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Effect of Feeding Dried Apple Pomace on Ruminal Fermentation, Methane Emission, and Biohydrogenation of Unsaturated Fatty Acids in Dairy Cows

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Citation: Gadulrab, K.; Sidoruk, P.; Kozłowska, M.; Szumacher-Strabel, M.; Lechniak, D.; Kołodziejski, P.; Pytlewski, J.; Strzałkowska, N.; Horbańczuk, J.O.; Jóźwik, A.; et al. Effect of Feeding Dried Apple Pomace on Ruminal Fermentation, Methane Emission, and Biohydrogenation of Unsaturated Fatty Acids in Dairy Cows. *Agriculture* **2023**, *13*, 2032. https://doi.org/10.3390/agriculture13102032

Academic Editor: Secundino López

Received: 21 September 2023
Revised: 11 October 2023
Accepted: 19 October 2023
Published: 21 October 2023

Abstract: Industrial fruit by-products are now being utilized as animal feeds for several reasons. They may substitute the conventional cereal feeds, and also offer economic and environmental benefits. One of the most important industrial fruit by-products is apple pomace, which can be used as a source of energy in the ration of ruminant species, including dairy cattle. The aim of the present study was to evaluate the effect of feeding dried apple pomace to dairy cattle on ruminal fermentation, fatty acid concentration, microbial populations, and methane production. The experiment lasted 64 days and was conducted with 4 cannulated commercial dairy cows. The control animals received a standard diet, while the experimental animals was fed a standard diet supplemented with 150 g/kg DM dried apple pomace. Ruminal fluid samples were collected at three different time intervals. The samples were obtained at 0-, 3-, and 6-h post-feeding. The ruminal fluid was used to assess the ammonia concentration, pH, volatile fatty acids (VFA), long-chain fatty acids (FA), microbial population. A number of ruminal fermentation variables changed as a result of the addition of dried apple pomace to the standard diet. Ruminal pH slightly increased ($p < 0.01$) while the ammonia concentration decreased ($p < 0.01$) by 46%. There was a significant decrease in total protozoa count ($p < 0.01$) and an increase ($p < 0.01$) in total volatile fatty acids. In addition, there was a decline in methane emission ($p = 0.05$) by 8% due to dried apple pomace feeding. To sum up, this study demonstrated a positive effect of 150 g/kg DM dietary dried apple pomace on ruminal metabolism including a decrease in ammonia concentration and methane emissions, alongside with an increase in total ruminal VFAs, higher nutrient digestibility, and milk production. Also, beneficial changes to the ruminal fatty acid profile resulting from

reduced biohydrogenation were observed although a decreased content of the C18:2 *cis* 9 *trans* 11 isomer was also noticed. The dietary inclusion of DAP can serve as a valuable, sustainable, and environmentally friendly dietary component for dairy cows.

Keywords: apple pomace; dairy cows; methane emission; ruminal fermentation; ruminal biohydrogenation; polyphenols

1. Introduction

With the expected increase in human population to over 9 billion by 2050, there is a growing concern about food production and security [1]. To promote sustainability in animal feed production, the use of agro-food by-products including grape pomace, tomato pomace, citrus pulp, apple pomace, guava pomace, and date pressed cake as animal feed components should be considered [2,3]. These industrial fruit by-products can substitute the conventional cereal feeds, but also can offer some economic and environmental benefits such as lower costs of animal feeding, reduction of environmental footprint of food products, and an improvement in the quality and sustainability of animal products [4–6]. One possible byproduct that can be used as a feed component is dried apple pomace (DAP). The research on apple pomace has already been conducted due to its nutritional value and the abundance of apples in Europe especially in Poland, the world's second-largest apple producer [7,8]. Apple pomace consists of flesh and peels (95%), seeds (3%), and stems. Generally, DAP or ensiled apple pomace (EAP) are shown as a sustainable and affordable substitute for energy-giving feeds with crude protein content ranging from 19 to 65 g/kg, metabolizable energy between 7.7 to 9.1 MJ/kg, neutral detergent fiber (NDF) content ranging from 300 to 482 g/kg, and acid detergent fiber content ranging from 250 to 420 g/kg on dry matter (DM) basis [9,10]. Its high moisture and fermentable sugar content make fresh apple pomace susceptible to rapid spoilage. Therefore, it needs to be ensilaged or dehydrated for longer preservation [11]. Ensiled apple pomace can be utilized for feeding ruminants as an energy feed replacing conventional cereals including maize in the diets [11]. Cereals and other high energy-giving feed deteriorates rumen health as they lower the pH whereas the effect of EAP on pH is slighter [11]. Moreover, the use of EAP in ruminant's diet changes the ruminal volatile fatty acid (VFA) proportion (increases acetate to propionate ratio by changing cellulolytic microflora and proteolytic activity) as well as reduces branched-chain VFA [12]. The inclusion of EAP in the diet for lactating dairy cows in the early stage of lactation effectively improves dry matter intake resulting in an increase in milk production. However, higher proportion (<10% of DM) of DAP in the diet of dairy cows reduces milk production and feed intake due to the high concentration of fermentable carbohydrates and low content of proteins [13]. Therefore, using the proper amount of DAP as a byproduct in a ruminant's diet is highly required. Previously published reports showed that apple pomace can be included in the diets of lactating dairy cows up to 13.5 kg/day fresh or ensiled, or 3 kg dehydrated, which represented approximately 15% of dry matter intake DMI [14]. The apple pomace contains also a variety of bioactive compounds primarily found in peels, especially polyphenols, flavonoids, flavones, and phenolic acids [15]. Its polyphenol content ranges from 2.62 to 8.56 g/kg DM [16]. Implementing polyphenols into the ruminant's diets may affect ruminal bacteria involved in fermentation, and methanogenesis and biohydrogenation (BH) processes. The reduction of ruminal BH by polyphenols causes an increase in LNA/n-3 PUFA content in ruminal fluid and finally improves milk and meat quality [17,18]. Moreover, previous in vivo studies using polyphenols (3.10 or 8.44 g/kg DM of powdered paulownia leaves or paulownia leaves extract, respectively) reduced methane production without affecting feed digestibility and volatile fatty acid concentrations in ruminal fluid and increased level of n-3 FA proportion reducing ruminal BH [19,20]. Also, the same source of polyphenols incorporated in the diets of high milk-producing cows demonstrated a beneficial effect on

ruminal fermentation processes and fatty acid profile of milk without a negative effect on milk production performance and simultaneously lowered methane emission [17]. Methane is one of the greenhouse gases (GHGs) that contribute to the current climate change crisis. High milk-producing cows produce 30% of the methane emitted from the livestock sector [21]. Methane is produced in the rumen when the carbon dioxide is reduced by methanogenic Archaea. Methane emission does not only affect the environment but also reduces feed efficiency as it is also linked with dietary energy loss [22]. Animal scientists have attempted to formulate diets with alternative feed components rich in plant bioactive compounds (e.g., polyphenols) in order to modulate the ruminal microbial populations' [23], to improve ruminal fermentation, reduce biohydrogenation processes of FA, mitigate methane emission as well as increase dairy cows' productivity. The hypothesis of this study is that, due to the high content of bioactive ingredients, apple pomace could modulate the population of microorganisms in the rumen, thus limiting methane emission and the biohydrogenation process. The objective of this experiment was to study the addition of apple pomace to TMR ration on in vivo ruminal fermentation, ruminal fatty acid composition, microbial population, and methane emission in dairy cows.

2. Materials and Methods

2.1. Experimental Animals, Design, and Sampling

The experiment was carried out at a commercial dairy cows farm. This experiment was conducted using 4 rumen-cannulated Polish Holstein–Friesian dairy cows in the same physiological state and condition in a replicated 2 × 2 crossover design. The cow's body weights were 630 kg ± 30 kg and the animals were 4–5 months in lactation. Cows were set apart from the general herd and housed in separate boxes. The cows were cared for in accordance with the guidelines of the Local Ethical Commission for Investigations on Animals (permit no. 27/2009) and the National Ethical Commission for Animal Research (Ministry of Science and Higher Education, Poland).

The cannulated cows were fed two dietary treatments, the control TMR (TMR CON) and the experimental diet (TMR DAP). The TMR CON consisted of a standard TMR and the experimental cows were fed a standard TMR with 150 g DAP/kg DM (Table 1).

Table 1. Ingredient components and chemical composition of control diet (TMR CON), experimental diet (TMR DAP), and dried apple pomace (DAP) used in in vivo experiments.

Items	TMR CON	TMR DAP	DAP
Particular components, g/kg DM			
Corn silage	388	329	-
Alfalfa silage	82	70	-
Grass silage	91	76	-
Beet pulp	103	88	-
Brewer's grain	95	81	-
Concentrate [1]	39	33	-
Farmer's grain with Maxammon [2]	81	69	-
Rapeseed meal	108	92	-
Mineral and vitamin premix [3]	14	12	-
Dried apple pomace	-	150	-
Forage to concentrate ratio	76:24	64:36	-
Chemical composition [4], g/kg DM			
DM, g/kg as fed	425	487	993
OM	895	907	985
aNDF	342	354	437
CP	164	158	116
EE	29.4	31.9	51

Phenolic acids, g GEA/kg DM [5]	6.67	59.2	14.4
VEM [6]	952	971	1100

[1] Concentrate contains (as g/kg of DM) OM (915), aNDFom (238), CP (180), and EE (34). [2] Contained a mixture of ground triticale and barley grains (60:40) treated with urea and urease additive, containing 178 g CP/kg of DM. [3] Mineral and vitamin premix contains (g/kg) Na (123), Ca (100), Mg (45), P (42), K (20), S (18), Co (14), Cu (5.0), Zn (2.8), Mn (1.4), Fe (1.05), F (0.42), I (0.028), Se (0.018), biotin (0.008); (IU/kg), vitamin A (200,000), vitamin D3 (40,000), and vitamin E (1200). [4] DM: dry matter; OM: organic matter; aNDF: neutral detergent fiber analyzed with α-amylase; CP: crude protein; EE: ether extract. [5] The content of phenolic acids (GEA—gallic acid equivalent) has been calculated based on a previous study [8]. [6] VEM = feed unit net energy lactation; calculated using the FeedExpert software (1.2.9, Smart Feed Ltd., Pilawy, Poland).

The four cows were used in a replicated 2 × 2 crossover design with 2 periods and 2 dietary groups (TMR CON and TMR DAP). The experiment lasted 64 days. Each period lasted 32 days including 21 days of adaptation and 11 days of sample collection. Each cow spent 3 days in the respiratory chamber to measure methane production, 5 days for ruminal fluid collection, and another 3 days for the feces samples collection. During the adaptation period of the experiment, the rumen microbial population has adapted and stabilized to the changes caused by the modification of used diets (TMR CON or TMR DAP). The cows were fed twice a day, at 6 a.m. and at 6 p.m. The cannulated cows were housed in rubber floor mat tie stalls which supported individual feeding and the cows had ad libitum access to water and salt blocks. Daily feed offered, orts remaining, and feces amount from individual cows during the sampling period (3 days) in tie stalls were recorded to determine the total tract nutrient digestibility. The subsamples (about 5% wt/wt) of feed and feces were preserved at −20 °C for analysis of DM, organic matter (OM), neutral detergent fiber (NDF), and crude protein (CP). The total tract nutrient digestibility was calculated using the formula [(nutrient intake − nutrient in feces)/nutrient intake] × 100. Milk production was recorded daily in the tie stalls using a milk meter (WB Ezi-Test Meter 33 kg; Tru-Test Limited, Manukau, New Zealand).

Approximately 400 g of ruminal content was collected from each cannulated cow, from the dorsal, midpoint, and ventral regions of the midventral sac of the rumen. Samples were taken at 3 different periods of feeding, at 0, 3, and 6 h after morning feeding [24]. The ruminal content was filtered through a two-layer cheesecloth and stored at −20 °C for further analysis of pH value, ammonia, volatile fatty acid (VFA), and fatty acid (FA) concentrations. For protozoa counting, 1 mL of ruminal fluid was mixed with 7 mL of 4% formaldehyde solution in a 10 mL polypropylene tube with a screw cup. For microbial analysis, ruminal fluid collected 3 h after morning feeding was used [25]. For the quantification and analysis of microbes, about 300 g of the ruminal fluid was filtered with two layered cheesecloths into a 100 mL polypropylene box and mixed. The mixture was then transferred into two 4.5 mL cryotubes and a 2 mL Eppendorf tube and immediately frozen in liquid nitrogen. The samples were stored at −80 °C until microbial analysis.

2.2. Sample Analysis

The chemical composition of the diet, feces and orts was determined using AOAC methods [26]. Method no. 934.01 was used to analyze dry matter. Crude ash was analyzed using method no. 942.05, while crude protein using Kjel-Foss Automatic analyzer (Foss Electric, Hillerod, Denmark), method no. 976.05. The ether extract was analyzed according to method no. 973.18 (Soxhlet System HT analyzer; Foss Electric, Hillerod, Denmark). Neutral detergent fiber abbreviated as aNDF was analyzed using Fibertech 1020 Analyzer (Foss, Analytical AB, Hoganas, Sweden) with addition of amylase and sodium sulfite and expressed without ash [27]. Organic matter was calculated by subtracting ash from dry matter.

As soon as the ruminal fluid was collected, pH meter (Elmetron, Type CP-104, Zabrze, Poland) was used to determine pH. Concentration of ammonia was measured using the colorimetric Nessler method and gas chromatography (GC Varian CP 3380, Sugarland,

TX, USA) was used to determine VFA concentration [25]. A light microscope (Primo Star 5, Zeiss, Jena, Germany) was used to quantify protozoa. Counting was done under different volumes for the different groups of protozoa (10 µL for *Ophryoscolecidae* and 100 µL for *Isotrichidae*). Fatty acids of feed and ruminal fluid were analyzed using a gas chromatograph (456-GC, Bruker, Fulton, USA) fitted with a fused-silica capillary column (100 m × 0.25 mm; overlaid with 0.25 µm; Agilent HP; Chrompack CP7420; Agilent Technologies, Santa Clara, CA, USA) and a flame ionization detector [25]. Methane production was measured using two open-circuit respiration chambers (SPA System, Ltd., Wroclaw, Poland) with dimensions of 300 cm × 400 cm × 220 cm in width, length, and height, respectively, were used [17]. The methane concentration was analyzed using two NDIR analyzers operating in the near-infrared spectrum (SERVOMEX 4100, SERVOMEX Ltd., UK, detector 1210 Gfx; [17]). Daily milk production was recorded using a milk meter (WB Ezi-Test Meter 33 kg; True-Test, Manukau, New Zealand).

Relative changes in abundances of selected ruminal bacterial species (*Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Butyrivibrio proteoclasticus*, *Butyrivibrio fibrisolvens*, *Streptococcus bovis*, and *Megasphaera elsdenii*) and two genera (*Prevotella* spp. and *Lactobacillus* spp.) were also determined by quantitative real-time PCR (qPCR) based on the protocol described previously [25]. Species- or genera-specific primers are presented in Table 2.

Table 2. The specific sequences of primers to the analyzed bacteria species.

Species	Primer Sequences (5′ to 3′)	Reference
Ruminococcus flavefaciens	F: CGAACGGAGATAATTTGAGTTTACTTAGG R: CGGTCTCTGTATGTTATGAGGTATTACC	[28]
Fibrobacter succinogenes	F: GTTCGGAATTACTGGGCGTAAA R: CGCCTGCCCCTGAACTATC	[29]
Streptococcus bovis	F: TTCCTAGAGATAGGAAGTTTCTTCGG R: ATGATGGCAACTAACAATAGGGGT	[30]
Butyrivibrio proteoclasticus	F: TCCTAGTGTAGCGGTGAAATG R: TTAGCGACGGCACTGAATGCCTA	[31]
Ruminococcus albus	F: CCCTAAAAGCAGTCTTAGTTCG R: CCTCCTTGCGGTTAGAACA	[32]
Butyrivibrio fibrisolvens	F: ACACACCGCCCGTCACA R: TCCTTACGGTTGGGTCACAGA	[33]
Megasphaera elsdenii	F: AGATGGGGACAACAGCTGGA R: CGAAAGCTCCGAAGAGCCT	[30]
Prevotella spp.	F: GAAGGTCCCCCACATTG R: CAATCGGAGTTCTTCGTG	[30]
Lactobacillus spp.	F: TATGGTAATTGTGTGNCAGCMGCCGCGGTAA R: AGTCAGTCAGCCGGATACHVGGGTWTCTAAT	[34]

The relative 16S rRNA gene copy abundances of specific bacterial species and genera were expressed as gene copy abundances of a target bacterium with respect to the total bacteria and then assigned to an arbitrary unit relative to the control values. The $2^{-\Delta\Delta Ct}$ formula was used to calculate the relative level of 16 S rRNA copies of each bacteria in relation to all bacterial species according to our previous studies [35]. The population sizes of total bacteria, total methanogens, *Methanobacteriales*, and *Methanomicrobiales* were quantified by fluorescence in situ hybridization, according to [25]. The following oligonucleotide probes were used: S-D-Arch-0915-a-A-20 (total methanogens), S-O-Mmic-1200-a-A-21 (*Methanobacteriales*), and S-F-Mbac-0310-a-A-22 (*Methanobacteriales*). The 4′,6-diamidino-2-phenylindole (DAPI) from anti-fading agent (VectaShield H- 1000, Vector laboratories) to distinguish the population sizes of total bacteria from methanogens was used.

2.3. Statistic Description

A repeated measure analysis using PROC MIXED procedure was performed in SAS with the main effect of the group for dry matter intake, lactation performance, nutrient digestibility, microorganisms' characteristics, CH_4, and CO_2 emissions. The period effect and its interaction with the group were examined as fixed effects while the animals were considered as a random effect. The collection time was set in the repeated statement. In this part of statistical analysis, period and period x group effects were not significant; therefore, they were excluded from the tables. Data of fatty acid profile were analyzed using repeated measures of PROC MIXED in SAS (v9.4, SAS Institute Inc., Cary, NC, USA) using the following model:

$$Yijklm = \mu + \text{Si} + \text{Tj} + (\text{Si} \times \text{Tj}) + Dk + \text{P}m + \text{A}l(\text{P}m) + \quad + eijklm$$

where *Yijklm* is the response variable due to animal *l*, treatment *i*, time collection *j*, day *k*, and period *m*; μ is the overall mean; Si is the fixed effect due to treatment *i*; Tj is the fixed effect due to time collection *j* (0, 3, 6 h); *Dk* is the fixed effect due to day collection (*k* = 1 to 5); P*m* is the fixed effect due to period *m* (*m* = 1, 2); Si × Tj is the fixed interaction effect of treatment and collection time; A*l*(P*m*) is the random effect of animal *l* nested within period *m*; and *eijklm* is the residual error of the terms. The effects were considered significant at *p* values < 0.05.

3. Results

Dry matter intake (DMI), milk yield, and nutrient digestibility are shown in Table 3. Inclusion of DAP in the diet resulted in significant increases of DMI ($p = 0.01$) and milk yield ($p = 0.04$). Also, significant increases in digestibility of DM ($p = 0.02$), OM ($p = 0.01$), aNDF ($p = 0.06$), CP ($p = 0.01$), and EE ($p = 0.02$) were observed in the experimental diet.

Table 3. The effect of dried apple pomace-containing total mixed ration (TMR) on lactation performance and nutrient digestibility.

Items [1]	Treatments [2]		SEM [3]	*p*-Value [4]
	TMR CON	TMR DAP		
DMI [4], kg/d	23.9	24.4	0.09	0.01
Milk yield, kg/d	30.6	31.9	0.34	0.04
Total-tract digestibility [5], g/kg DM				
DM	645	667	5.14	0.02
OM	673	712	7.71	0.01
NDF	512	551	10.5	0.06
CP	564	651	12.5	0.01
EE	645	667	5.14	0.02

[1] DMI: Dry matter intake.[2] CON: control; DAP: addition of dried apple pomace. [3] SEM: standard error of the means. [4] At a *p*-Value of ≤ 0.05 the results are significantly different. [5] DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber.

The results of changes in fermentation parameters in ruminal fluid and changes in the microorganism's populations are shown in Table 4. A significant increase of pH ($p < 0.01$) and a decline of ammonia concentration ($p < 0.01$) were noted in the TMR DAP diet. The concentrations of acetic ($p = 0.06$), propionic ($p < 0.01$), and butyric acids ($p < 0.01$) increased when DAP was used as a dietary component. Molar proportion of acetate decreased ($p < 0.01$) while the molar proportion of propionate increased ($p < 0.01$) by DAP supplementation. Also, the molar proportions of isobutyric and isovaleric acids differed significantly between the groups. An increase in isobutyric acid ($p < 0.05$) was found in the experimental group. Isovaleric acid significantly decreased ($p < 0.01$) due to DAP feeding. Total VFA concentration increased ($p < 0.01$) when animals were fed the experimental diet

compared to the control group. Additionally, decreased population sizes of total methanogen as well as *Methanobacteriales* and *Methanomicrobiales* ($p < 0.001$) translated to lowered methane production by 8% in the TMR DAP group ($p = 0.05$). A significant decrease ($p < 0.01$) in *Ophryoscolecidae* and total protozoa population was noted in the TMR DAP group.

The in vivo ruminal bacteria population results are presented in Table 5 as an arbitrary unit relative to the total bacterial gene copy abundance. The addition of 150 g DAP/kg DM caused an increase in *Streptococcus bovis* ($p \leq 0.001$), *Megasphaera elsdenii* ($p \leq 0.001$), and *Ruminococcus albus* abundances ($p \leq 0.01$). The abundances of *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, *Prevotella* spp., *Butyrivibrio proteoclasticus*, *Butyrivibrio fibrisolvens*, and *Lactobacillus* spp. were not altered by TMR + DAP feeding to dairy cows.

Table 4. The effect of dried apple pomace-containing total mixed ration (TMR) on the ruminal fermentation characteristics, protozoa and methanogens counts, and CH_4 and CO_2 emission.

Composition	Treatments [1]		SEM [2]	*p*-Value [3]
	TMR CON	TMR DAP		
pH	6.01	6.18	0.02	0.001
NH_3-N, mM	13.0	6.97	0.44	0.001
Total VFA [4], mM	91.7	103	1.59	0.001
VFA [4], mM				
Acetic (A)	57.8	60.4	0.68	0.060
Propionic (P)	17.5	24.4	0.85	0.001
Isobutyric	0.58	0.84	0.06	0.040
Butyric	11.9	14.1	0.271	0.001
Isovaleric	2.04	1.13	0.136	0.001
Valeric	1.85	2.13	0.121	0.260
VFA [4], %				
Acetic (A)	63.1	58.7	0.482	0.001
Propionic (P)	19.1	23.6	0.526	0.001
Isobutyric	0.64	0.84	0.067	0.130
Butyric	13.0	13.7	0.211	0.070
Isovaleric	2.23	1.10	0.155	0.001
Valeric	2.01	2.04	0.106	0.870
A/P	3.30	2.50	0.090	0.001
Total bacteria, $\times 10^9$/mL	8.36	10.5	0.157	<0.001
Archaea, $\times 10^7$/mL	5.19	4.20	0.116	<0.001
Methanobacteriales, $\times 10^7$/mL	1.84	0.58	0.061	<0.001
Methanomicrobiales, $\times 10^7$/mL	2.17	1.79	0.061	<0.001
Total protozoa, $\times 10^3$/mL	636	289	56.9	0.01
Isotrichidae, $\times 10^3$/mL	6.34	3.24	0.976	0.115
Ophryoscolecidae, $\times 10^3$/mL	631	287	56.24	0.01
CH_4 g/d	451	417	8.62	0.05
CH_4, g/kg DMI	18.7	16.1	0.49	0.01
CO_2, g/d	12.127	11.426	308	0.26
CO_2, g/kg DMI	502	472	17.5	0.42

[1] CON: control; DAP: addition of dried apple pomace. [2] SEM: standard error of the mean. [3] At a *p*-Value of ≤ 0.05 the results are significantly different. [4] Volatile fatty acids.

Table 5. The effect of dried apple pomace-containing total mixed ration (TMR) on ruminal bacteria populations.

Parameters	Treatments [1]		SEM [2]	*p*-Value [3]
	TMR CON	TMR DAP		
Ruminococcus flavefaciens *	0.41	0.16	0.08	0.12
Ruminococcus albus *	4.16	11.05	1.34	0.01
Fibrobacter succinogenes *	2.34	3.00	0.53	0.54
Butyrivibrio fibrisolvens *	3.54	1.55	0.65	0.13
Butyrivibrio proteoclasticus *	2.47	2.59	0.42	0.89
Streptococcus bovis *	0.20	0.70	0.08	<0.001
Megasphaera elsdenii *	0.04	0.17	0.02	<0.001
Prevotella spp. *	6.51	6.82	0.70	0.83
Lactobacillus spp. *	0.10	0.40	0.14	0.32

[1] CON: control; DAP: addition of dried apple pomace. [2] SEM: standard error of the mean. [3] At a *p*-Value of ≤ 0.05 the results are significantly different. * Expressed as an arbitrary unit relative to the total bacterial gene copy abundance.

The level of C16:0, C18:0, and C18:2 *cis*-9, *trans*-11 decreased ($p = 0.01$), whereas the level of C12:0, C14:0, C15:0, and C16:1 increased ($p \leq 0.01$) in the ruminal fluid (Table 6). The proportion of C18:1 *trans*-10, C18:1 *cis*-9, C18:2 *cis*-9, *cis*-12, and C18:3 n-3 increased in time-dependent ($p \leq 0.05$) manners in TMR DAP group. Concentrations of total unsaturated fatty acids (UFA), polyunsaturated fatty acids (PUFA), and monounsaturated fatty acids (MUFA) increased ($p \leq 0.01$) in the TMR DAP group, which resulted in a lower concentration of total SFA ($p < 0.01$). Also, in the TMR DAP group lower n6/n3 ratio, and PUFA/SFA ratio were observed ($p = 0.01$).

Table 6. The effect of dried apple pomace-containing total mixed ration (TMR) on fatty acid profile in ruminal fluid (g/100 g of fatty acids).

FA Profile (g/100 g FA) [1]	Treatments [2]		SEM [3]	*p*-Value [4]		
	TMR CON	TMR DAP		Group	Time	Group × Time
C12:0	0.30	0.46	0.010	<0.001	<0.001	0.241
C14:0	0.88	1.49	0.037	<0.001	0.129	0.328
C14:1	0.51	0.52	0.007	0.660	<0.001	0.018
C15:0	1.28	1.32	0.009	<0.001	<0.001	0.033
C15:1	0.47	0.49	0.010	0.032	0.049	0.015
C16:0	21.46	20.75	0.132	<0.001	<0.001	0.965
C16:1	0.23	0.27	0.005	<0.001	<0.001	0.061
C17:0	0.59	0.59	0.004	0.545	0.029	0.048
C17:1	0.04	0.04	0.002	0.429	<0.001	0.506
C18:0	53.80	51.53	0.316	<0.001	<0.001	0.001
C18:1 t10	1.12	1.59	0.043	<0.001	<0.001	0.764
C18:1 t11	3.00	2.89	0.056	0.060	0.001	0.002
C18:1 *cis*9	8.11	8.84	0.099	<0.001	<0.001	0.033
C18:2 c9c12	6.29	7.19	0.207	<0.001	<0.001	<0.001
C18:3 c9c12c15	0.94	1.17	0.041	<0.001	<0.001	0.122
C18:2 c9t11	0.62	0.50	0.042	0.004	<0.001	0.009
C18:2 t10c12	0.17	0.18	0.006	0.088	<0.001	0.082
C18:3 n6	0.20	0.19	0.006	0.693	0.035	0.008
SFA	78.31	76.13	0.296	<0.001	<0.001	<0.001
UFA	21.69	23.87	0.296	<0.001	<0.001	<0.001

MUFA	13.46	14.64	0.116	<0.001	<0.001	0.052
PUFA	8.21	9.23	0.216	<0.001	<0.001	<0.001
n-6	12.77	14.58	0.413	<0.001	<0.001	<0.001
n-3	0.94	1.17	0.041	<0.001	<0.001	0.121
n6/n3	14.01	12.75	0.494	0.011	0.021	0.054
PUFA/SFA	0.11	0.12	0.003	<0.001	<0.001	<0.001

[1] SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. [2] CON: control; DAP: addition of dried apple pomace. [3] SEM: pooled standard error of the means. [4] *p*-value of treatment groups, time (0, 3, 6 h), and interaction effect of groups and time.

4. Discussion

The present experiment was conducted to assess dried apple pomace (DAP) as a component of TMR fed to dairy cows on performance, nutrient digestion, ruminal fermentation characteristics, including the process of methanogenesis, microbial population, and unsaturated fatty acids biohydrogenation. The obtained results confirmed the positive effect of 150 g DAP/kg of DM in TMR on ruminal fermentation parameters like pH, total VFA as well as particular VFA. The increase in ruminal pH of animals fed the diet with apple pomace was within the physiological range of 6.1 to 6.6 [27] as apple pomace is a rich source of fiber [9,36]. The use of feed with high fiber content (i.e., pectin) has a tendency to increase ruminal pH as it stimulates the production of saliva which contains bicarbonate that increases ruminal pH [37]. Polyphenolic compounds also have a tendency to increase pH value in the rumen ecosystem [23]. Moreover, in Orzuna-Orzuna et al. [38] study, feeding a diet rich in flavonoids increased the pH value. The pH and ammonia results were similar to those obtained when Paulownia leaves, also rich in polyphenols, were used in the diets of dairy cows [17]. This phenomenon is explained by the potential of plant bioactive compounds, i.e., polyphenols, to modulate ruminal microbial populations. An increase in lactate-consuming bacteria (e.g., *M. elsdenii*) and propionate-producing bacteria might lower A/P ratio, thus preventing an unfavorable pH decrease [39,40]. Similar to the study of Huang et al. [17] an increase in *M. elsdenii* population in the current study was noted. Also, *R. albus* and *S. bovis* populations increased in the DAP groups. No statistically significant difference in the abundance of the other major fibrolytic bacteria was observed. The increased population of some fibrolytic bacterial populations including *R. albus* ($p = 0.01$), is attributed to the increase in DM digestibility and VFA concentration [41] which was also observed in the presented study. The higher VFA concentration was not only due to the higher fermentable energy content in the used ration but was also linked to improved DMI, DM, and OM digestibility, which has positively impacted the overall digestive efficiency. These findings, together with improved ruminal protein-to-energy balance resulting in lower ammonia concentrations (13.0 vs. 6.97 mM), contribute to higher milk production (23.9 vs. 24.4 kg/day). Another finding of the research is the higher CP digestibility. The microbial breakdown of nitrogen-containing compounds in the rumen produces ammonia [42]. Ammonia level is influenced by the number and species composition of ruminal microorganisms. The reduced protozoa populations (636 vs. 289 × 10^9/mL) were accompanied by an increased total bacterial population in the ruminal fluid (8.36 vs. 10.5 × 10^3/mL). However, no increase in proteolytic bacteria such as *Prevotella* spp., *B. proteoclasticus*, and *B. fibrisolvens* was observed. This result may be an effect of polyphenols found in TMR DAP (59.2 g GEA/kg DM) that inhibit the growth of microorganisms. Furthermore, Zhang et al. [43] concluded that increased propionate and reduced acetate proportion in the ruminal fluid negatively affected the population size of *F. succinogenes* and *B. fibrisolvens*, which is consistent with our results. As commonly known, VFA are products of the fermentation of non-structural and structural carbohydrates in the presence of different bacteria in the rumen [44]. The results of the presented study reported a substantial increase in propionate and butyrate concentrations (by 23% and

5%, respectively), while the increase in acetate concentration was not statistically significant. The increase in propionic and butyric acid concentrations is attributed to the high non-fiber carbohydrates (up to 20–57 g/110 g) found in apple pomace [16]. The total VFA increased by 12% when feeding the experimental diet in comparison with the control group. However, Kim et al. [45] recorded a decrease in total VFA when the standard diet was supplemented with a plant extract rich in flavonoids. These differences confirmed the different effects of individual polyphenols and flavonoids on the rumen environment depending on their sources. As previously reviewed, different profile of bioactive compounds in plants would be differently metabolized in the rumen [46,47], primarily due to their fermentability and bactericidal characteristics. Flavonoids with strong antimicrobial activity would unfavorably disrupt ruminal fermentation by suppressing microbial populations, thus decreasing fermentation rate. This includes flavone, kaempferol, and myricetin that were reported to decrease VFA concentration and DM digestibility [48] due to their strong antibacterial effects. On the other hand, some types of flavonoids from *Solidago vigaurea* extract and quercetin are rapidly degraded in the rumen [49,50], which can provide an alternative carbon source. In apple pomace, quercetin is one of the most abundant flavonoids. A recent study reported that numerous beneficial metabolites including pyruvate, glucose, tyrosine, proline, and glucose-6-phosphate were enriched [51]. Additionally, agricultural byproducts such as grape pomace rich in polyphenols exhibited nutrigenomics effect, i.e., positively altered genes functionally associated with inflammation, antioxidant, and antimicrobial effects [52]. These supporting reasons, together with the higher OM digestibility in our study, can plausibly explain the greater VFA production and milk production in this study. However, as the data on the flavonoid profile of apple pomace are lacking in our study, it is imperative to characterize the bioactive compounds of apple pomace.

As mentioned above, there was a significant decrease of 54% of the total protozoa population in DAP-fed cows. Kim et al. [45] also reported a decrease in protozoa count by over 60% when flavonoid-rich plant extracts were evaluated in vitro, thus partially supporting our experimental results. The decrease in protozoa count is a result of the antimicrobial properties of flavonoids found in different plants, including apple pomace that affect cytoplasmic membrane function [48]. A decrease in the protozoa population reduces the digestion of cellulose contributed by the protozoa [45]. But the reduced protozoal number can increase total bacterial populations as observed in the study, including fibrolytic bacteria, which can increase fiber digestibility to some extent. This explains the tendency of an increase in acetate which is a product of fiber fermentation [53]. A flavonoid extract from *A. sissoo* reduced the population of protozoa, resulting in increased propionic acid and a marked decline in methane production, which was comparable with the results of this study [54]. Studies using extracts containing quercetin (the main polyphenol in DAP) have shown a reduction in protozoa, inhibition of methanogen populations, and changes in VFA profiles [55] especially reducing acetate to propionate ratio, which was noticed also in the current study. The inclusion of dietary Indian gooseberry fruit pomace, which is rich in polyphenolic compounds, resulted in lowered methane production in lactating buffaloes [56]. These results support our observations of decreased methanogens due to the limiting effect of the bioactive substances contained in DAP. In previous studies, attention was paid not only to mitigating methanogens numbers but also to protozoa count as the cause of the methane reduction, which could also have occurred in the presented studies [17].

In the present study, the TMR DAP caused an overall reduction of the biohydrogenation process by ruminal microbiota, which resulted in greater UFA and lower content of SFA in ruminal fluid. The decreased concentration of C18:2 *cis*-9, *trans*-11, and no change in the concentration of C18:1 *trans*-11 was found. This can be explained by the lower activity of *B. fibrisolvens*, which is responsible for the synthesis of these two isomers [57]. In the study of Singh et al. [57], the population of *B. fibrisolvens* was numerically reduced. Thus, the lack of changes in C18:1 *trans*-11 concentration might result from a decrease in

the concentration of C18:0 acid. This can be attributed to decreased *B. proteoclasticus* activity, which is one of the bacteria responsible for the conversion of C18:1 *trans*-11 to C18:0 [17]. The linear increase in C18:2 *cis*-9, *cis*-12, and C18:3 n-3 also confirms the limiting effect of DAP on biohydrogenation, resulting in an increase in ruminal PUFA and n-3 fatty acid concentrations. Previous studies have shown that the fatty acid profile (especially PUFA, linoleic acids isomers, and n-3 FA) in the ruminal fluid is associated with an increase in their content in milk [17]. However, attention should be also paid to the decreased concentration of C18:2 *cis* 9 *trans* 11 isomer, a beneficial FA for human health, in the rumen fluid, which may result in its lower content in milk.

One of the limitations of this study is the low number of replicates for milk production performance. Further study using DAP should be evaluated using a large number of animals in practical commercial farm conditions to recommend it for feeding dairy cows.

5. Conclusions

Inclusion of the dried apple pomace (150 g/kg DM) in dairy cows' diet modulated the ruminal microorganism populations and thus reduced methane emission, improved nutrient digestibility, and ruminal volatile fatty acid concentration. Also, beneficial changes (greater PUFA content) to the ruminal fatty acid profile resulting from reduced biohydrogenation were observed although the content of C18:2 *cis* 9 *trans* 11 isomer was decreased by DAP. This study indicated that DAP could serve as a valuable, sustainable, and environmentally friendly dietary component for dairy cows.

Further research on dairy cows with DAP-based diets is highly required to demonstrate whether its beneficial effect on FA profile in the ruminal fluid, i.e., a higher PUFA and n-3 content is translated to the milk.

Author Contributions: K.G.: investigation, methodology, data curation, and writing—Original draft. P.S.: investigation, data curation. M.K.: investigation, data curation. M.S.-S.: investigation, data curation. D.L.: investigation, data curation. P.K.: data curation. J.P.: data curation. N.S.: data curation. J.O.H.: project administration, data curation. A.J.: conceptualization, data curation. Y.R.Y.: data curation. A.I.: data curation, statistical analysis, writing—Review & editing. A.K.P.: validation, writing—Review & editing. A.C.: supervision, project administration, methodology, validation, writing—Review & editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by European Union's Horizon 2020 Research and Innovation Program under Grant Agreement No 696356 for research carried out within the ERA-GAS/ERA-NET SUSAN/ICT-AGRI project CCC farming (SUSAN/II/CCCFARMING/03/202); National Science Center, Poland (Grant No. 267659/7/NCBR/2015), and by statutory funding from the Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences (PULS), by the Department of Animal Nutrition (no. 506.533.04.00). We also thank AREX Company for providing dried apple pomace to this study as part of the project POIR.01.01.01-00-0593/18.

Institutional Review Board Statement: The study was performed according to the guidelines framed by the National Ethical Commission for Animal Research (Ministry of Science and Higher Education, Poland). Additionally, the Local Ethical Commission for Animal Research (permission no. 27/2009) approved the presented study.

Data Availability Statement: Data are presented in tables and available upon reasonable request to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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