neutral detergent fiber | 419 |
| Chemical composition, g/kg DM | | Acid detergent fiber | 297 |
| Total ash | 90.0 | Hemicellulose | 122 |
| Organic matter | 910 | Gross energy, MJ/kg | 17.0 |
| Crude protein | 145 | Active components, g/kg DM | |
| Ether extract | 40.2 | Total phenolics | 224 |
| Neutral detergent fiber | 492 | Condensed tannin | 1.50 |
| Acid detergent fiber | 367 | Saponins | 25.3 |
| Acid detergent lignin | 27.0 | Flavonoids | 7.40 |
| Cellulose | 340 | Vitamin C | 29.0 |
| Hemicellulose | 125 | DPPH scavenging activity, % | 36.0 |
| Gross energy, MJ/kg | 18.5 | | |

[1] Concentrate mixture contained (g/kg as feed basis): maize grain, 250; bajra grain, 50; barley grain, 60; mustard cake, 150; cotton seed cake, 70; soybean meal, 70; deoiled rice bran, 100; rice bran, 50; wheat bran, 42; bypass fat, 10; dried distillers grains, 50; guar korma, 30; mineral mixture, 20; sodium salt, 20; limestone powder, 10; sodium bicarbonate, 5; dicalcium phosphate, 4; Yea-Sacc$^{R1026}$ (*Saccharomyces cerevisiae* strain with a minimum concentration of $1 \times 10^9$ counts/g; Alltech Biotechnology Pvt. Ltd., India), 2; Toxfin™ 300 (toxin binder contains hybrid nano silicates, activated clays and organic acids; Kemin Industries, Inc. USA), 1; and Bioplex Dairy Max (contains zinc proteinate 80 g/kg, manganese proteinate 40 g/kg, copper proteinate 25 g/kg, ethylenediamine dihydroiodide 4.0 g/kg, selenium yeast 0.6 g/kg, chromium yeast 0.4 g/kg; Alltech Biotechnology Pvt. Ltd. India)

*DM* dry matter; *DPPH* 2, 2-diphenyl-1-picrylhydrazyl

of EFP, i.e., 0, 10, 20, 30, 40, and 50 g/kg of substrate. The control substrate (without EFP) was mixed with the respective doses of EFP to obtain six treatments of 6 different doses.

### Preparation of buffered ruminal inoculum

Ruminal fluid was collected from three fistulated Murrah buffalo bulls (body weight of 516 ± 12.6 kg and age of 6–7 years) maintained on a standard diet (Table 1) to meet their nutrient requirement as per ICAR (2013). Ruminal contents were collected before feed offering to animals at 0800 h in a thermos flask flushed with $CO_2$ and maintained at 39 °C. The ruminal contents were strained through four layers of muslin cloth, and the ruminal fluid was pooled together with equal volumes. Buffered ruminal inoculum was prepared by mixing ruminal fluid with buffer in a ratio of 1:2, and was continuously gassed with $CO_2$ at 39 °C as per the standard procedure (Menke and Steingass 1988). The buffer solution contained 960-mL distilled water, 660-mL bicarbonate buffer, 330-mL macromineral solution, 0.16-mL micromineral solution, and 1.6-mL (1 g/L) resazurin (Menke and Steingass 1988).

### Gas production, degradability, and methane production

Weighed quantity of moisture free substrate (around 375 mg) along with different doses of EFP (as per treatment) was taken in 100-mL calibrated glass syringes (Haberle Labortechnik, Germany) in triplicates with blank syringes (without any substrates) in three sets, and incubated with 30 mL of buffered ruminal inoculum for 24 h in a water bath maintained at 39 °C and swirled every 1 h over a 24-h incubation period. If the gas volume in the syringes exceeded 70 mL after 8 h, the volume of gas produced was recorded, and the gas was released (Menke and Steingass 1988). A reference standard feed (dried berseem fodder) was used. After 24 h of incubation, the volume of gas produced in each syringe was recorded, and fermented fluid samples (1 mL) were taken and preserved for volatile fatty acid (VFA) analysis (Cottyn and Boucque 1968). The contents of syringes were then transferred to spout-less beakers, and refluxed with 20-mL neutral detergent solution for 1 h to assess the in vitro true organic matter (IVTOMD) and neutral detergent fiber degradability (IVNDFD). The beaker content was then filtered through preweighed sintered crucibles (grade 1). The dry matter

content of the residue was weighed and in vitro true degradability of feeds was calculated (Blummel et al. 1997). Blank-corrected net gas production, partitioning factor (PF), and microbial biomass production (MBP) were calculated as described previously (Blummel et al. 1997).

To determine the gas production kinetics, the substrates (around 200 mg) in 6 treatments were incubated for 96 h and gas volumes were recorded at 2, 4, 6, 8, 10, 12, 24, 36, 48, 72, and 96 h of incubation. The gas production data at different hours were fitted to the graph-pad prism program to determine gas production kinetics using the model with inclusion of lag time as described previously (Pal et al. 2015).

For determination of methane production, 200 mg of substrates in six different treatments was incubated with buffered ruminal fluid until $T_{½}$ of each treatment. The volume of gas produced in each syringe was recorded at $T_{½}$, and gas samples were obtained from the headspace of syringe in an airtight syringe and injected into a gas chromatograph (Netchrom 9100, Netel (India) Ltd., Navi Mumbai, India) equipped with flame ionization detector and stainless steel column packed with Porapak-Q. A 50/50 mixture of methane and $CO_2$ (Spancan; Spantech Products Ltd., England) was used as a standard. The VFA were analyzed using a gas chromatograph (Netchrom 9100, Netel (India) Ltd., Navi Mumbai, India) equipped with glass column and flame ionization detector (Cottyn and Boucque 1968).

## In vivo experiment

### Animals, treatment, and feeding management

Ten lactating Murrah buffaloes (body weight of 558 ± 12.2 kg; milk yield of 9.01 ± 0.57 kg/d and parity of 2–4) were selected and randomly divided into two treatment groups in a completely randomized design. All animals were fed individually a control diet (total mixed ration with forage to concentrate ratio of 62:38; Table 1) or a control diet supplemented with EFP at 20 g/kg of dry matter intake (DMI) for 120 days as per ICAR (2013) nutrient requirements of crude protein and digestible energy for lactating buffaloes. Both control and experiment groups were kept under similar management conditions and were fed as per existing feeding practices of the institute dairy farm. After adaptation period of 10 days, the EFP at 20 g/kg of total DM intake was mixed with 1 kg of concentrate mixture and offered at the time of milking (morning) to the experiment group individually, whereas the control group received same concentrate mixture without EFP. It was ensured that all animals consumed feed amounts provided in the milking parlor. The rest of the concentrate mixture and forage were mixed and offered as a total mixed ration to all buffaloes individually. The buffaloes were housed in a concrete shed and were stall fed individually at 0900 h daily. The animals were weighed for 3 consecutive days at monthly intervals on a digital electronic weighing balance (Swift, New Delhi, India), and the feeding schedule was revised accordingly. The animals were provided drinking water twice a day ad libitum and were taken out in the yard for 1-h exercise daily.

### Quantification of enteric methane emission

Enteric methane emission from lactating buffaloes was quantified using the sulfur hexafluoride ($SF_6$) tracer technique (Johnson et al. 1994). The methane was quantified from day 35th to 50th of the feeding experiment and minimum 5 and maximum 6 observations were recorded from each buffalo for determination of enteric methane production. In the breath samples of animals, $SF_6$ and methane concentrations were measured using a gas chromatograph (Ultima-2100, Netel Ltd., Navi Mumbai, India), which was equipped with electron capture and flame ionization detectors. Methane emission rate was calculated as the product of the ratio of methane to $SF_6$ concentration in the sample and the emission rate of permeation tube. The gas chromatograph was equipped with a Porapak Q column made of stainless steel with 2-mm inner diameter and 1.5 m length for methane determination, and 3.3-m molecular sieve with inner diameter of 0.32 mm for $SF_6$ determination. The temperatures of oven, injector, and detector were set at 52 °C, 40 °C, 100 °C, and 50 °C, 40 °C, 220 °C for methane and $SF_6$, respectively. The nitrogen flow rate (carrier gas) for both $SF_6$ and methane was set at 30 mL/min. The standards for methane (200.4 ppm) and $SF_6$ (232 ppt) gases (Chemix Speciality Gases and Equipments, Bangalore, India) were used to standardize the gas chromatograph. Methane production was calculated as per the method described previously (Johnson et al. 1994; Singh et al. 2020).

### Digestibility trial

A digestibility trial was conducted with a 6-day total collection period between 51 and 57 days of feeding. During the trial period, feed intake and orts were recorded daily at 0900 h before morning feeding. Total feces were collected daily in plastic buckets and weighed individually. A portion of fresh feces (5 g/kg of total weight) was collected in plastic containers containing 25 mL of $H_2SO_4$ solution (200 mL in 1 L distilled water), mixed and stored for analysis of nitrogen. The other portion of feces (50 g/kg of total weight) was dried at 60 °C for 48 h in a forced air oven (NSW-148B Tray drier, Narang Scientific Works, New Delhi, India) for determination of DM, and then composited together followed by grinding through a 1-mm screen and stored for further analysis. The digestibility of each chemical component of the diet was calculated for each animal by using the average individual DMI and fecal outputs.

### Blood samples

Blood samples from individual animal (4 h postprandial) were collected in heparinized tubes at the end of digestibility trial by puncturing jugular vein. The plasma was separated after centrifugation at 1602*g* for 15 min and stored at −20 °C until analyzed for different biochemical constituents.

### Sample analysis

Feeds, EFP, orts, and fecal samples were dried at 60 °C in a hot air oven till a constant weight; ground in a Wiley mill (Macro Scientific Works Pvt. Ltd., Delhi) to pass a 1-mm sieve, and analyzed (AOAC International 2007) for DM (method no. 934.01), total ash (method no. 942.05), ether extract (method no. 973.18), and crude protein (method no. 976.05) concentrations. Organic matter was calculated by subtracting ash from DM concentration. The concentrations of neutral detergent fiber (NDF; with sodium sulfite and without amylase in the solution), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined following the method of Robertson and Van Soest (1981), and the composition was expressed without residual ash. Concentrations of hemicelluloses and cellulose were calculated by deducting ADF from NDF and ADL from ADF, respectively. The gross energy (GE) contents of EFP and TMR were determined by using bomb calorimeter (Toshniwal Bros, Delhi, India). The energy content of methane was taken as 55.4 KJ/g (Brouwer 1965).

The plasma samples were analyzed for different biochemical constituents, i.e., total protein, albumin, globulin, cholesterol, triglycerides, glucose, blood urea nitrogen (BUN), urea, uric acid, bilirubin, aspartate aminotransaminase (AST), and alanine aminotransaminase (ALT) using a semiautomatic analyzer (Model-Erbachem 5×, Transasia, Mumbai, India) by using Erba diagnostic kits (Transasia Bio-Medical Ltd., Solan, H.P. India) as per the procedures described in the their protocols.

Milk yield of all animals was recorded daily, and milk samples were collected fortnightly for two consecutive days (morning and evening) throughout the experimental period and for fat, protein, solid not fat, and lactose (milk analyzer 'Lactoscan LA' from Milktronic LTD, Bulgaria). The somatic cell count (SCC) was performed using Direct Cell Counter (DeLaval, Tumba, Sweden), which was based on counting the stained DNA by means of charged-coupled device camera. Results were expressed as number of cells/mL of milk. Fat and protein-corrected milk (FPCM) yield was calculated as per the following formula (CVB 2008): FPCM (kg/d) = milk yield (kg/day) × [0.337 + 0.0116 × fat (g/kg) + 0.006 × protein (g/kg)].

For FA analysis, milk samples were collected at fortnightly intervals from days 31 to 120 and pooled together in equal volumes. The pooled milk samples were processed for FA analysis. Fat was extracted from 100 g of milk sample using n-hexane, and this extracted fat was esterified by saponification method using a trans-methylene mixture. Samples of TMR and milk fat were analyzed for FA content according to method of Ranganna (1986) using FA methyl esters prepared by liquid partitioning with petroleum ether and distilled water. On a Perkin-Elmer chromatograph equipped with a flame ionization detector, the FA was separated by using a capillary column (CP-Sil 88 fused silica capillary column; 100 m × 0.25-mm internal diameter; 0.25-μm film thickness). The initial temperature of oven was set at140 °C, which was raised to 250 °C at a rate of 4 °C/min and held for 15 min. The injector and detector temperatures were 260 °C. The injection volume was 1 μL with a split ratio 1:100. Helium was used as carrier gas at a flow rate of 0.5 mL/min. Individual FA methyl esters were identified by retention time with reference to the methyl esters standards (FAME-37 MIX, Supelco, Sigma–Aldrich). The atherogenic and thrombogenic indices were calculated as described previously (Bryszak et al. 2019).

### Statistical analysis

The data obtained from the in vitro and in vivo studies were analyzed for various variables in a completely randomized design using the general linear model procedure of SPSS version 16.0 (2007). The following statistical model used was used:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where $Y_{ij}$ is each observation, $\mu$ is overall mean, $T_i$ is effect of *i*th treatment and $\varepsilon_{ij}$ is residual error. When treatment was significant ($P \leq 0.05$) in the in vitro study, Tukey's test was used to find out the significant differences among the treatments (i.e., different doses of EFP). Significance was considered at $P \leq 0.05$, and a trend at $0.05 < P \leq 0.10$.

## Results

### Composition of *Emblica* fruit pomace

The EFP had low amount of crude protein and ether extract content, but moderate amount of NDF content. It was rich in plant secondary metabolites, i.e., total phenolics (224 g/kg DM), condensed tannins (1.50224 g/kg DM), saponins (25.3224 g/kg DM), flavonoids (7.40224 g/kg DM), and vitamin C (29.0224 g/kg DM) with high DPPH scavenging activity (36.0%).

**Table 2** Effect of supplementation of *Emblica* fruit pomace (EFP) on in vitro total gas production and kinetics, substrate degradability, partitioning factor and methane production (in vitro experiment)

| Item | Dose of EFP, g/kg | | | | | | SEM | *P* value |
|---|---|---|---|---|---|---|---|---|
| | Control | 10 | 20 | 30 | 40 | 50 | | |
| NGP, mL/g at 24 h | 137 | 139 | 144 | 143 | 141 | 142 | 0.70 | 0.13 |
| *L*, h | 0.82 | 1.00 | 0.90 | 0.95 | 0.89 | 0.96 | 0.048 | 0.15 |
| $Y_{max}$, mL/g DM | 179 | 185 | 180 | 190 | 189 | 187 | 3.14 | 0.12 |
| *K*, per h | 0.056 | 0.055 | 0.055 | 0.056 | 0.056 | 0.053 | 0.001 | 0.41 |
| $T_{½}$, h | 12.5 | 12.5 | 12.7 | 12.3 | 12.3 | 13.0 | 0.25 | 0.41 |
| IVTOMD, g/kg | $696^{bc}$ | $717^{d}$ | $701^{cd}$ | $690^{ab}$ | $681^{ab}$ | $677^{a}$ | 1.58 | < 0.001 |
| IVNDFD, g/kg | $534^{ab}$ | $552^{c}$ | $548^{bc}$ | $532^{a}$ | $523^{a}$ | $525^{a}$ | 2.04 | 0.006 |
| PF, mg/mL | $5.10^{c}$ | $5.13^{c}$ | $4.93^{b}$ | $4.83^{ab}$ | $4.80^{ab}$ | $4.77^{a}$ | 0.015 | < 0.001 |
| MBP, mg/g DM | $395^{b}$ | $410^{c}$ | $393^{b}$ | $376^{a}$ | $370^{a}$ | $364^{a}$ | 1.58 | < 0.001 |
| $CH_4$, mL/g DM | $43.1^{c}$ | $41.5^{bc}$ | $38.5^{b}$ | $33.8^{a}$ | $32.6^{a}$ | $31.2^{a}$ | 0.36 | < 0.001 |
| $CH_4$, mL/g IVTDDM | $63.9^{d}$ | $60.3^{cd}$ | $56.2^{bc}$ | $50.5^{ab}$ | $51.9^{ab}$ | $48.1^{a}$ | 0.56 | < 0.001 |
| $CH_4$, mL/g IVTDOM | $69.1^{c}$ | $65.3^{bc}$ | $61.4^{b}$ | $55.4^{a}$ | $56.6^{a}$ | $52.0^{a}$ | 0.60 | < 0.001 |

*NGP* net gas production; *IVNDFD* in vitro neutral detergent fiber degradability; *IVTOMD* in vitro true organic matter degradability; *PF* partitioning factor; *MBP* microbial biomass production; *DM* dry matter; $Y_{max}$ maximum gas production; *K* rate of gas production; $Y_{min}$ minimum gas production; $T_{½}$ time at which half of the maximum gas production occurs; *IVTDDM* in vitro true degraded dry matter; *IVTDOM* in vitro true degraded organic matter; *SEM* pooled standard error of mean

[a,b,c,d] Means with different superscripts in a row differ significantly ($P < 0.05$)

## Gas production kinetics, degradability of substrate, and methane production (in vitro experiment)

Net gas production and any of the gas production kinetics such as $Y_{max}$, *L*, *K*, and $T_{½}$ were not affected by the treatment (Table 2). The degradability of NDF was affected ($P = 0.006$) by treatments, which was greater at 10 g/kg of EFP than the control, but was not affected in other treatments compared with the control. Degradability of true OM was also greater at 10 g/kg than the control, but decreased at 50 g/kg of EFP compared with the control. Partitioning factor decreased at 20 g/kg or greater concentrations with the lowest at 50 g/kg compared with the control, but it was similar between the control and 10 g/kg of EFP. However, MBP was greater at 10 g/kg of EFP than the control, and was similar between the control and 20 g/kg of EFP, but was lower at 30 g/kg or greater doses than the control. Methane production expressed as g/kg DM and g/kg in vitro true digested DM and OM lowered ($P < 0.001$) at 20 g/kg and other greater doses of EFP with the greatest suppressing effect at 50 g/kg of EFP in the substrate.

## Ruminal volatile fatty acids (in vitro experiment)

Total VFA concentration ($P = 0.012$) at 50 g/kg of EFP, acetate concentration ($P < 0.05$) at 20 g/kg or greater doses of EFP, and acetate to propionate ratio ($P < 0.05$) at 30 g/kg or greater doses decreased compared with the control (Table 3). Concentrations of propionate and butyrate were not affected by the EFP. The concentration of branched chain volatile fatty acid (BCVFA) increased at 20 and 30 g/kg of EFP compared with the control, but it was similar at 40 and 50 g/kg of EFP to the control.

Proportion of acetate decreased ($P = 0.002$) at 20 g/kg or greater doses with the lowest proportion at 50 g/kg of EFP, whereas proportion of propionate ($P = 0.003$) increased at 40 and 50 g/kg of EFP and proportion of BCVFA increased ($P = 0.007$) at 20, 30, and 50 g/kg of EFP compared with the control. The proportion of butyrate was not affected ($P = 0.079$) by the EFP in the substrate compared with the control.

## Feed intake, milk production, and digestibility (in vivo experiment)

Feeding of EFP to buffaloes did not affect DMI (Table 4). However, milk yield, FPCM milk yield, and milk production efficiency (kg milk/kg DMI) were increased ($P < 0.015$) by EFP feeding. Concentrations of fat and lactose in milk were not affected by EFP, but the concentration of protein tended ($P = 0.07$) to increase and that of SNF increased ($P = 0.032$) due to the EFP feeding. Yields (kg/day) of milk fat ($P = 0.026$), SNF ($P = 0.011$), and protein ($P = 0.002$) were greater in the EFP diet than the control. Somatic cell count in milk was lower ($P = 0.032$) due to EFP feeding compared with the control diet.

The digestibility of ether extract ($P < 0.001$) increased, and DM ($P = 0.077$ and OM ($P = 0.051$) tended to increase due to EFP supplementation compared with the control diet

**Table 3** Effect of supplementation of *Emblica* fruit pomace (EFP) on in vitro ruminal volatile fatty acid concentration and proportion (in vitro experiment)

| Item | Dose of EFP, g/kg | | | | | | SEM | P value |
|---|---|---|---|---|---|---|---|---|
| | Control | 10 | 20 | 30 | 40 | 50 | | |
| VFA concentration, mM | | | | | | | | |
| TVFA | 72.4$^{b}$ | 71.5$^{b}$ | 70.9$^{b}$ | 68.9$^{b}$ | 66.2$^{ab}$ | 61.2$^{a}$ | 0.60 | 0.012 |
| Acetate | 43.5$^{d}$ | 40.7$^{cd}$ | 37.7$^{bc}$ | 36.6$^{bc}$ | 32.9$^{ab}$ | 29.2$^{a}$ | 0.46 | 0.001 |
| Propionate | 21.7 | 22.5 | 22.6 | 22.9 | 24.9 | 23.4 | 0.36 | 0.33 |
| Butyrate | 4.89 | 4.69 | 4.34 | 4.54 | 4.84 | 4.28 | 0.07 | 0.23 |
| BCVFA | 2.30$^{a}$ | 3.62$^{ab}$ | 6.34$^{c}$ | 4.77$^{bc}$ | 3.55$^{ab}$ | 4.12$^{ab}$ | 0.18 | 0.008 |
| A:P | 2.01$^{d}$ | 1.81$^{cd}$ | 1.67$^{bcd}$ | 1.60$^{abc}$ | 1.33$^{ab}$ | 1.25$^{a}$ | 0.031 | 0.003 |
| Relative molar proportion of VFA, % | | | | | | | | |
| Acetate | 60.1$^{c}$ | 56.9$^{bc}$ | 53.1$^{ab}$ | 53.1$^{ab}$ | 49.8$^{a}$ | 47.6$^{a}$ | 0.45 | 0.002 |
| Propionate | 29.9$^{a}$ | 31.4$^{a}$ | 31.8$^{a}$ | 33.4$^{ab}$ | 37.6$^{bc}$ | 38.1$^{c}$ | 0.39 | 0.003 |
| Butyrate | 6.74 | 6.56 | 6.12 | 6.59 | 7.30 | 6.99 | 0.088 | 0.079 |
| BCVFA | 3.18$^{a}$ | 5.09$^{ab}$ | 8.94$^{c}$ | 6.92$^{bc}$ | 5.37$^{ab}$ | 6.72$^{bc}$ | 0.252 | 0.007 |

*TVFA* total volatile fatty acids; *A:P* acetate to propionate ratio; *BCVFA* total branched-chain volatile fatty acids (isovalerate and isobutyrate); *SEM* pooled standard error of mean

[a,b,c] Means with different superscripts in a row differ significantly ($P < 0.05$)

(Table 4). Digestibility of CP and different fiber fractions was not affected by EFP supplementation.

### Methane production (in vivo experiment)

Methane production (g/d or MJ/d), yield (g/kg DM intake or g/kg digestible organic matter intake), and intensity (methane production per unit of products, i.e., g/L milk, g/kg milk fat, g/kg milk protein, g/kg milk SNF or g/kg FPCM) were significantly ($P < 0{,}001$) lower for the EFP-supplemented diet that the control diet (Table 5). Methane production expressed to percent of gross energy intake was also lower for the EFP-containing diet than the control diet.

### Blood metabolites and enzymes (in vivo experiment)

Any blood metabolites such as glucose, total protein, albumin, globulin, triglyceride, urea, creatinine, uric acid, and bilirubin were not affected by EFP supplementation except for cholesterol that tended to increase ($P = 0.086$) due to EFP feeding (Table 6). Serum enzymes such as AST and ALT were also not affected due to the addition of EFP in the TMR.

### Fatty acid profile in milk (in vivo experiment)

Among the saturated fatty acids (SFA) in milk, C12:0 ($P = 0.094$) and C15:0 ($P = 0.073$) proportions tended to increase, and C23:0 ($P = 0.090$) tended to decrease due to the feeding of EFP compared with the control (Table 7). Among the monounsaturated fatty acids (MUFA), only proportion of C18:1 *trans*-9 FA was lower ($P = 0.049$) in the EFP-fed buffaloes than control-diet fed buffaloes. Proportions of any individual polyunsaturated fatty acids (PUFA) were not affected by EFP feeding. Total SFA proportion tended to be greater ($P = 0.057$) in the EFP group that the control group, but total n-6, n-3, MUFA, and PUFA proportions were similar in both groups. Ratios of total SFA to total unsaturated FA ($P = 0.083$) and total SFA to total MUFA ($P = 0.098$) tended to be greater for the EFP group than the control group, but the ratios of n-6 to n-3 FA, and total SFA to total PUFA were unaffected by the EFP feeding. Total long-chain FA (C18 to C24) proportion was similar between the groups, but total short- and medium-chain FA proportion increased ($P = 0.045$) due to EFP feeding. Desaturation indices were similar between the groups. Atherogenic index in milk from buffaloes fed EFP showed a tendency ($P = 0.077$) to be greater, but the thrombogenic index was similar between the groups.

## Discussion

The concentration of phenolic compounds in EFP was very high (224 g/kg DM). *Emblica* fruit contains an array of numerous phytochemicals including high concentrations of bioactive polyphenolic compounds such as ellagic acid, gallic acid, chebulinic acid, chlorogenic acid, different tannins, flavonoids (for example, rutin, and quercetin), and antioxidants including vitamin C (Variya et al. 2016). Among tannins, it is especially high in hydrolyzable tannins such as emblicanin A, emblicanin B, punigluconin, and pedunculagin (Variya et al. 2016). Major nutrients such as CP, EE, and total ash content in EFP are low compared with the common feedstuffs fed to

**Table 4** Effect of supplementation of *Emblica* fruit pomace (EFP) to a total mixed ration on feed intake, milk yield and composition and digestibility of nutrients in lactating buffaloes (in vivo experiment)

| Item | Control | EFP | SEM | *P* value |
|---|---|---|---|---|
| Body weight (kg) | 564 | 551 | 12.2 | 0.50 |
| DMI, kg/d | 11.2 | 11.1 | 0.57 | 0.47 |
| GE intake, MJ/d | 205 | 210 | 4.32 | < 0.001 |
| Milk production | | | | |
| Initial, kg/d | 9.00 | 9.02 | 0.578 | 0.98 |
| Average milk yield, kg/d | 7.24 | 8.25 | 0.084 | < 0.001 |
| Milk, kg/kg DMI | 0.66 | 0.73 | 0.014 | < 0.001 |
| FPCM, kg/d | 10.7 | 12.5 | 0.48 | 0.015 |
| FPCM, kg/kg DMI | 0.97 | 1.11 | 0.034 | < 0.001 |
| Milk composition and yield | | | | |
| Fat, g/kg | 77.5 | 78.8 | 3.25 | 0.78 |
| SNF, g/kg | 109 | 113 | 1.0 | 0.011 |
| Protein, g/kg | 42.1 | 44.5 | 0.87 | 0.071 |
| Lactose, g/kg | 58.8 | 60.0 | 0.086 | 0.30 |
| Fat, kg/d | 0.55 | 0.64 | 0.028 | 0.026 |
| SNF, kg/d | 0.80 | 0.93 | 0.043 | 0.032 |
| Protein, kg/d | 0.30 | 0.37 | 0.013 | 0.002 |
| Lactose, kg/d | 0.43 | 0.50 | 0.113 | 0.101 |
| SCC, × 1000 cells/mL | 82.0 | 62.1 | 5.51 | 0.023 |
| Digestibility of nutrients, g/kg | | | | |
| Dry matter | 643 | 675 | 1.21 | 0.077 |
| Organic matter | 666 | 698 | 1.12 | 0.051 |
| Crude protein | 719 | 704 | 0.67 | 0.13 |
| Ether extract | 686 | 751 | 1.13 | < 0.001 |
| Neutral detergent fiber | 526 | 557 | 1.57 | 0.18 |
| Acid detergent fiber | 547 | 582 | 1.59 | 0.13 |
| Cellulose | 603 | 628 | 1.46 | 0.22 |

*DMI* dry matter intake; *FPCM* fat and protein corrected milk; *SNF* solid not fat; *SCC* somatic cell counts; *SEM* pooled standard error of mean

ruminants, but it contains moderate amount of fiber components (Kumari et al. 2012; Chaturvedi et al. 2014; Wadhwa et al. 2015).

In vitro degradability of OM and NDF increased at a dose of 10 g/kg; whereas, it decreased at 50 g/kg DM. It clearly indicates the dose-dependent effect of EFP in the degradability of feed. Generally in line with the in vitro study, the in vivo digestibility of OM tended ($P = 0.051$) to increase due to feeding of EFP at 20 g/kg diet. Inclusion of *Emblica* fruit also increased degradability of OM and DM in an in vitro study (Kumar et al. 2016). Pomegranate peel extract, which contains similar phytochemicals (i.e., soluble phenolics, ellagic acid, and punicalagins A and B) as that of EFP, improved the digestibility of DM, CP, an NDF in lactating cattle at 40 g/kg, but not at the lower (10 and 20 g/kg DM) doses (Jami et al. 2012). Polyphenolics, especially tannins, inhibit ruminal

**Table 5** Effect of supplementing *Emblica* fruit pomace (EFP) to the total mixed ration on enteric methane ($CH_4$) production in lactating buffaloes (in vivo experiment)

| Item | Control | EFP | SEM | *P* value |
|---|---|---|---|---|
| DM intake, kg | 11.1 | 11.1 | 0.23 | 0.47 |
| $CH_4$, g/d | 260 | 186 | 7.41 | < 0.001 |
| $CH_4$, g/kg DMI | 23.4 | 16.8 | 0.52 | < 0.001 |
| $CH_4$, g/kg DOMI | 39.5 | 26.7 | 1.20 | < 0.001 |
| $CH_4$, g/kg milk | 36.2 | 23.6 | 1.32 | < 0.001 |
| $CH_4$, g/kg milk fat | 469 | 298 | 14.1 | < 0.001 |
| $CH_4$, g/kg milk protein | 332 | 212 | 12.1 | < 0.001 |
| $CH_4$, g/kg milk SNF | 864 | 527 | 28.1 | < 0.001 |
| $CH_4$, g/kg FPCM | 24.3 | 15.4 | 0.74 | < 0.001 |
| Methane energy, MJ/d | 14.4 | 10.3 | 0.41 | < 0.001 |
| Methane, % of GE | 7.01 | 4.92 | 0.154 | < 0.001 |

*DMI* dry matter intake; *DOMI* digestible organic matter intake; *FPCM* fat and protein corrected milk; *SNF* solid not fat; and *GE* gross energy; *SEM* pooled standard error of mean

microorganisms at greater doses (> 40 g/kg diet) depending upon the type of chemical structures and molecular weights (Mueller-Harvey 2006; Patra et al. 2012). In this study, phenolics in EFP at low doses seem to favor the growth of some categories of microbiota, preferably, fiber-degrading populations, which was further substantiated from increased MBP at the low dose in the in vitro study. The antioxidant activities of EFP may be responsible for the increased microbial activities and protein synthesis at the low dose (Tagliapietra et al. 2013),

**Table 6** Effect of supplementation of *Emblica* fruit pomace (EFP) to the total mixed ration on the blood profile of lactating buffaloes (in vivo experiment)

| Parameters | Control | EFP | SEM | *P* value |
|---|---|---|---|---|
| Glucose, mg/L | 646 | 656 | 20.8 | 0.74 |
| Total protein, g/L | 83.2 | 81.0 | 3.75 | 0.69 |
| Albumin (A), g/L | 28.0 | 31.7 | 2.15 | 0.26 |
| Globulin (G), g/L | 55.2 | 49.2 | 4.28 | 0.36 |
| A:G | 0.53 | 0.67 | 0.075 | 0.23 |
| Cholesterol, mg/L | 574 | 744 | 59.9 | 0.086 |
| Triglycerides, mg/L | 95.0 | 129 | 14.3 | 0.13 |
| Urea, mg/L | 325 | 363 | 30.7 | 0.44 |
| Creatinine, mg/L | 14.1 | 15.3 | 0.50 | 0.13 |
| Uric acid, mg/L | 4.78 | 6.68 | 1.17 | 0.29 |
| Bilirubin total, mg/L | 2.36 | 2.86 | 0.31 | 0.36 |
| Bilirubin direct, mg/L | 1.60 | 2.10 | 0.029 | 0.34 |
| AST, IU/L | 109 | 120 | 7.14 | 0.28 |
| ALT, IU/L | 50.0 | 52.6 | 4.96 | 0.73 |

*AST* aspartate aminotransferase; *ALT* alanine aminotransferase; *SEM* pooled standard error of mean

**Table 7** Effect of supplementation of *Emblica* fruit pomace (EFP) to the total mixed ration on fatty acid (FA) profile (g/100 g FA) of buffalo milk (in vivo experiment)

| Fatty acid | Control | EFP | SEM | *P* value |
|---|---|---|---|---|
| Saturated FA | | | | |
| C4:0 | 0.013 | 0.057 | 0.011 | 0.11 |
| C6:0 | 0.70 | 0.81 | 0.212 | 0.73 |
| C8:0 | 0.97 | 1.10 | 0.139 | 0.54 |
| C10:0 | 1.79 | 2.42 | 0.244 | 0.14 |
| C10:0 | 0.055 | 0.077 | 0.021 | 0.53 |
| C12:0 | 2.44 | 3.09 | 0.231 | 0.094 |
| C13:0 | 0.083 | 0.14 | 0.017 | 0.18 |
| C14:0 | 11.1 | 13.9 | 0.986 | 0.12 |
| C15:0 | 0.87 | 1.19 | 0.100 | 0.073 |
| C16:0 | 30.9 | 29.6 | 1.49 | 0.58 |
| C17:0 | 1.60 | 1.69 | 0.494 | 0.90 |
| C18:0 | 14.7 | 15.0 | 0.703 | 0.72 |
| C20:0 | 0.45 | 0.54 | 0.109 | 0.58 |
| C20:0 | 0.20 | 0.21 | 0.113 | 0.95 |
| C22:0 | 0.37 | 0.46 | 0.172 | 0.73 |
| C23:0 | 0.37 | 0.26 | 0.120 | 0.52 |
| C23:0 | 0.13 | 0.038 | 0.026 | 0.090 |
| Monounsaturated FA | | | | |
| C14:1 *cis*-9 | 0.54 | 0.55 | 0.043 | 0.89 |
| C15:1 *cis*-10 | 0.84 | 0.17 | 0.262 | 0.13 |
| C16:1 *cis*-9 | 1.13 | 0.98 | 0.272 | 0.66 |
| C17:1 *cis*-10 | 0.55 | 0.26 | 0.113 | 0.20 |
| C18:1 *cis*-9 | 25.0 | 23.2 | 1.13 | 0.28 |
| C18:1 *trans*-11 | 0.82 | 0.010 | 0.093 | 0.049 |
| C20:1 *cis*-11 | 0.33 | 0.37 | 0.130 | 0.84 |
| C22:1n-9 | 0.39 | 0.28 | 0.076 | 0.41 |
| C24:1n-9 | 0.072 | 0.050 | 0.015 | 0.36 |
| Polyunsaturated FA | | | | |
| C18:2n-6 | 1.80 | 1.77 | 0.17 | 0.92 |
| C18:2 *trans*-9, *trans*-12 | 0.095 | 0.15 | 0.047 | 0.37 |
| C18:3n-6 | 0.037 | 0.18 | 0.082 | 0.50 |
| C18:3n-3 | 0.49 | 0.43 | 0.113 | 0.72 |
| C20:4n-6 | 0.19 | 0.32 | 0.138 | 0.53 |
| C20:2n-6 | 0.45 | 0.17 | 0.117 | 0.14 |
| C20:3n-6 | 0.13 | 0.16 | 0.066 | 0.78 |
| C20:3n-3 | 0.34 | 0.53 | 0.141 | 0.38 |
| C22:2n-6 | 0.18 | 0.082 | 0.068 | 0.35 |
| C20:5n-3 | 0.13 | 0.13 | 0.031 | 1.00 |
| C22:6n-3 | 0.060 | 0.030 | 0.026 | 0.61 |
| Fatty acid group | | | | |
| Total n-3 FA | 0.99 | 1.08 | 0.145 | 0.69 |
| Total n-6 FA | 2.82 | 2.78 | 0.313 | 0.94 |
| Total SFA | 66.7 | 70.6 | 1.49 | 0.057 |
| Total MUFA | 28.1 | 25.5 | 1.31 | 0.19 |
| Total PUFA | 3.74 | 3.70 | 0.356 | 0.94 |
| Total unsaturated FA | 32.7 | 29.4 | 1.24 | 0.12 |
| Total LCFA (C18–C24) | 45.9 | 44.1 | 1.68 | 0.47 |
| Total SMCFA (C4–14) | 16.5 | 21.6 | 1.45 | 0.046 |
| Fatty acid ratio | | | | |
| SFA/USFA | 2.07 | 2.43 | 0.134 | 0.083 |
| SFA/MUFA | 2.38 | 2.81 | 0.163 | 0.098 |
| SFA/PUFA | 19.5 | 19.4 | 2.04 | 0.98 |
| n-6/n-3 | 3.30 | 2.80 | 0.663 | 0.62 |
| DI C14 | 0.049 | 0.038 | 0.010 | 0.52 |
| DI C16 | 0.037 | 0.033 | 0.009 | 0.76 |
| DI C18 | 0.63 | 0.61 | 0.011 | 0.15 |
| Atherogenic index | 2.44 | 3.05 | 0.218 | 0.077 |
| Thrombogenic index | 3.06 | 3.36 | 0.232 | 0.39 |

*SFA* saturated fatty acids; *MUFA* monounsaturated fatty acids; *PUFA* polyunsaturated fatty acids; *USFA* unsaturated fatty acids; *LCA* long-chain fatty acids; *SMCFA* short- and medium-chain fatty acids; *DI* desaturation index; *SEM* pooled standard error of mean

DI C14 = C14:1 *cis*-9/(C14:0 + C14:1 *cis*-9); DI C16 = C16:1 *cis*-9/(C16:0 + C16:1 *cis*-9); DI C18 = C18:1 *cis*-9/(C18:0 + C18:1 *cis*-9); Atherogenic index = (C12:0 + (4 × C14:0) + C16:0)/(MUFA + (n-6) + (n-3)); Thrombogenic index = (C14:0 + C 16:0 + C18:0)/(0.5 × MUFA + (0.5 × n-6) + (3 × n-3) + (n-3/n-6))

whereas the antimicrobial activities of phenolics at the high doses reduced nutrient digestibility (Patra et al. 2012). Also, tannins at a low concentration may stimulate the growth of bacteria. It has been speculated due to the interaction of tannins with the cell surface proteins of bacteria, which facilitates cell transport functions or improves enzyme activities (Mole and Waterman 1985; Krause et al. 2005). Therefore, a fine tuning is required when plant extracts or plant materials with antioxidant and phenolic compounds are used for improving ruminal fermentation.

Total VFA concentrations in the buffered media reduced at the highest dose, which was mainly due to reduction in acetate production by the ruminal microbiota. Acetate is mainly produced from NDF digestibility by the cellulolytic bacterial populations, but NDF degradability was not affected in the in vitro study. Concentration of propionate was not changed, but proportion of propionate increased due to reduction of total VFA concentration at the higher doses. Thus, noncellulolytic microorganisms that produce acetate were perhaps suppressed by the bioactive compounds in EFP. It is also possible that, due to inhibition of methanogenesis by EFP, rechanneling of hydrogen occurred towards propionate metabolic pathways (Patra et al. 2017). This should be investigated using molecular microbiology tools to uncover the bacterial populations altered by the EFP. It should be noted here that though true degradability of OM increased, total VFA concentration did not alter at the lowest dose of EFP. This might be due to

redirection of degraded nutrients for MBP that was indeed improved in the in vitro study at the lowest dose. The concentration and proportion of BCVFA were substantially increased, especially at 20 and 30 g/kg of EFP. These BCVFA are mainly produced from the deamination of amino acids with some contribution from recycling of microbial protein (Miura et al. 1980). Therefore, EFP might stimulate the microorganisms responsible for degradation of protein and deamination of amino acids in the rumen. Tannins depending upon molecular weights and chemical structures may form tannin-protein complexes, thus lowering the protein degradation in the rumen. But EFP contains mainly phenolic acids and hydrolyzable tannins that seems confer less protection against protein degradation in the rumen (Patra et al. 2012).

Supplementation of EFP did not influence feed intake in buffaloes, which indicates that plant bioactive compounds, especially phenolic compounds, in EFP at 20 g/kg DM have no adverse effect on the feed consumption of feeds containing EFP. High concentrations of polyphenolic compounds including tannins depending upon the nature of the tannins may limit the feed intake due to astringency of the compounds and impairing ruminal function (Mueller-Harvey 2006; Patra et al. 2012). In this study, the concentration of polyphenolic compounds in the TMR was low, which may be below the tolerance limit to cause astringency effect and to lower ruminal feed digestion (Patra et al. 2012). There appears no study on feeding of EFP or *Emblica* fruit in ruminants, but the studies on non-ruminant species suggested that *Emblica* fruit or its pomace as feed additives had no effect on feed intake at 2.5 and 7.5 g/kg diet (Kumari et al. 2012), 2.5 g/kg (Dalal et al. 2018), 4 and 8 g/kg diet (Patel et al. 2016) and 2.5 g/kg diet (Aswal et al. 2017) or decreased feed intake at 5 to 10 g/kg diet (Dalal et al. 2018) in poultry.

Although feed intake was not affected, milk production along with milk components and its efficiency improved due to supplementation of EFP, which might be due to improved nutrient digestibility and better health status of the animals owing to rich in antioxidants. Feeding of EFP tended to enhance OM degradability in the present study. Supplementation of quebracho condensed tannins to lactating cows at 30 g/kg DM did not alter milk production and nutrient digestibility, but improved production efficiency due to lower feed intake (Dschaak et al. 2011). A concentrated pomegranate-residue extract, which contains phytochemicals similar type to *Emblica* fruits such as punicalagins A and B (26.5 g/kg DM), and ellagic acid (2.5 g/kg DM), and total soluble phenolics (63 g/kg DM), increased milk production at doses of 10, 20, or 40 g/kg diet (Jami et al. 2012). Similarly, pomegranate peel extract increased milk production and efficiency in lactating cows fed at a dose of 800 mL extract (equivalent to 11 g/kg DM), but not at a low (400 mL/day) or a high (1200 mL/day) dose (Abarghuei et al. 2013). In our study, fat composition in milk was not affected due to EFP feeding, which was similar to the study of Abarghuei et al. (2013), but protein composition tended to increase, which was similar to the finding of Jami et al. (2012). Because BCVFA concentration was increased by EFP, tannins present in EFP, it seems, did not confer protein-binding action in the rumen (Mueller-Harvey 2006; Patra et al. 2012), and thus, it is unlikely that ruminal escape protein increased milk protein concentration. Digestibility of CP was similar between the control and EFP groups. Therefore, increased protein synthesis in the rumen, which was also indicative in this in vitro study at the low doses, may increase concentration and yield of milk protein in this study. The greater yields of fat, protein, and SNF due to EFP feeding may be attributed to increased milk production. Feeding of EFP decreased SCC in milk, which indicates the better udder health of buffaloes in the EFP group because high SCC indicate inflammation of udders and deteriorate the hygienic milk quality (Suriyasathaporn et al. 2006). Oxidative stress may increase the lipid peroxidation and increased malondialdehyde concentration, which was positively to SCC in raw milk (Suriyasathaporn et al. 2006). High antioxidant properties of the EFP may thus reduce oxidative stress and SCC in milk. Supplementation of condensed tannins also alleviated oxidative stress and SCC in milk of lactating cows (Liu et al. 2013).

Inclusion of EFP lowered in vitro methane production at the doses greater than 10 g/kg substrate in the present study. Also, feeding of EFP decreased methane production (g/d), yield (g/kg DM intake), and methane intensity (g/kg milk production). Different bioactive compounds such as phenolic acids, tannins, flavonoids, and saponins exhibit antimethanogenic activity (Patra and Saxena 2010; Patra 2016), which are present in *Emblica* fruit and pomace in high concentrations (Variya et al. 2016). An in vitro study using ruminal fluid from buffaloes, methanolic, and ethanolic extracts of *Emblica* fruit decreased methane production by 28% and 20%, respectively at 0.50 mL compared with the control, but the low dose (0.25 mL) of the extracts as well as water extract of the fruit did not exert any effect (Patra et al. 2006), suggesting the active bioactive compounds in *Emblica* fruit for antimethanogenic activity being mainly soluble in ethanol and methanol. Also, *Emblica* fruit powder (10 to 20 g/kg substrate) lowered in vitro methane production significantly at the dose rate of 20 to 30 g/kg substrate (42 versus 34 mL/kg substrate) with buffalo ruminal fluid (Kumar et al. 2016). *Emblica* fruit also decreased methane production in other in vitro study (Singh et al. 2018). *Emblica* fruit contains high concentration of phenolic compounds, mostly hydrolyzable tannins, and flavonoids (Wadhwa et al. 2015; Variya et al. 2016), which presumably directly inhibited methanogenesis as the nutrient digestibility along with NDF digestibility was not affected due to EFP feeding. Pomegranate peel extract that contains similar type of phenolic compounds also suppressed methane production in

buffaloes (Hundal et al. 2019). The in vitro studies from reported literature and the present in vivo study confirm that EFP feeding at 20 g/kg DM intake in ruminants including buffaloes may decrease methane production without affecting nutrient digestibility. For sustainable adoption of methane mitigation by farmers in ruminant production, methane mitigation strategies should improve animal production, and it should not create disquiets on animal health and residues in food products (Patra et al. 2017). Compared with many studies that aim to mitigate methane production (Patra et al. 2017), the additional advantage of EFP feeding is that it increased milk production and reduced methane intensity. The combined effects of methane mitigation and improved milk production decreased direct carbon footprint (carbon emission per unit of food product) of milk production markedly by 35%, which is important to lower the carbon footprint of food products from animal origins (Patra 2017).

Any blood metabolites were not changed due to EFP feeding in lactation buffaloes and the values were within the normal ranges (Kaneko et al. 1997; Abd Ellah et al. 2013), indicating EFP had no adverse effect on health of buffaloes. Total cholesterol tended to be higher ($P = 0.089$) in buffaloes fed EFP. These metabolite concentrations in blood were normal (Kaneko et al. 1997; Abd Ellah et al. 2013). The feeding of *Emblica* fruit to rabbits (Mishra et al. 1981) and rats (Kim et al. 2005) has been shown to reduce blood cholesterol due to alteration of cholesterol absorption, increased enzymatic degradation of cholesterol in liver or other tissues, and inhibition of 3-hydroxy 3-methylglutaryl coenzyme A reductase (Variya et al. 2016). The reason of higher concentrations of blood cholesterol in the EFP-fed buffaloes than the control buffaloes is not clear in this study. The digestibility of EE was enhanced by EFP feeding, which may increase the cholesterol and triglyceride concentrations in blood.

The concentration of fat in buffalo milk is high, but their FA profiles are usually similar to cow milk (Varricchio et al. 2007). The proportions of SFA, MUFA, and PUFA in cow milk fat represent for approximately 70%, 25%, and 2.3%, respectively of the total FA (Månsson 2008). In the present study, proportions of SFA, MUFA and PUFA were 68.3%, 26.8%, and 3.72% in buffalo milk fat, respectively, which was similar to other study on buffaloes (65.5%, 27%, and 4.5%, respectively; Varricchio et al. 2007). Feeding of EFP modified only a few FA profile such as C12:0, C15:0, total short- and medium-chain FA, and total SFA in milk. Even number short-medium chain FA including C12:0 in milk are synthesized de novo in the mammary glands by the activity of the lipogenic enzymes from acetate and β-hydroxybutyrate precursors mainly (Varricchio et al. 2007; Urrutia and Harvatine 2017). Increased acetate proportion may stimulate milk fat synthesis (Urrutia and Harvatine, 2017). It is possible that EFP with rich in phenolic compounds might modulate ruminal fermentation towards acetate production. For example, propolis extract rich in phenolics increased ruminal acetate and butyrate proportions in buffaloes (Costa et al. 2012; De Paula et al. 2016). However, it seems ruminal acetate production in vivo was not increased as evidenced from the in vitro ruminal VFA profile in this study. Thus, increased proportions of these FA in milk due to EFP feeding suggest that bioactive compounds in EFP may increase expression of lipogenic genes. The increased synthesis of short- and medium chain FA contributed to greater proportion of total SFA in milk. The increased proportion of C15:0 in milk is a suggestive of changes in microbial populations in the rumen due to EFP feeding because C15:0 is mainly synthesized by the ruminal bacteria (Fievez et al. 2012; Vazirigohar et al. 2018). This is also evident from the increased microbial protein synthesis in the present in vitro experiment. Although phenolic compounds depending upon the concentrations and type can modulate the ruminal biohydrogenation process of the unsaturated FA by altering the FA biohydrogenating bacteria (Patra et al. 2012), desaturation indices for different FA were not altered, which indicates that ruminal biohydrogenation or de novo desaturation was not altered by EFP feeding due to low concentration of phenolic compounds present in the TMR. Overall, the milk FA profile suggests that feeding of EFP in lactating buffaloes have no major effects except the changes in the total concentrations of short- and medium chain FA, which have been shown health benefits in humans, particularly with cardiac diseases (Labarthe et al. 2008).

## Conclusion

In the vitro experiment, inclusion of EFP at 20 g/kg substrate decreased methane production without adversely affecting ruminal fermentation. Supplementation of EFP (20 g/kg DM) to the diet of lactating buffaloes increased milk and its component yields and milk production efficiency. Moreover, methane production, yield and intensity, and methane conversion rate were reduced by EFP feeding. There were no major changes in milk fatty acid profile except for the increased proportion of total short- and medium-chain fatty acids in the EFP group. Therefore, feeding of EFP at 20 g/kg DM intake may serve a win–win situation by improving milk production performance and decreasing carbon footprint of milk production and EFP residue disposal problems without impacting milk quality and health of buffaloes.

**Authors' contributions** JS Hundal, M Wadhwa, and A Singla conceived and designed the experiments. A Singla, JS Hundal, M Wadhwa, V Nagarajappa, and P Malhotra performed the experiments and collected the data. JS Hundal and AK Patra analyzed the data. AK Patra and JS Hundal wrote the paper, interpreted the data, and revised the paper. All authors read and approved the final manuscript.

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**Data availability** All mean data are presented in the tables of this paper. The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interests.

**Ethical approval** All experimental protocols were validated and compliant with the recommendations authorized by Institutional Animal Ethics Committee (IAEC/2018/1025-1060 dated May 3, 2018).

**Consent to participate** Not applicable.

**Consent to publish** All authors have consent for the publication of the manuscript.

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