Effects of berry seed residues on ruminal fermentation, methane concentration, milk production, and fatty acid proportions in the rumen and milk of dairy cows

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ABSTRACT

Strawberry (SB), black currant (BC), and raspberry seed (RB) residues were used in 3 experiments to study their effects on ruminal fermentation, methane concentration, and fatty acid (FA) proportions in the ruminal fluid and milk of dairy cows. Initially, a batch fermentation in vitro study (experiment 1) was performed to investigate the effects of the 3 berry residues on basic ruminal fermentation parameters. Total volatile fatty acid concentrations, including acetate, propionate, and butyrate, increased in the BC group compared with other treatments. Based on the preliminary in vitro results, 2 consecutive in vivo experiments were conducted using 4 Polish Holstein-Friesian cows fitted with rumen cannulas (experiment 2) and 30 lactating Polish Holstein-Friesian dairy cows (experiment 3) in a replicated 2 × 2 crossover design. Cows in both experiments received a partial mixed ration (PMR) in 2 variants: (1) a control diet of PMR + 2 kg of concentrate (control); (2) PMR + 2 kg of BC seed residues (BC). The BC diet did not mitigate ruminal methane production. Ruminal fermentation (experiment 2) was not affected by the BC diet; however, the concentrations of C18:1 *trans*-11 and C18:2 *cis*-9,*trans*-11 increased significantly by 91 and 131%, respectively. Likewise, concentrations of total *trans* C18:1 and total monounsaturated FA in ruminal fluid were increased significantly by BC seed residues. In experiment 3, BC significantly increased milk fat C18:1 *trans*-11, C18:2 *cis*-9,*trans*-11, n-3, n-6, and polyunsaturated FA concentrations without affecting milk production performance. In conclusion, the amount

(2 kg/d) of BC used in this study did not adversely affect ruminal fermentation or milk production and composition. However, using BC increased proportions of unsaturated FA and conjugated linoleic acid in milk. Although dietary BC did not exert a strong methane inhibition effect, it could represent an inexpensive alternative concentrate to improve beneficial FA in milk without negative effects on rumen fermentation and production parameters in dairy cows. Incorporation of berry seed residues in diets would be profitable economically and nutritionally for dairy cattle production. **Key words:** berry, dairy cow, fermentation, fatty acid composition, milk production

INTRODUCTION

Poland represents one of the largest producers of berries in the world. According to the Food and Agriculture Organization of the United Nations, Poland produced 196,972 t of strawberries (*Fragaria × ananassa* Duchesne), 166,110 t of currants (*Ribes nigrum* and *Ribes rubrum*), and 129,063 t of raspberries (*Rubus idaeus*) in 2016 (FAOSTAT, 2016). It is estimated that the total production of all types of berries is about 523,443 t in cultivated areas of 143,953 ha (FAOSTAT, 2016). During processing of berry fruits, large amounts of waste by-products are produced and used for composting or combustion (Roj et al., 2009; Szumacher-Strabel et al., 2011).

The EU Directive 75/442/EEC for a common European Union policy on the management and utilization of bio-wastes encourages the recovery of waste materials including fruit industry waste by-products to conserve natural resources and reduce environmental pollution. In recent years, a lot of attention has been paid to issues related to the presence of natural and biologically active substances in animal feeds, which would

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enrich products of animal origins and at the same time improve animal health. Berry fruits and their by-products contain many biologically active substances such as tannins, saponins, flavones, phenolic acids, ellagic acid, vitamins C and E, folic acid, and β -sitosterol that can be exploited in animal nutrition (Roj et al., 2009). In ruminants, these biologically active substances may stimulate microbial metabolism in the rumen (Broudiscou et al., 2002) and increase the digestibility of nutrients (Patra, 2010). Moreover, they may improve ruminal fermentation and reduce incidences of ruminal acidosis by increasing the numbers of lactate-consuming bacteria (e.g., *Megasphaera elsdenii*) in the rumen (Balcells et al., 2012). The biologically active substances modulating microbial populations and fermentation in the rumen (Lazze et al., 2003; Szumacher-Strabel and Cieslak, 2010) may also be considered as an effective strategy in mitigation of methane production (Wang et al., 2009; Patra and Saxena, 2010).

The bioactive substances may also modulate ruminal fatty acid (FA) biohydrogenation leading to changes in rumen and milk FA profile (Henke et al., 2017). For instance, quebracho condensed tannins inhibit one of the bacterial species involved in ruminal biohydrogenation, *Butyrivibrio proteoclasticus* (Buccioni et al., 2015) and consequently may improve the quality of milk by increasing UFA contents (Henke et al., 2017). The possibility of using berry by-products in animal nutrition may allow for the production of healthy animal-derived foods and mitigation of enteric methane emission, hence an environmentally friendly production system. Therefore, the present experiments were undertaken to evaluate the effects of berry fruit seed residues on in vitro and in vivo ruminal fermentation processes, milk production, and composition. We hypothesized that the berry seed residues may replace a part of the concentrate in diets of dairy cows while beneficially modulating rumen fermentation, decreasing methane production, and improving the FA proportions in the ruminal fluid and milk of dairy cows.

MATERIALS AND METHODS

The residues of strawberry (SB), black currant (BC), and raspberry (RB) seeds were investigated for their effects on ruminal fermentation and milk production performance of cows in 3 successive experiments: an in vitro ruminal fermentation study (experiment 1), an in vivo ruminal fermentation study using 4 cannulated cows (experiment 2), and a milk production performance study using 30 lactating dairy cows on a commercial farm (experiment 3). All experimental procedures were performed in accordance with the guidelines of the National Ethical Commission for Animal

Research (Ministry of Science and Higher Education, Poland).

Experimental Materials

Fresh fruits of SB, BC, and RB harvested from the south-east region of Poland were used for concentrated juice production at Alpex company (Łęczeszyce 120, Belsk Duży, Poland). The dry fruit seeds were collected after separation from dried pomace and were used for extraction of oil using supercritical CO₂ as a solvent in a pilot plant (New Chemical Syntheses Institute, Puławy, Poland). Briefly, the seeds of SB, BC, and RB were crushed separately and loaded into an extractor. Subsequently, the extraction was carried out in a supercritical state using CO₂ as a solvent. The extracted oil fraction of each fruit was collected in a one-stage separator. After extraction, the solid residues of SB, BC and RB were separately driven by a pneumatic transporter to a storage tank, where they were collected for investigation in this study.

Experimental Design, Diet, and Management

In Vitro Experiment. An in vitro batch culture fermentation experiment was carried out to study the effect of partially replacing a concentrate with seed residues of SB, BC, and RB on the ruminal fermentation characteristics and methane production (experiment 1). The fresh ruminal inoculum was collected from 4 Polish Holstein-Friesian dairy cows (650 $\pm$ 30 kg) fitted with ruminal cannula before morning feeding. One month before the start of the in vitro experiment, the dairy cows were offered a TMR (Table 1). The average feed intake was controlled daily and each cow was fed 21 kg of DM. The fresh TMR was provided twice a day at 0600 and 1800 h.

Ruminal content (approximately 400 g) from each cow was collected from the top, bottom, and middle parts of the rumen. The rumen content for each cow was separately squeezed through 4 layers of cheesecloth, pooled in equal proportions, and again filtered through 4 layers of cheesecloth into a bottle (Schott North America Inc., Elmsford, NY) without headspace for inoculum. The ruminal fluid was quickly transported to the laboratory, keeping the bottle in a water bath maintained at 39°C. The in vitro fermentation experiment was completed in 2 separate runs on different days with 6 incubation bottles per run for each substrate as described previously (Szumacher-Strabel et al., 2011). Additionally, 6 bottles in each run containing buffered ruminal fluid only were included as a blank control. In brief, ruminal fluid was diluted with a buffer solution at a ratio of 1:4. Then, the buffered ruminal fluid

Table 1. Ingredient and chemical composition of diets and supplements fed to dairy cows¹

Item	TMR	PMR	Concentrate
Ingredient composition, g/kg of DM			
Maize silage	392	429	—
Lucerne silage	70	77	—
Grass silage	103	112	—
Beet pulp	112	122	—
Brewer's grain	84	92	—
Extracted rapeseed meal	42	46	—
Concentrate	187	112	—
Mineral-vitamin mixture	10	10	—
Chemical composition, g/kg of DM			
OM	905	911	913
Ash	92.2	89.7	87.3
CP	167	157	210
EE	25.5	23.2	29.1
aNDF	366	392	303
Fatty acid (FA), g/100 g of FA			
C14:0	0.82	0.78	0.29
C16:0	21.3	20.9	17.8
C18:0	4.35	4.73	3.25
C18:1 <i>cis</i> -9	19.6	18.9	26.8
C18:2 <i>cis</i> -9, <i>cis</i> -12	41.1	40.7	43.4
C18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	8.83	9.58	3.29
Other FA ²	3.62	4.62	5.17
SFA	29.0	29.0	24.4
UFA	71.0	71.0	75.6
MUFA	20.1	19.4	27.4
PUFA	50.9	51.6	48.2
n-6 FA	41.5	42.2	44.3
n-3 FA	9.44	10.2	4.04

¹PMR = partial mixed ration; EE = ether extract; aNDF = neutral detergent fiber determined with amylase.

²Other FA include C12:0, C14:1, C16:1, C18:1 *cis*-11, C18:1 *cis*-15, and C20:3n-6.

(40 mL) was transferred into 125-mL glass incubation bottles (Midland Scientific Inc., Omaha, NE) containing 400 mg of the substrate (DM basis). The control group consisted of a homogeneous mixture of ground meadow hay (240 mg of DM) and ground barley meal (160 mg of DM). In the experimental groups, ground barley grains (8 mg; i.e., 5% barley) were replaced by equivalent amounts (8 mg) of seed residues of SB, BC, and RB, respectively. Subsequently, 400 mg of the dried substrate was added to each bottle. After the buffered fluid was transferred to the bottles, they were filled with CO₂, closed with a rubber stopper, sealed with aluminum covers, and incubated in an incubator (Galaxy 170R, Eppendorf North America Inc., Hauppauge, NY) for 48 h anaerobically at a temperature of 39°C. The bottles were automatically agitated every 30 min during incubation period. Fermented fluid samples were obtained at 8, 24, and 48 h of incubation to determine concentrations of VFA (1 mL) and ammonia (5 mL), and number of protozoa (1 mL) and bacteria (20 µL). The gas samples (500 µL) were collected from the headspace of the bottles in a gas-tight syringe for measurement of methane concentration in gas samples. The *in vitro* dry matter digestibility (IVDMD) was determined after 48 h of incubation.

In Vivo Experiment Using Cannulated Dairy Cows. In experiment 1, BC seed residues did not show any adverse effects on ruminal fermentation characteristics; thus, it was selected for *in vivo* study on ruminal fermentation and microbial population in lactating dairy cows (experiment 2). Four ruminal cannulated Polish Holstein-Friesian cows (multiparous, 650 ± 30 kg of BW; 5–6 mo in lactation) were allocated to 2 dietary groups (control and BC seed residues; n = 4) in a replicated 2 × 2 crossover design of 2 periods. Each period lasted 26 d, with a 21-d adaptation and a 5-d sampling period. The control group was fed a partial mixed ration (**PMR**) along with 2 kg/d of concentrate feed (Table 1). The concentrate feed was fed separately. In the experimental group, cows were fed the same PMR and 2 kg/d of BC seed residues. Feeds were fed twice daily at 0600 and 1800 h. During the first 8 d of feeding, PMR was fed for *ad libitum* intake in both groups. Thereafter, all cows were provided restricted amounts of feeds, which was 95% of the daily feed intake based on previous 5 d to avoid effects of DMI on ruminal fermentation. During the experimental period, cows consumed 21.0 ± 0.4 kg of DM daily. Drinking water was offered *ad libitum*. The rumen fluid was obtained before the morning feeding

from each cannulated cow for determination of ruminal fermentation characteristics and FA profile and estimation of methane production. Additionally, during the sampling period, total feces were collected and weighed and 100 g of feces was sampled for determination of DM and CP digestibility.

In Vivo Experiment Using Dairy Cows Kept Under Commercial Conditions (Experiment 3).

In experiment 3, the period (total 26 d with a 21-d adaptation and a 5-d sampling) and organization (replicated 2×2 crossover design) were the same as in experiment 2. Thirty multiparous Polish Holstein-Friesian lactating dairy cows (650 ± 45 kg of BW; 5–6 mo in lactation) were equally and randomly allocated to 2 dietary groups (control and BC; $n = 30$; 15 in each group). The diets were the same as used in experiment 2, where cows in the BC group were fed 2 kg/d of BC seed residue instead of the 2 kg/d of concentrate offered to the control group. Fresh PMR was offered twice daily at 0600 and 1800 h, whereas both the concentrate and BC seed residue were supplied by a computer controlled feeder station (type FP 204, De Laval, Tumba, Sweden). The concentrate and BC residues were completely consumed by cows. The daily DMI for each group was maintained at 21.0 ± 0.4 kg during the experiment. Cows were usually milked twice daily at 0530 and 1730 h and daily milk production was measured using a milk meter (WB Ezi-Test Meter 33 kg; Tru-Test Limited, Manukau, New Zealand). During the sampling days (last 5 d of each period), milk samples were obtained at each milking and daily milk samples were pooled proportionately based on morning and evening milk production. The daily pooled milk samples for each cow were again composited for 5 d. Finally, the 5-d composite samples for each cow were divided in 2 parts: one part for analysis of basic milk constituents directly and other part for determination of milk FA composition, which was stored at -20°C until analysis.

Laboratory Analyses

Chemical Composition of Feeds. Samples of TMR, PMR, concentrate, SB, BC, and RB seed residues were analyzed following the procedures of AOAC (2007) for DM (method no. 934.01), CP (using a Kjeld-Foss Automatic 16210 analyzer; method no. 976.05), ash (method no. 942.05), crude fiber (**CF**; using FOSS Tecator, Fibertec System, method no. 962.09), and ether extract (**EE**; using a Soxhlet System HT analyzer; method no. 973.18) concentrations. Organic matter was calculated by subtracting ash concentration from DM content. Nitrogen-free extract was estimated by

deducting concentrations of crude fiber, CP, EE, and ash from DM content.

The extracts containing phenolic compounds were prepared from powdered berry seed residues (Stochmal and Oleszek, 2007) to analyze tannin, flavonoid, and anthocyanin content. The flavonoids and anthocyanins in the extracts were determined on a HPLC with kaempferol glucoside as a standard (Janda et al., 2007). The liquid chromatography-MS technique was also used for identification and determination of tannins (Janda et al., 2007). The amount of tannins was calculated using (+)-catechin as an external standard.

Determination of Ruminal Fermentation Characteristics. Immediately after sample collection, pH values in ruminal fluid samples were determined using a pH meter (Type CP-104, Elmetron, Zabrze, Poland). The ammonia concentration was measured following a colorimetric Nessler method described previously (Szumacher-Strabel et al., 2011). For determination of IVDMD, the contents of the incubation flasks was transferred to previously weighed crucibles at the end of incubation. The residues were washed with distilled water thoroughly and dried at 105°C for 3 d. The percentage loss in weight of the feed DM in microbial fermentation process was expressed as IVDMD. The VFA concentrations were determined following a method described by Tangerman and Nagengast (1996) with some modifications. In brief, 3.6-mL ruminal fluid samples were preserved immediately after collection with 0.4 mL of HgCl_2 solution (46 mM) and stored at -20°C until analysis of VFA on a gas chromatograph (GC Varian CP 3380, Sugarland, TX) equipped with a capillary column (30 m $\times$ 0.25 mm; Agilent HP-Innowax, 19091N-133, Agilent Technologies, Santa Clara, CA) and a flame ionization detector. The concentrations of individual VFA were determined using an external VFA standard prepared by mixing individual VFA (Sigma-Aldrich, St. Louis, MO).

Methane Production. Methane concentration in experiment 1 was measured by a gas chromatograph (SRI PeakSimple model 310; Alltech Inc, Nicholasville, KY) using appropriate gas standards (mix gases of 5.63% CO_2 , 5.56% CH_4 , 5./d10% H_2 and rest N_2 , Multax, Zielonki-Parcela, Poland) using PeakSimple ver. 3.29 (Szumacher-Strabel et al., 2011). In experiment 2, methane generation during microbial fermentation was estimated based on theoretical stoichiometry of ruminal molar proportions of individual VFA and OM degraded in the rumen (Wolin, 1960). Methane production in experiment 3 was continuously measured by infrared methane analyzers (Servomex 4000 Series, Servomex Ltd., Jarvis Brook, UK) and employing 1,210 Gfx modules using the gas filter correlation technique during

the feeding of concentrate in the feeder station. Two infrared methane analyzers were used with a range of 0 to 500 ppm (0–625 mg/m³) for ambient air and 0 to 1,000 ppm (0–1,250 mg/m³) for feeding station where concentrates were supplied. Air samples were continuously collected from the feed bins in the feeder stations at 15 L/min via an 8-mm diameter polyethylene tube approximately 25 m in length and connected to a gas panel. The gas samples were distributed to the inlet port of the analyzer with a flow rate of 0.6 L/min. To decrease the system response time, an additional gas sample was driven by a by-pass line. An external pump was used to draw air between the gas inlet to the sample panel and the outlet of the feeding station. Methane concentrations were measured at 2-s intervals and data were stored on a computer using software with a database system (RS 232; AnaGaz, Wroclaw, Poland). Before measurement of methane concentrations in gas samples, the analyzers were calibrated using a standard calibration gas (Multax, Zielonki-Parcela, Poland) containing 1,210 ppm of methane in nitrogen gas (99.99%).

Fatty Acid Analysis. The FA concentrations in samples were determined using a gas chromatograph (Szczechowiak et al., 2016). Briefly, 3 mL of 2 M NaOH solution was added to 2,500, 100, and 500 mg of ruminal fluid, feed, and milk samples, respectively, in screw-cap Teflon-stoppered tubes (glass, 15 mL) for hydrolysis of fats. Hydrolyzed FA samples were incubated on a block heater at 90°C for 40 min. Samples were then extracted and trans-esterified using 0.5 M NaOH in methanol borontrifluoride (Fluka, Sigma-Aldrich) to FAME. Analysis of FAME was performed on a gas chromatograph (GC Bruker 456-GC, Billerica, MA) equipped with a capillary column (100-m fused-silica, 0.25 mm i.d., 0.25 µm film thickness; Chrompack CP7420, Agilent HP) and a flame ionization detector using 1 µL of sample injected into the column. Temperatures in injector and detector were maintained at 200 and 250°C, respectively. The oven temperature was initially set at 120°C for 7 min, then increased at a rate of 7°C/min to 140°C, held for 10 min, and then increased at a rate of 4°C/min to 240°C. Hydrogen gas was used as a carrier gas at a flow rate of 1.3 mL/min. Fatty acids were identified and quantified with appropriate FAME standards (37 FAME Mix, Sigma-Aldrich). The concentrations of CLA were determined using a CLA standard (a mixture of *cis*- and *trans* 9,11 and 10,12-octadecadienoic acid methyl esters; Sigma-Aldrich) using a Galaxie Work Station 10.1 (Varian, CA).

The desaturase indices (Brojna et al., 2011), atherogenic index (Chilliard et al., 2003), thrombogenic index (Ulbricht and Southgate, 1991), and milk energy

(Tyrrell and Reid, 1965) values were estimated using the appropriate equations.

Analysis of Chemical Composition of Milk. Composite milk samples were analyzed for basic milk constituents using an infrared analyzer (Milko-Scan 255 A/S N; Foss Electric, Hillerød, Denmark). Urea concentration in milk was determined in an accredited milk quality laboratory by means of infrared spectrometry using a CombiFoss 6000 analyzer (Foss Electric).

Feed Cost Calculation

The FeedExpert (Rovecom, Hoogeveen, the Netherlands) software was used to calculate feeding costs of dairy cows. The cost was calculated based on the actual prices paid during the duration of the *in vivo* experiment. It was assumed that the cost of 1 kg of concentrate feed was approximately €0.21, whereas the cost of BC residue was €0.02/kg.

Statistical Analysis

In experiment 1, data were obtained only at one time point (8 or 24 or 48 h after start of the *in vitro* culture) for each replicate. Data within each time point were analyzed in one-way ANOVA with treatment or time as a main factor. Statistical analyses were conducted with Sigma Plot version 11.0. In experiment 2, ruminal fermentation data were analyzed using PROC GLM univariate ANOVA (ver. 9.4, SAS Institute Inc., Cary, NC) for a crossover design with a model containing group (dietary treatment sequence), period, and treatment as main effects, time as repeated measures, and cow as a random effect. The model for the FA analysis contained group, period, and treatment as a main effect. In experiment 3, data were analyzed with the model containing treatment, group, period as fixed effects and cows within a group as a random effect. Statistical analyses for the *in vivo* experiments were performed using JMP software (SAS Institute Inc.). All pairwise multiple comparisons were performed using a Holm-Sidak test. The results were considered significant when $P < 0.05$. All values are shown as the means with pooled standard errors of means.

RESULTS

Basic Nutrient, Fatty Acid, Phytochemical Contents in Berry Residues, and Cost of Diets

The BC seed residues contained 262, 58, and 287 g/kg of DM of CP, EE, and nitrogen-free extract, respectively (Table 2). The SB and RB seed residues

Table 2. Chemical composition of the berry seed residues¹

Item	Seed residue		
	SB	BC	RB
Chemical composition, g/kg of DM			
OM	926	911	949
Ash	74.1	89.4	51.1
CP	167	262	110
CF	526	304	585
EE	33.8	57.9	36.3
NFE	199	287	218
Fatty acids (FA), g/100 g of FA			
C14:0	0.17	0.13	0.16
C16:0	6.99	7.75	4.77
C16:1	0.29	0.11	0.33
C18:0	2.33	2.09	1.42
C18:1 <i>cis</i> -9	15.5	11.3	9.74
C18:2n-6	46.4	45.9	48.1
C18:3n-3	25.8	14.6	33.1
C18:3n-6	0.01	12.6	0.11
C20:5n-3	0.26	0.15	0.30
SFA	9.94	12.9	6.81
UFA	90.1	87.1	93.2
MUFA	17.5	13.8	11.5
PUFA	72.6	73.3	81.7
Tannins, flavonoids, and anthocyanins, mg/100 g			
Tannins ²	87.9	2.54	150
Flavonoids ³	317	18.3	240
Anthocyanins	ND	37.6	ND

¹SB = strawberry; BC = black currant; RB = raspberry; CF = crude fiber; EE = ether extract; NFE = nitrogen-free extract; ND = not detected.

²The tannins concentration was based on the (+) catechin as a standard.

³The flavonoids concentration was based on kaempferol as a standard.

contained high amounts of crude fiber (526 and 585 g/kg of DM, respectively). The EE in seed residues were mainly of UFA type (90.1, 87.1, and 93.2 g/100 g of FA in SB, BC, and RB seed residues, respectively). Linoleic and linolenic acids were the major FA among the UFA. Total MUFA, which mainly consisted of oleic acid, ranged from 11.5, through 13.8 to 17.5 g/100 g of FA in SB, BC, and RB, respectively. Flavonoid concentrations in SB seed residues were high, whereas RB seed residue was rich in both flavonoids and tannins. The BC contained anthocyanins that were not detected in SB and RB samples.

The daily cost of feed was lower in the BC group compared with the control (€2.22 vs. €2.70 per day, respectively).

In Vitro Experiment

In experiment 1, changes in all variables were time-dependent and the effects were not always permanent (Table 3). Significant differences in holotrich number, pH values, and ammonia concentration were observed after the first 8 h of incubation. The entodiniomorph population and hydrogen recovery were not altered at 8 h of incubation. Greater ammonia concentration was

observed at 8 h of incubation in RB treatment compared with the control (Table 3). Significant changes in ammonia were also found after 24 h of incubation. The changes in entodiniomorph counts occurred at 24 h of incubation in BC and RB groups. The rise in hydrogen recovery occurred in the group supplemented with the RB seed residue compared with the control at 48 h of fermentation. Several time-dependent changes in VFA proportions were also noticed (Table 4). The highest concentration of the total VFA was found in the BC group after 48 h fermentation. This was likely due to the increased concentration of 3 VFA (propionate, butyrate, and isovalerate; Table 4).

In Vivo Experiments

In experiment 2, total bacteria, total protozoa, holotrichs, and entodiniomorph counts were unaffected in the BC group compared with the control group (Table 5). Ammonia concentration increased ($P < 0.05$) at 6 h postfeeding in the BC group compared with the control. Many of the VFA profiles were unaffected by treatments. However, isovalerate concentration at 3-h postfeeding was negatively affected by BC ($P < 0.05$) compared with the control (Table 6). Methane produc-

Table 3. Effects of berry seed residues on microbial counts and some ruminal fermentation characteristics in a batch culture system (experiment 1)

Item ¹	Incubation time	Treatment ²				SEM	P-value
		Control	SB	BC	RB		
Total bacteria, $\times 10^8$ /mL	8 h	14.1 ^C	20.7 ^B	21.7 ^B	20.8 ^B	1.25	0.09
	24 h	32.1 ^A	32.7 ^A	36.8 ^A	31.3 ^A	0.84	0.07
	48 h	25.7 ^B	29.0 ^A	27.8 ^B	26.9 ^A	0.92	0.68
	SEM	2.66	2.08	2.45	1.71		
	P-value	0.001	0.02	0.01	0.01		
Total protozoa, $\times 10^3$ /mL	8 h	10.0 ^B	10.1 ^C	9.04 ^C	8.63 ^C	0.43	0.32
	24 h	11.4 ^{B,b}	8.11 ^{B,ab}	12.9 ^{B,a}	11.2 ^{B,b}	0.71	0.09
	48 h	20.4 ^A	19.4 ^A	20.2 ^A	21.5 ^A	0.90	0.14
	SEM	1.64	1.59	1.66	1.99		
	P-value	<0.001	<0.001	<0.001	<0.001		
Holotrichs, $\times 10^2$ /mL	8 h	5.53 ^{A,a}	4.42 ^b	4.67 ^{ab}	5.09 ^a	0.15	0.001
	24 h	4.95 ^A	4.87	4.93 ^A	4.96 ^A	0.83	0.99
	48 h	3.97 ^B	3.45	4.28 ^C	4.04 ^B	0.15	0.08
	SEM	0.26	0.29	0.10	0.18		
	P-value	0.008	0.110	0.002	<0.001		
Entodiniomorphs, $\times 10^3$ /mL	8 h	9.45 ^B	9.62 ^C	8.57 ^C	8.29 ^C	0.30	0.36
	24 h	10.9 ^{ab}	11.7 ^{B,ab}	12.4 ^{B,a}	10.7 ^{B,b}	0.27	0.03
	48 h	19.9 ^A	19.0 ^A	19.8 ^A	21.1 ^A	0.32	0.15
	SEM	1.66	1.61	1.66	1.99		
	P-value	<0.001	<0.001	<0.001	0.001		
Methane, mM	8 h	4.53 ^C	4.44 ^C	4.81 ^C	4.74 ^C	0.07	0.21
	24 h	5.69 ^B	5.25 ^B	5.41 ^B	5.30 ^B	0.08	0.19
	48 h	6.54 ^A	6.08 ^A	6.38 ^A	6.17 ^A	0.08	0.10
	SEM	0.31	0.24	0.23	0.22		
	P-value	0.001	0.001	0.001	0.001		
pH	8 h	6.42 ^{Aa}	6.39 ^{A,ab}	6.37 ^{ab}	6.35 ^{A,b}	0.01	0.04
	24 h	6.26 ^B	6.24 ^B	6.22 ^B	6.22 ^B	0.007	0.10
	48 h	6.17 ^{C,b}	6.14 ^b	6.15 ^{C,bc}	6.21 ^{B,a}	0.008	0.03
	SEM	0.037	0.036	0.032	0.023		
	P-value	0.001	0.001	0.001	0.001		
Ammonia, mM	8 h	4.42 ^{C,b}	4.96 ^{ab}	4.87 ^{ab}	5.21 ^{C,a}	0.01	0.01
	24 h	9.89 ^{B,a}	9.74 ^{ab}	9.07 ^{B,c}	10.4 ^{B,a}	0.01	0.002
	48 h	13.5 ^A	13.7 ^A	13.5 ^A	14.3 ^A	0.02	0.09
	SEM	1.33	1.27	1.24	1.32		
	P-value	0.001	0.001	0.001	0.001		
Hrec, %	8 h	57.3 ^A	56.1 ^A	55.3 ^A	56.0 ^A	0.62	0.69
	24 h	48.9 ^B	50.5 ^B	50.2 ^B	51.0 ^B	0.36	0.17
	48 h	48.8 ^{B,b}	50.5 ^{ab}	50.1 ^{B,ab}	50.9 ^{B,a}	0.30	0.04
	SEM	1.61	1.00	0.87	0.87		
	P-value	0.010	0.002	0.001	0.001		
IVDMD, %	8 h	36.3 ^C	34.6 ^C	42.6 ^B	34.1 ^C	1.33	0.05
	24 h	47.9 ^B	46.8 ^B	46.1 ^B	44.9 ^B	0.73	0.60
	48 h	58.4 ^A	54.9 ^A	55.7 ^A	60.9 ^A	0.96	0.08
	SEM	3.26	3.25	2.06	3.98		
	P-value	0.001	0.003	0.005	0.001		

^{A-C}Means within a column for a variable with different uppercase superscripts differ ($P < 0.05$).

^{a-c}Means within a row with different lowercase superscripts differ ($P < 0.05$).

¹Hrec = hydrogen recovery; IVDMD = in vitro dry matter digestibility.

²SB = control diet with strawberry seed residues; BC = control diet with black currant seed residues; RB = control diet with raspberry seed residues.

tion was time dependent ($P < 0.05$) and was not affected by the BC feeding. The whole-tract digestibilities of DM and CP were unaffected by BC compared with the control group (Figure 1). As shown in Table 7, BC diet reduced the concentration of C14:0, C15:0, C17:0, and C17:1 in ruminal fluid. In contrast, the concentrations of C18:1 *trans*-11 and C18:2 *cis*-9,*trans*-11 significantly increased by 91 and 131%, respectively. Likewise, concentrations of total *trans* C18:1 and MUFA in ruminal

fluid increased in experimental groups. Feeding of BC decreased n-6 FA content in ruminal fluid. Sum of odd-(**OBCFA**) and branched-chain FA (**BCFA**) concentrations decreased after supplementation of BC due to their decreased proportions after 3 and 6 h postfeeding (Table 7). Time of sample collection significantly affected concentration of most FA profiles.

In experiment 3, although milk fat content, milk yield, urea concentration, and methane emission were

not affected by diets (Table 8), the presence of BC seed residue altered the FA proportions in milk (Table 9). Feeding of BC led to an increased ($P < 0.05$) proportion of the biohydrogenation intermediates; vacenic acid (C18:1, *trans*-11; **VA**), C18:2, *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers as well as C18:2 FA. Proportion of both C20:4n-6 (arachidonic acid) and C22:6n-3 (docosahexaenoic acid) increased ($P < 0.05$) in BC compared with the control. Feeding of BC did not affect SFA proportion, but increased ($P < 0.05$) total PUFA concentrations including both n-6 and n-3, which consequently resulted in increased ($P < 0.05$) PUFA/SFA ratio compared with the control. Among the desaturases indices, BC decreased ($P < 0.05$) only

C18:1 desaturase index (Table 10). Thrombogenic and atherogenic indices of milk were not changed by BC supplementation.

DISCUSSION

The dynamically developing food industries specializing in the production of fruit and vegetable juices produce large quantities of plant residues, which might adversely affect the environment. Supercritical CO₂ extraction of berry seeds produces high-quality oil rich in UFA (Helbig et al., 2008). The high nutrient contents of the by-products make them for proper valorization, bringing both ecological and economic benefits (Helbig

Table 4. Effects of berry seed residues on VFA concentrations in rumen fluid incubated in a batch culture system (experiment 1)

Item	Incubation time	Treatment ¹				SEM	P-value
		Control	SB	BC	RB		
Total VFA, mM	8 h	39.0 ^C	38.2 ^C	38.6	37.6 ^C	0.26	0.64
	24 h	57.5 ^{B,a}	56.2 ^{B,b}	58.6 ^a	55.7 ^{B,a}	0.04	0.002
	48 h	70.1 ^{A,b}	69.2 ^{ab}	73.6 ^a	69.4 ^{A,b}	0.06	0.003
	SEM	4.52	4.50	5.08	4.60		
	P-value	0.001	0.001	0.001	0.001		
Acetate (A), mM	8 h	26.2 ^{C,a}	24.9 ^{C,b}	25.9 ^{C,a}	24.9 ^{C,b}	0.02	0.01
	24 h	39.1 ^{B,a}	36.7 ^{B,b}	38.5 ^{B,a}	36.4 ^{B,b}	0.04	0.001
	48 h	46.6 ^{A,a}	44.1 ^{A,b}	46.9 ^{A,a}	43.9 ^{A,b}	0.04	0.001
	SEM	2.98	2.81	3.06	2.75		
	P-value	0.001	0.001	0.001	0.001		
Propionate (P), mM	8 h	6.78 ^C	7.29 ^C	6.66 ^C	6.61 ^B	0.159	0.43
	24 h	8.28 ^{B,c}	9.30 ^{ab}	9.67 ^{Ba}	9.25 ^{A,b}	0.015	0.001
	48 h	10.4 ^{A,b}	11.6 ^b	12.3 ^{A,a}	11.6 ^{A,a}	0.020	0.005
	SEM	0.54	0.65	0.81	0.44		
	P-value	0.001	0.001	0.001	0.001		
Butyrate, mM	8 h	4.87 ^C	4.91 ^C	4.90 ^C	4.95 ^C	0.028	0.83
	24 h	7.94 ^B	8.02 ^B	8.12 ^B	7.87 ^B	0.053	0.44
	48 h	10.0 ^{A,b}	10.2 ^{A,b}	10.9 ^A	10.5 ^{A,ab}	0.02	0.01
	SEM	0.75	0.76	0.87	0.81		
	P-value	0.001	0.001	0.001	0.001		
Isobutyrate, mM	8 h	0.35 ^{C,b}	0.33 ^{C,b}	0.33 ^{C,b}	0.37 ^{C,a}	0.005	0.04
	24 h	0.47 ^B	0.46 ^B	0.48 ^B	0.46 ^B	0.004	0.57
	48 h	0.63 ^A	0.68 ^A	0.70 ^A	0.66 ^A	0.013	0.24
	SEM	0.042	0.051	0.055	0.043		
	P-value	0.001	0.001	0.001	0.001		
Isovalerate, mM	8 h	0.44 ^C	0.44 ^C	0.46 ^C	0.44 ^C	0.008	0.09
	24 h	0.73 ^B	0.74 ^B	0.78 ^B	0.74 ^B	0.010	0.42
	48 h	1.23 ^{A,b}	1.40 ^{A,a}	1.49 ^{A,a}	1.40 ^{ab}	0.017	0.009
	SEM	0.115	0.143	0.153	0.143		
	P-value	0.001	0.001	0.001	0.001		
Valerate, mM	8 h	0.36 ^{C,a}	0.36 ^{C,a}	0.34 ^{C,a}	0.30 ^{C,b}	0.003	0.003
	24 h	1.02 ^B	0.96 ^B	1.03 ^B	0.99 ^B	0.014	0.35
	48 h	1.21 ^A	1.25 ^A	1.31 ^A	1.24 ^A	0.033	0.22
	SEM	0.111	0.132	0.144	0.140		
	P-value	0.001	0.001	0.001	0.001		
A/P ratio	8 h	3.87 ^B	3.90 ^B	3.89	3.89 ^B	0.011	0.86
	24 h	4.01 ^A	4.03 ^A	3.98	4.05 ^A	0.015	0.41
	48 h	3.95 ^{AB}	3.93 ^B	3.84	3.94 ^B	0.02	0.11
	SEM	0.02	0.02	0.03	0.03		
	P-value	0.03	0.02	0.09	0.01		

^{A-C}Means within a column for a variable with different uppercase superscripts differ ($P < 0.05$).

^{a-c}Different lowercase superscripts in a row show differences ($P < 0.05$) among treatments.

¹SB = control diet with strawberry seed residues; BC = control diet with black currant seed residues; RB = control diet with raspberry seed residues.

et al., 2008; Chedea et al., 2017). One of the methods allowing reuse of residues is their application in animal feed industries. However, most of the fruit industry by-products are either composited or combusted, with only a small amounts used in animal diets. The oil and residues contain nonnutritive phytochemicals that can be used to modulate the fermentation process (Mirzaei-Aghsaghali and Maheri-Sis, 2008; Cieřlak et al., 2013). The oils are mainly use in pharmaceutical industry. The main phytochemicals in beery seed residues are tannins and flavonoids. Because of their antimicrobial properties, they have been shown to lower ruminal methane production in vitro and in vivo (Bodas et al., 2009; Patra and Saxena, 2010). The amount of energy lost as methane in ruminant animals may account for 6 to 12% of gross energy intake, or 8 to 14% of digestible energy intake (Czerkawski, 1969; Johnson and Ward, 1996). For mitigation of methane production in ruminants, it is crucial to develop a strategy that decreases methane-producing microbial activities and proliferation without limiting beneficial rumen function (Foiklang et al., 2016; Patra, 2016). Berry seed residues were considered as potential factors for modulating rumen microflora populations responsible for methanogenesis. However, each type of phytochemicals such as saponins, tannins, and essential oils has a different effect on rumen microbial populations and methane production (Szumacher-Strabel and Cieřlak, 2010; Wencelová et al., 2015, 2016). These effects

are generally multifaceted, affecting microorganisms participating in the production of hydrogen as well as utilizing hydrogen to produce methane. We did not confirm the hypothesis that berry seed residues, in the amount used, have strong potential to mitigate rumen methane production; however, methane produced with berry seed residues in in vitro and in vivo studies was numerically lower than the controls.

In the present study, berry seed residues did not generally show antimicrobial activity; however, BC residues modified in vitro ruminal VFA profile. A meta-analysis studying the effects of phytochemicals on ruminal fermentation characteristics concluded that for tannins, for example, a decrease in methane emission is not always related to changes in acetate and propionate concentrations, although acetate to propionate ratio decreased quadratically with inhibition of methane (Patra, 2010). Studies investigating the influence of berry seed by-products on ruminal fermentation are scarce; however, information is available on the effect of various other plant materials containing bioactive compounds on ruminal fermentation, including ciliate protozoal counts. It should be noted that the amount and type of phytobactors have an important influence on modulation of ruminal microbial metabolism (Ohene-Adjei et al., 2008; Wang et al., 2016).

No effects of berry seed residues on methane concentration and acetate-to-propionate ratio were observed. The average concentration of the in vitro total VFA

Table 5. Effect of feeding black currant (BC) seed residues to cannulated dairy cows on basic rumen parameters (experiment 2)

Item	Treatment	Total	0 h	3 h	6 h	SEM	P-value
Total bacteria, $\times 10^8$ /mL	Control	123	127	115	126	5.28	0.30
	BC	126	128	118	132	4.50	0.08
	SEM	2.99	4.60	4.50	5.50		
	P-value	0.44	0.83	0.65	0.44		
Total protozoa, $\times 10^4$ /mL	Control	69.5	69.2	73.9	65.4	0.565	0.35
	BC	68.5	61.2	71.2	73.2	0.899	0.89
	SEM	0.43	0.93	0.52	0.76		
	P-value	0.89	0.54	0.73	0.30		
Holotrichs, $\times 10^4$ /mL	Control	1.02	0.82 ^b	1.52 ^a	0.73 ^b	0.152	0.04
	BC	0.84 ^b	0.63 ^b	1.14 ^a	0.76 ^b	0.181	0.001
	SEM	0.09	0.09	0.22	0.11		
	P-value	0.27	0.21	0.18	0.82		
Entodiniomorphs, $\times 10^5$ /mL	Control	6.84	6.83	7.24	6.46	0.563	0.37
	BC	6.77	6.05	7.01	7.25	0.899	0.92
	SEM	0.43	0.94	0.53	0.76		
	P-value	0.92	0.55	0.78	0.30		
pH	Control	6.04	6.39 ^a	5.98 ^b	5.75 ^c	0.061	0.001
	BC	6.04	6.33 ^a	6.08 ^b	5.71 ^c	0.071	0.001
	SEM	0.04	0.04	0.03	0.04		
	P-value	0.96	0.53	0.12	0.71		
NH ₃ , mM	Control	9.07 ^{A,b}	6.71 ^c	13.7 ^a	6.77 ^{A,b}	1.11	0.001
	BC	10.3 ^{B,a}	7.39 ^c	12.9 ^a	10.7 ^{B,c}	1.07	0.001
	SEM	0.13	0.34	0.96	0.90		
	P-value	0.02	0.15	0.21	0.04		

^{A,B}Means in a column within a variable with different uppercase superscripts differ ($P < 0.05$).

^{a-c}Means in a row with different lowercase superscripts differ ($P < 0.05$).

Table 6. Effects of feeding of black currant (BC) seed residues on VFA concentration in ruminal fluid and methane production in cannulated dairy cows (experiment 2)

Item	Treatment	Total	0 h	3 h	6 h	SEM	P-value
Total VFA, mM	Control	107	94.4 ^b	112 ^a	116 ^a	1.80	0.01
	BC	110	101 ^b	108 ^b	123 ^a	1.95	0.02
	SEM	1.7	2.8	1.8	2.2		
	P-value	0.31	0.25	0.27	0.11		
Acetate (A), mM	Control	68.6	61.3 ^b	71.4 ^a	73.1 ^a	0.29	0.001
	BC	71.1	65.9 ^b	69.6 ^b	78.0 ^a	0.28	0.001
	SEM	0.20	0.33	0.37	0.22		
	P-value	0.16	0.16	0.33	0.07		
Propionate (P), mM	Control	23.3	19.6 ^b	24.3 ^a	26.1 ^a	0.29	0.001
	BC	24.1	21.9 ^b	22.9 ^b	27.5 ^a	0.25	0.001
	SEM	0.19	0.34	0.34	0.24		
	P-value	0.45	0.16	0.32	0.28		
Isobutyrate, mM	Control	0.78	0.75	0.80	0.79	0.01	0.10
	BC	0.82	0.77	0.83	0.85	0.01	0.30
	SEM	0.01	0.02	0.01	0.01		
	P-value	0.08	0.71	0.36	0.11		
Butyrate, mM	Control	11.7 ^b	10.3 ^c	12.2 ^b	12.7 ^a	0.13	0.001
	BC	11.6 ^a	10.0 ^b	11.5 ^{ab}	13.2 ^a	0.15	0.05
	SEM	0.10	0.24	0.12	0.14		
	P-value	0.74	0.73	0.16	0.30		
Isovalerate, mM	Control	1.45 ^A	1.25 ^b	1.58 ^{A,a}	1.51 ^a	0.02	0.006
	BC	1.32 ^B	1.09 ^b	1.40 ^{B,a}	1.46 ^a	0.02	0.003
	SEM	0.02	0.03	0.03	0.02		
	P-value	0.05	0.09	0.02	0.44		
Valerate, mM	Control	1.57	1.27 ^b	1.73 ^a	1.72 ^a	0.03	0.001
	BC	1.72	1.34 ^b	1.80 ^a	2.02 ^a	0.03	0.001
	SEM	0.02	0.03	0.02	0.03		
	P-value	0.06	0.55	0.24	0.001		
A/P ratio	Control	3.00	3.22	2.96	2.82	0.05	0.025
	BC	2.99	3.02	3.10	2.85	0.06	0.013
	SEM	0.040	0.064	0.085	0.038		
	P-value	0.93	0.15	0.41	0.75		
Methane, mM	Control	0.61	0.56 ^b	0.60 ^a	0.67 ^a	0.01	0.015
	BC	0.59	0.53 ^b	0.62 ^{ab}	0.63 ^a	0.01	0.012
	SEM	0.01	0.01	0.01	0.01		
	P-value	0.25	0.32	0.28	0.07		
Methane, mM/M of VFA	Control	5.70	5.93	5.36	5.78	0.02	0.11
	BC	5.36 ^{ab}	5.25 ^a	5.74 ^{ab}	5.12 ^b	0.03	0.02
	SEM	0.03	0.04	0.02	0.03		
	P-value	0.17	0.15	0.10	0.17		

^{A,B}Means in a column within a variable with different uppercase superscripts differ ($P < 0.05$).

^{a-c}Means in a row with different lowercase superscripts differ ($P < 0.05$).

Figure 1. Effect of black currant seed residues on whole-tract digestibility (g/kg) of DM and CP in dairy cows. BC = control diet with black currant residue. Error bars represent SEM.

was higher for the BC diet compared with the control, which was probably as a result of increased concentrations of propionate, butyrate, and isovalerate. Increased VFA concentration, unaltered rumen microbial populations and methane concentration at 24 and 48 h of incubation may suggest that the amount of berry seed by-products used had no negative effects on fermentation in the rumen. This was further confirmed in the in vivo study because no changes were noticed in VFA concentrations, basic rumen parameters, and DM digestibility due to BC supplementation.

In the present experiment, we showed that the berry seed residues could be a significant source of phytochemicals. The SB seed residue was a rich source of flavonoids (317 mg/100 g), whereas RB seed residue was rich in flavonoids and tannins (240 and 150 mg/100 g,

Table 7. Fatty acid (FA) concentration (g/100 g of FA) in ruminal fluid of cannulated dairy cows fed a diet with black currant (BC) seed residues (experiment 3)

Item	Treatment	Total	0 h	3 h	6 h	SEM	P-value
C8:0	Control	0.05	0.04	0.04	0.05	0.002	0.09
	BC	0.05	0.05	0.06	0.05	0.002	0.47
	SEM	0.002	0.004	0.003	0.003		
	P-value	0.11	0.27	0.22	0.57		
C10:0	Control	0.08	0.08	0.11	0.06	0.008	0.23
	BC	0.07	0.06	0.06	0.08	0.005	0.39
	SEM	0.007	0.007	0.02	0.005		
	P-value	0.16	0.15	0.18	0.07		
C12:0	Control	0.43	0.39	0.45	0.42	0.01	0.67
	BC	0.49 ^{ab}	0.37 ^b	0.56 ^a	0.52 ^a	0.02	0.01
	SEM	0.02	0.01	0.04	0.03		
	P-value	0.17	0.54	0.14	0.05		
C14:0	Control	1.21 ^A	1.22 ^A	1.23	1.17	0.03	0.87
	BC	1.05 ^{B,ab}	0.97 ^{B,b}	1.07 ^a	1.09 ^a	0.01	0.01
	SEM	0.03	0.05	0.04	0.02		
	P-value	0.04	0.009	0.08	0.08		
C14:1	Control	1.32	1.30	1.33	1.36	0.02	0.49
	BC	1.30	1.27	1.32	1.31	0.01	0.47
	SEM	0.01	0.02	0.02	0.02		
	P-value	0.52	0.51	0.82	0.17		
C15:0	Control	1.28 ^A	1.28 ^A	1.29 ^A	1.31 ^A	0.02	0.59
	BC	1.11 ^B	1.14 ^B	1.07 ^B	1.11 ^B	0.009	0.15
	SEM	0.03	0.03	0.04	0.03		
	P-value	0.001	0.02	0.001	0.003		
C15:1	Control	0.48	0.49	0.50	0.50	0.01	0.92
	BC	0.49	0.49	0.50	0.49	0.005	0.98
	SEM	0.01	0.015	0.015	0.013		
	P-value	0.49	0.85	0.67	0.84		
C16:0	Control	18.49 ^A	18.22	18.68	18.58 ^A	0.07	0.07
	BC	18.92 ^{B,a}	18.24 ^b	19.35 ^a	19.17 ^{B,a}	0.11	0.001
	SEM	0.09	0.04	0.18	0.10		
	P-value	0.01	0.85	0.06	0.001		
C16:1	Control	0.34	0.33	0.36 ^A	0.33	0.006	0.27
	BC	0.31	0.30	0.34 ^B	0.29	0.008	0.34
	SEM	0.008	0.01	0.007	0.01		
	P-value	0.13	0.23	0.04	0.23		
C17:0	Control	0.54 ^A	0.55 ^A	0.56 ^A	0.53 ^A	0.004	0.08
	BC	0.51 ^B	0.52 ^B	0.50 ^B	0.50 ^B	0.004	0.05
	SEM	0.006	0.007	0.01	0.006		
	P-value	0.001	0.04	0.002	0.006		
C17:1	Control	0.10 ^A	0.11 ^A	0.10	0.11 ^A	0.003	0.61
	BC	0.09 ^B	0.09 ^B	0.09	0.09 ^B	0.003	0.92
	SEM	0.004	0.005	0.007	0.004		
	P-value	0.05	0.02	0.50	0.01		
C18:0	Control	47.8 ^{ab}	50.8 ^a	46.5 ^b	45.7 ^b	0.70	0.01
	BC	46.5 ^b	51.0 ^a	43.9 ^c	44.9 ^{bc}	0.62	0.001
	SEM	0.51	0.39	1.06	0.35		
	P-value	0.30	0.76	0.27	0.30		
C18:1 <i>trans</i> -10	Control	0.20	0.26	0.25	0.04	0.05	0.31
	BC	0.15	0.46	0.00	0.00	0.08	0.17
	SEM	0.06	0.15	0.08	0.02		
	P-value	0.70	0.54	0.18	0.36		
C18:1 <i>trans</i> -11	Control	3.09 ^{A,b}	3.44 ^{A,a}	3.52 ^{A,a}	2.39 ^{A,c}	0.10	0.001
	BC	5.01 ^B	5.35 ^B	4.62 ^B	4.95 ^B	0.17	0.56
	SEM	0.31	0.29	0.21	0.49		
	P-value	0.001	0.001	0.001	0.01		
C18:1 <i>cis</i> -9	Control	5.79	4.93	6.32	6.11	0.22	0.08
	BC	5.90 ^b	4.65 ^c	6.59 ^a	6.36 ^{ab}	0.17	0.001
	SEM	0.15	0.10	0.36	0.07		
	P-value	0.75	0.19	0.74	0.08		
C18:2 <i>cis</i> -9, <i>cis</i> -12	Control	3.78 ^b	2.42 ^{A,c}	3.33 ^{bc}	5.69 ^{A,a}	0.32	0.001
	BC	2.94 ^{ab}	1.92 ^{B,b}	3.19 ^{ab}	3.70 ^{B,a}	0.22	0.02
	SEM	0.22	0.11	0.32	0.43		
	P-value	0.06	0.02	0.77	0.02		

Continued

Table 7 (Continued). Fatty acid (FA) concentration (g/100 g of FA) in ruminal fluid of cannulated dairy cows fed a diet with black currant (BC) seed residues (experiment 3)

Item	Treatment	Total	0 h	3 h	6 h	SEM	<i>P</i> -value
C18:2 <i>cis</i> -9, <i>trans</i> -11	Control	0.57 ^{A,b}	0.28 ^b	0.43 ^{A,b}	1.02 ^a	0.08	0.001
	BC	1.33 ^{B,b}	0.20 ^c	2.40 ^{B,a}	1.36 ^b	0.18	0.001
	SEM	0.12	0.04	0.35	0.16		
	<i>P</i> -value	0.001	0.40	0.001	0.32		
C18:2 <i>trans</i> -10, <i>cis</i> -12	Control	0.57 ^{ab}	0.25 ^b	0.47 ^b	0.99 ^{A,a}	0.08	0.009
	BC	0.38 ^a	0.09 ^b	0.57 ^a	0.48 ^{B,a}	0.05	0.001
	SEM	0.06	0.04	0.12	0.09		
	<i>P</i> -value	0.10	0.06	0.70	0.001		
C18:3n-6	Control	0.175 ^{A,ab}	0.159 ^b	0.180 ^{A,ab}	0.187 ^a	0.003	0.02
	BC	0.192 ^{B,a}	0.165 ^b	0.208 ^{B,a}	0.205 ^a	0.005	0.001
	SEM	0.004	0.004	0.006	0.007		
	<i>P</i> -value	0.01	0.48	0.01	0.20		
C18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	Control	0.68	0.60	0.85	0.60	0.06	0.52
	BC	0.51	0.49	0.49	0.55	0.01	0.07
	SEM	0.05	0.04	0.12	0.02		
	<i>P</i> -value	0.19	0.16	0.20	0.27		
C20:4n-6	Control	0.16	0.16	0.15	0.18	0.005	0.13
	BC	0.16	0.17	0.16	0.17	0.003	0.34
	SEM	0.004	0.005	0.006	0.007		
	<i>P</i> -value	0.44	0.36	0.50	0.37		
C20:5n-3	Control	0.01	0.02	0.03	0.04	0.003	0.12
	BC	0.04	0.03	0.04	0.03	0.004	0.81
	SEM	0.004	0.005	0.007	0.005		
	<i>P</i> -value	0.19	0.27	0.25	0.67		
C22:6n-3	Control	0.41	0.40	0.40	0.44	0.01	0.58
	BC	0.41	0.42	0.40	0.41	0.01	0.97
	SEM	0.02	0.01	0.02	0.02		
	<i>P</i> -value	0.94	0.49	0.93	0.48		
Sum of other FA ¹	Control	12.7	12.5	13.2	12.4	0.16	0.26
	BC	12.3	11.7	12.7	12.4	0.18	0.24
	SEM	0.17	0.23	0.19	0.29		
	<i>P</i> -value	0.21	0.09	0.18	0.92		
Sum of SFA	Control	72.5 ^{ab}	74.9 ^a	71.5 ^b	70.7 ^b	0.54	0.008
	BC	71.5 ^b	74.8 ^a	69.9 ^c	70.2 ^{bc}	0.46	0.001
	SEM	0.38	0.31	0.79	0.21		
	<i>P</i> -value	0.29	0.87	0.35	0.28		
Sum of UFA	Control	25.3 ^a	23.1 ^b	26.1 ^a	27.1 ^a	0.48	0.004
	BC	26.5 ^a	23.3 ^b	28.1 ^a	27.9 ^a	0.45	0.001
	SEM	0.37	0.31	0.71	0.22		
	<i>P</i> -value	0.11	0.75	0.19	0.066		
Sum of MUFA	Control	19.2 ^{A,b}	19.0 ^{bc}	20.7 ^a	18.1 ^{A,c}	0.25	0.001
	BC	20.6 ^B	19.9	20.6	21.0 ^B	0.25	0.43
	SEM	0.25	0.29	0.33	0.57		
	<i>P</i> -value	0.007	0.17	0.93	0.01		
Sum of PUFA	Control	7.97 ^b	5.55 ^{A,c}	7.35 ^{bc}	11.23 ^{A,a}	0.54	0.001
	BC	7.46 ^a	4.67 ^{B,b}	9.14 ^a	8.49 ^{B,a}	0.42	0.001
	SEM	0.28	0.20	0.57	0.58		
	<i>P</i> -value	0.43	0.04	0.16	0.02		
Sum of n-6 FA	Control	8.94 ^{A,b}	6.15 ^{A,b}	7.99 ^b	12.87 ^{A,a}	0.65	0.001
	BC	7.18 ^{B,ab}	4.97 ^{B,b}	7.62 ^{ab}	8.75 ^{B,a}	0.44	0.009
	SEM	0.46	0.23	0.63	0.87		
	<i>P</i> -value	0.05	0.01	0.79	0.02		
Sum of n-3 FA	Control	1.59	1.48	1.75	1.58	0.06	0.54
	BC	1.46	1.43	1.48	1.49	0.02	0.85
	SEM	0.06	0.04	0.12	0.04		
	<i>P</i> -value	0.35	0.58	0.29	0.31		
Sum of <i>trans</i> C18:1	Control	6.55 ^{A,b}	7.36 ^{A,a}	7.28 ^{ab}	4.96 ^{A,c}	0.23	0.001
	BC	7.99 ^B	8.79 ^B	7.22	7.87 ^B	0.24	0.18
	SEM	0.24	0.28	0.17	0.58		
	<i>P</i> -value	0.001	0.004	0.88	0.01		
Sum of medium-chain FA	Control	26.8	26.2	27.4	27.0	0.21	0.14
	BC	26.9 ^a	25.7 ^b	27.9 ^a	27.2 ^a	0.21	0.001
	SEM	0.18	0.20	0.33	0.19		
	<i>P</i> -value	0.72	0.27	0.47	0.73		

Continued

Table 7 (Continued). Fatty acid (FA) concentration (g/100 g of FA) in ruminal fluid of cannulated dairy cows fed a diet with black currant (BC) seed residues (experiment 3)

Item	Treatment	Total	0 h	3 h	6 h	SEM	P-value
Sum of long-chain FA	Control	72.7	73.4	72.1	72.4	0.22	0.10
	BC	72.7 ^b	74.0 ^a	71.6 ^b	72.4 ^b	0.22	0.001
	SEM	0.19	0.21	0.35	0.18		
	P-value	0.92	0.20	0.54	0.96		
Sum of BCFA ²	Control	1.15 ^A	1.07	1.26 ^A	1.10 ^A	0.03	0.22
	BC	1.04 ^B	1.04	1.06 ^B	1.02 ^B	0.02	0.66
	SEM	0.03	0.04	0.05	0.04		
	P-value	0.02	0.42	0.04	0.07		
Sum of OBCFA ²	Control	2.45 ^A	2.46	2.50 ^A	2.39 ^A	0.03	0.54
	BC	2.27 ^B	2.33	2.24 ^B	2.24 ^B	0.02	0.45
	SEM	0.04	0.05	0.05	0.04		
	P-value	0.01	0.18	0.002	0.03		
PUFA/SFA ratio	Control	0.11 ^b	0.07 ^{A,c}	0.10 ^{bc}	0.16 ^{A,a}	0.008	0.001
	BC	0.11 ^a	0.06 ^{B,b}	0.13 ^a	0.12 ^{B,a}	0.006	0.001
	SEM	0.004	0.003	0.009	0.008		
	P-value	0.55	0.05	0.17	0.02		
n-6/n-3 FA ratio	Control	5.65 ^b	4.16 ^{A,c}	4.50 ^{A,c}	8.20 ^{A,a}	0.35	0.001
	BC	4.93 ^{ab}	3.50 ^{B,b}	5.21 ^{B,ab}	5.95 ^{B,a}	0.31	0.02
	SEM	0.24	0.12	0.17	0.54		
	P-value	0.14	0.003	0.03	0.05		

^{A,B}Means in a column within a variable with different uppercase superscripts differ ($P < 0.05$).

^{a-c}Means in a row with different lowercase superscripts differ ($P < 0.05$).

¹Other FA include C6, C10:1, C11:0, C12 *iso*, C12 *anteiso*, C13:0, C14 *anteiso*, C15 *anteiso*, C16 *anteiso*, C16 *iso*, C16:1 *trans*, C17 *iso*, C17 *anteiso*, C18:1 *trans*-5, C18:1 *trans*-6–8, C18:1 *trans*-9, C18:1 *trans*-15, C18:1 *cis*-11, C18:1 *cis*-12, C18:1 *cis*-13, C18:1 *cis*-14, C19:0, C18:1 *cis*-15, C18:2 *cis*-9, *cis*-12, C18:2 *cis*-9, *cis*-15, C20:0, C20:1 *trans*, C18:3n-6, C21:0, C20:2, C22:0, C20:3n-6, C22:1n-9, C20:3n-3, C23:0, C22:2, C24:0, and C24:1.

²BCFA = branched-chain fatty acids; OBCFA = odd and branched-chain fatty acids.

respectively). The lowest content of phytofactores was found in BC seed residue; however, BC was a source of anthocyanins (tannins 2.54 mg/100 g; flavonoids 18 mg/100 g; and anthocyanins 38 mg/100 g). Increased VFA concentration and unaltered rumen microbial populations and methane concentration at 24 and 48 h of incubation may suggest that the amount of berry seed residues used had no negative effects on fermentation in the rumen.

Phytofactores including tannins can reduce the number of protozoa and microorganisms actively influencing rumen fermentations (Makkar et al., 1995; Szumacher-Strabel and Cieslak, 2010; Henke et al., 2017). It has been shown that increasing concentrations of condensed tannins from quebracho extract decreased entodiniomorph (0.2 mg of tannins/mL of rumen fluid) and holotrich (0.05 and 0.1 mg of tannins/mL of rumen fluid) populations (Carulla et al., 2005). The in vitro (Carulla

Table 8. Effect of feeding black currant (BC) seed residues to dairy cows on the milk production, yield, and composition

Item	Treatment		SEM	P-value
	Control	BC		
Milk production, kg	30.3	29.7	0.55	0.31
Milk composition				
Fat, g/kg	41.6	41.1	0.38	0.92
CP, g/kg	35.1	34.4	0.18	0.38
Lactose, g/kg	51.6	50.4	0.22	0.37
TS, g/kg	128	126	1.2	0.81
SNF, g/kg	86.7	84.8	0.33	0.37
Energy, Mcal/kg	0.75	0.74	0.004	0.87
Methane, μ L/L	324	317	3.3	0.68
Urea, mg/L	297	294	3.3	0.86
Yield				
Fat, g/d	1,244	1,196	19.9	0.23
CP, g/d	1,063	1,018	18.4	0.20
Lactose, g/d	1,562	1,494	27.0	0.19
Energy, Mcal/d	22.7	21.8	0.36	0.42

Table 9. Effect of feeding black currant (BC) seed residues to dairy cows on fatty acid (FA) concentrations (g/100 g of FA) in milk (experiment 3)

Item	Treatment		SEM	P-value
	Control	BC		
SFA				
C8:0	0.99	0.91	0.020	0.16
C10:0	2.81	2.70	0.035	0.18
C12:0	3.69	3.55	0.037	0.10
C14:0	11.2	11.2	0.083	0.89
C14:1	1.20	1.24	0.021	0.45
C15:0	1.31	1.32	0.012	0.77
C15:1	0.41	0.45	0.006	0.005
C16:0	29.4	28.9	0.16	0.33
C16:1	1.78	1.64	0.033	0.10
C17:0	0.61	0.62	0.006	0.27
C17:1	0.29	0.29	0.004	1.0
C18:0	10.7	11.2	0.13	0.039
MUFA				
C18:1 <i>trans</i> -10	0.47	0.49	0.008	0.75
C18:1 <i>trans</i> -11	1.26	1.35	0.004	0.016
C18:1 <i>cis</i> -9	22.5	21.9	0.79	0.54
PUFA				
C18:2 <i>cis</i> -9, <i>cis</i> -12	2.21	2.28	0.579	0.39
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.71	0.78	0.001	0.002
C18:2 <i>trans</i> -10, <i>cis</i> -12	0.19	0.22	0.003	0.039
C18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.44	0.49	0.019	0.020
C20:4n-6	0.10	0.12	0.003	0.017
C20:5n-3	0.09	0.10	0.004	0.29
C22:6n-3	0.11	0.14	0.004	0.002
Other FA ¹	40.2	43.6	0.74	0.017
Total FA				
Sum of SFA	64.3	64.0	0.20	0.47
Sum of UFA	35.7	36.0	0.19	0.85
Sum of MUFA	31.5	31.2	0.16	0.22
Sum of PUFA	4.23	4.76	0.074	0.001
Sum of n-6 FA	2.91	3.16	0.092	0.004
Sum of n-3 FA	0.74	0.90	0.024	0.003
Sum of <i>trans</i> -C18:1	2.39	2.66	0.029	0.001
Sum of medium-chain FA	50.0	49.2	0.24	0.097
Sum of long-chain FA	44.9	45.9	0.26	0.060
Sum of BCFA ²	1.82	1.82	0.015	0.91
Sum of OBCFA ²	3.29	3.30	0.026	0.80
PUFA/SFA ratio	0.066	0.075	0.002	0.004
n-6/n-3 FA ratio	4.18	3.86	0.107	0.11

¹Other FA include C6, C10:1, C11:0, C12 *iso*, C12 *anteiso*, C13:0, C14 *anteiso*, C15 *anteiso*, C16 *anteiso*, C16 *iso*, C16:1 *trans*, C17 *iso*, C17 *anteiso*, C18:1 *trans*-5, C18:1 *trans*-6–8, C18:1 *trans*-9, C18:1 *trans*-15, C18:1 *cis*-11, C18:1 *cis*-12, C18:1 *cis*-13, C18:1 *cis*-14, C19:0, C18:1 *cis*-15, C18:2 *cis*-9,*cis*-12, C18:2 *cis*-9,*cis*-15, C20:0, C20:1 *trans*, C18:3n-6, C21:0, C22:0, C20:3n-6, C22:1n-9, C20:3n-3, C23:0, C22:2, C24:0, and C24:1.

²BCFA = branched-chain fatty acids; OBCFA = odd and branched-chain fatty acids.

et al., 2005) and in vivo (Benchaar and Chouinard, 2009; Szczechowiak et al., 2016) studies suggested that holotrichs may be more sensitive to condensed tannins than entodiniomorphs. Among the berry seed residues, only SB reduced the holotrich populations after 8 h of fermentation. This may suggest that not only tannins, but also flavonoids can reduce the number or activity of this group of microorganisms. The 3 different bioactive compounds aforementioned showed combined effects in the rumen environment; however, the mode of action for individual compounds could not be clarified from this study. Most of the effects either on rumen

microorganisms or VFA were observed only in the in vitro scale. In vivo, the amount of berry seed residues fed (2 kg/d) to animals seemed to have a low effect on ruminal fermentation parameters. In the in vivo study (experiment 2 and 3), the BC diet significantly decreased proportion of OBCFA and BCFA in the rumen. Few rumen bacterial membranes are rich sources of BCFA and OBCFA (Vlaeminck et al., 2006; Alves et al., 2017), which are recommended to reflect changes in the ruminal microbial populations (Vazirigohar et al., 2018). However, none of the treatments affected the number of bacteria at either the in vitro or in vivo level.

Table 10. Effects of black currant (BC) seed residues on desaturation (DI), thrombogenic, and atherogenic indices of milk of dairy cows

Item	Treatment		SEM	P-value
	Control	BC		
DI ¹	0.32	0.32	0.002	0.46
DI C14:1 ¹	0.096	0.099	0.002	0.36
DI C16:1 ¹	0.057	0.053	0.001	0.22
DI C18:1 ¹	0.68	0.66	0.003	0.006
DI RA/(VA+RA) ¹	0.37	0.37	0.003	0.85
Thrombogenic index ²	2.48	2.40	0.024	0.22
Atherogenic index ³	2.21	2.18	0.022	0.61

¹According to Brogna et al. (2011). VA = vaccenic acid; RA = rumenic acid.

²According to Ulbricht and Southgate (1991).

³According to Chilliard et al. (2003).

Decreased concentrations of BCFA and OBCFA in the rumen were also not reflected in reducing DM or CP digestibility. Moreover, the lack of important changes in the rumen fermentation processes was supported by similar milk yield between the groups. Ammonia concentration decreased only at 24 h of incubation when BC diet was fed (experiment 1); however, no differences were observed at 48 h and the ammonia concentrations were within the physiological range.

The berry seed residues used in this study, especially BC, due to the lack of negative effects on ruminal fermentation processes both in vitro and in vivo, can be considered as feed components for ruminants. Related to the in vivo productive performance, no changes were noticed in milk production and composition. However, significant changes were tracked in the ruminal and milk FA profile. Several studies have highlighted different effective supplements (e.g., marine lipids and secondary plant metabolites) that can increase the accumulation of VA through inhibition of the last steps of rumen biohydrogenation (Szczechowiak et al., 2016). The changes in FA profile can be directly related to changes in microbial population involved in biohydrogenation process, which was, in the present study, partly supported by changes in BCFA and OBCFA proportions in the ruminal fluid. In the present study, increased proportion of VA (+62%) in the ruminal fluid could partly explain the increased proportion of VA (+7%) in milk. This process is of special importance because Δ^9 desaturase catalyzes the oxidation of VA in the mammary gland, resulting in production of 70 to 95% of C18:2 *cis-9,trans-11* present in the milk (Ebrahimi et al., 2017). In the present study, C18:2 *cis-9,trans-11* significantly increased in milk (+10%, compared with the control group); however, it was not directly related to the proportion of C18:2 *cis-9,trans-11* in the rumen fluid (+133%, compared with the control group). Our study showed that higher proportions of VA and CLA in the rumen were not directly linked to proportions of

these FA in milk. One possible reason for this is that FA proportions in the rumen fluid do not fully reflect those in the duodenum, which is the main absorption site for FA (Szczechowiak et al., 2016). The milk FA proportions also depend on the activity of mammary gland desaturases, especially Δ^9 desaturase, and genes participating in milk fat synthesis and secretion (Brzozowska and Oprządek, 2016; Bernard et al., 2018; Cappucci et al., 2018).

Polyunsaturated FA, including C18:3n-3; C18:3n-6; C20:4n-6, and C22:6n-3, PUFA n-6, and PUFA n-3, significantly increased in milk of cows fed BC-containing diet (+11, +16, +20, +27, +7, and +22%, respectively). Long-chain FA synthesis in the mammary gland indicated a considerable dependence on substrate availability [e.g., C18:0, C18:1 *trans-11*, C18:3 *cis-9,cis-12,cis-15* (Urrutia et al., 2015)], which suggested that in the present study, the BC supplement increased *in-ter alia* availability of the above FA in the mammary gland. This observation agrees with the results of the experiment by Szczechowiak et al. (2018), in which condensed tannins and fish-soybean oil blend mixture directly modulated the blood n-3 PUFA and finally more n-3 PUFA reached the mammary gland and were secreted in milk. Additionally, it should be also considered that downregulation of the genes involved in long-chain PUFA synthesis (e.g., *FADS1*, *FADS2*, *ELOVL2*, and *ELOV5*) by n-3 PUFA in long-term transcriptional regulation could be one of the mechanisms regulating this type of synthesis affecting final FA profile in milk and avoiding excessive levels of these FA (Urrutia et al., 2015).

In the present study, C20:4n-6 and C22:6n-3 significantly increased. The higher proportion of C20:4n-6 and C22:6n-3 in the milk likely resulted from the higher availability of their precursors, C18:2n-6 and C18:3n-6 (Bionaz and Looor, 2008; Cappucci et al., 2018) or from the increase in accessibility of precursors for FA elongation in the liver (Zachut et al.,

2010; Szczechowiak et al., 2018). Therefore, enhancing FA content in milk requires an understanding of the interrelationship among dietary lipid supply, rumen fermentation, and metabolic changes occurring in liver, blood, and finally in mammary gland (Brzozowska and Oprządek, 2016; Varadyova et al., 2018; Szczechowiak et al., 2018). Additionally, the cost associated with the BC residue supplementation was calculated considering the cost of the individual ingredients of the control and BC diets. The cost of daily BC supplemented diet was ~18% less than the cost of feeding cows the control diet, mainly due to the fact of replacement of 2 kg/d per cow concentrate by the same amount of BC residue.

CONCLUSIONS

The amount of BC seed residues used in this study did not affect in vivo ruminal fermentation parameters, methane production or milk production, and basic composition. However, the milk FA profile including increased C18:2 *cis*-9,*trans*-11 (CLA), arachidonic acid and docosahexaenoic acid, total PUFA, and n-6 FA and n-3 FA concentration, and PUFA to SFA ratio was better for the BC-supplemented group compared with control. Berry seed residues as an example of waste by-products can be successfully used as dietary components partly replacing more expensive concentrate feeds in dairy cow diets. Incorporation of berry seed residues in diets would be beneficial economically and nutritionally.

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