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In vitro ruminal fermentation and nutrient compositions of potato starch by-products

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ABSTRACT. The purpose of the present study was to determine the chemical composition and *in vitro* ruminal fermentation capacity of potato pulp (PP), potato pulp with peel (PPp) and potato peel (Pp), i.e. potato starch by-products. PP and PPp were applied at 5.0, 7.5 and 10.0% (on a dry matter basis) instead of corn flake and barley grain in dairy cow total mixed ration (TMR). The volumes of *in vitro* total gas and methane production, and metabolisable energy (ME) values of PP and PPp were higher compared to Pp ($P < 0.001$). The *in vitro* true dry matter digestion (tDMd) and *in vitro* true organic matter digestion (tOMd) values of PP using at 7.5% and 10% rates in TMR were decreased linearly according to that of control TMR value ($P < 0.05$). Therefore, the use of potato starch by-products in ruminant diet can be suggested due to the high and rapid fermentation potential, especially of potato pulp without peel. The study showed that PP could be applied at up to 5% and PPp at a level of up to 10% in dairy cattle TMR (on a DM basis). Further *in vitro* studies should be carried out using dried potato starch by-products.

Introduction

The three most important plant raw materials used in European starch production are potatoes, maize and wheat. Their share in the annual European starch production of 22 million tonnes is 38.4% of potatoes, 31.2% of maize and 30.4% of wheat. In the production of potato starch, 80% of potato end up as wastes and by-products (An et al., 2012). Potato starch processing wastes from the potato starch industry, which include effluents and potato residues, cause serious environmental problems. The potato plant (*Solanum tuberosum*) has approximately 5000 varieties and is native to South America. In the process of potato starch production, when raw potato tubers are brought to the factory, they are first washed and then the peel parts are separated. In this peeling process, the potatoes are subjected to heat treatment and their peels are largely separated from the tubers. This product is the part containing potato peels. After washing the raw material, followed by granitising and centrifugation, the production of potato starch and potato protein takes place in two separate stages. The production of potato starch begins with the separation of the large fibre fraction (Ratnayake and Jackson, 2003; Grommers and van der Krogt, 2009). Previous studies have reported that potato juice, which has a high lysine content as a by-product, has the potential to be used as a ruminant nutrient in the form of a more concentrated liquid. (Stearns et al., 1994; Kasapidou et al., 2014; Li et al., 2015; Özdemir and Basmacıoğlu-Malayoğlu, 2017).

On a global scale, the potato is the fourth most important food crop (the others being rice, wheat and maize). Potatoes are the most important food in the colder regions of the world. Asia and Europe are the world's major potato-producing regions, accounting for more than 80% of world production, and Europeans consume the most of this vegetable (Kasapidou et al., 2014). Potatoes are also an efficient livestock feed. The starch industry obtains starch from maize, potatoes and wheat, which all involve different production processes (Pęksa and Miedzianka, 2021).

Potato residue refers to a thin solid by-product obtained after starch extraction, which has been shown to contain 33 isolates (28 bacteria, 4 fungi, 1 yeast), and is difficult to store due to its high water content exceeding 80% (Gélinas and Barrette, 2007). Wastes from potato starch processing, which include effluents and potato residues, cause serious environmental problems (Li et al., 2015). One study found that potato pulp with peel contained on a DM basis 17% pectin, 37% starch, 14% hemicellulose, 4% protein, 4% ash and 17% cellulose (Stearns et al., 1994; Kasapidou et al., 2014; Li et al., 2015; Özdemir and Basmacıoğlu-Malayoğlu, 2017). Potato pulp without peel is a rich source of pectin (Li et al., 2015), and potato protein has been recognised as one of the most valuable non-animal proteins due its high content of essential amino acids (lysine and threonine) (Kowalczewski et al., 2019).

Cull potatoes which occur during potato harvest can spoil quickly and are not utilised for human consumption, but can be used in ruminant nutrition as an energy source due to their high starch content (Fiems et al., 2013; Li et al., 2015; Duynisveld and Charmley, 2018). However, there are not many studies concerning the use of potato starch by-products in ruminant nutrition (Dhingra et al., 2013). The hypothesis of the present study was that all potato starch by-products (especially potato pulp with peel and potato peel), which have the potential to pose environmental problems due to the easily degradable nutrients in their content, could potentially be used in ruminant diets due to their digestible and fermentable carbohydrate compositions.

The aim of the work was to determine the *in vitro* rumen fermentation parameters and nutritional composition of potato pulp (Pp), potato pulp with peel (PPp) and potato peel (Pp), i.e. potato starch by-products. Another objective of the present study was to investigate the effect of applying by-products with good *in vitro* fermentation values in dairy cattle rations (at 5.0, 7.5 and 10.0% DM), instead of corn flake and barley grain, on feed digestibility [*in vitro* cumulative gas production, *in vitro* true dry matter digestion (tDMd), *in vitro* true organic matter digestion (tOMd) and estimated fermentation parameters].

Material and methods

This study was submitted to the Erciyes University Animal Experiments Ethics Committee and was conducted in accordance with its guidelines.

Sampling area

Samples of potato starch by-products were collected at a local factory in the Nevsehir province, Türkiye. Potato (*Solanum tuberosum* L.) tubers with a diameter of 35–60 mm were used for planting, and the planting depth was usually 4–5 cm, depending on soil moisture and temperature, and the tubers were covered with at least 10–15 cm soil cover. Irrigation was carried out with a sprinkler system using artesian water. Potato plants and their tubers were harvested at the end of the 5th month. Tubers were processed at the plant to obtain potato starch.

During potato starch processing, potato skin and potato pulp were removed from the process separately. At the same time, a separate waste containing a certain amount of peel residue and potato pulp were released in the second step of the process. By-products (5 kg) collected from each of the three different waste products were transferred to the laboratory.

Determination of chemical compositions

DM contents in the samples (n = 5) were determined after incubation at 60 °C for 24 h, followed by 105 °C for 24 h. Dried samples were ground in a grinder mill (IKA Werke, Germany) to a maximum particle size of 1 mm. Crude ash (CA), crude protein (CP) (nitrogen × 6.25) and diethyl ether extract (EE) contents were determined according to the method reported by AOAC International (1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents were analysed according to Van-Soest et al. (1991). NDF was determined using sodium sulphite (0.5 g) and heat stable amylase (200 µl) (neutral detergent fibre determined using amylase, aNDF). NDF, ADF, and ADL did not include residual ash (aNDFom, ADFom, and ADL). The level of non-fibrous carbohydrate (NFC) was calculated using the equation given in NRC (2001); NFC = 100 − (aNDFom% + CP% + EE% + CA%). The hemicellulose (HemC) content was calculated using the following formula: HemC% = aNDFom% − ADFom%. The total starch content was determined by spectrophotometry using the Commercial Assay Kit (Megazyme, K-AMIAR 02/20, Wicklow, Ireland) (Table 1).

Table 1. Nutrient composition of potato starch by-products (DM %)

	PP	PPp	Pp
DM (wet basis)	22.07	15.63	16.51
Ash	6.20	11.10	49.76
OM	93.80	88.90	50.24
CP	6.04	13.97	10.79
EE	0.83	0.42	0.60
aNDFom	9.53	41.16	33.77
ADFom	5.70	29.30	25.12
ADL	1.42	16.01	12.53
HemC	3.82	11.86	8.65
NFC	78.03	33.38	5.28
Total starch	38.20	16.65	0.47

PP – potato pulp, PPp – potato pulp with peel, Pp – potato peel; DM – dry matter, OM – organic matter, CP – crude protein, EE – diethyl ether extract, aNDFom – neutral detergent fibre without ash, ADFom – acid detergent fibre without ash, HemC – hemicellulose, NFC – non-fibrous carbohydrate, ADL – acid detergent lignin

In vitro gas production technique

For *in vitro* fermentation, fresh rumen fluid collected by gastric tube from three beef cows was used. During the experiment, the animals were fed a diet containing forages (approx. 20% of the diet, DM basis) and concentrate feed (approx. 80% of the diet, DM basis). The TMR consisted of approx. 50% of maize silage, 20% meadow hay, 20% alfalfa hay and 10% wheat straw (DM basis). Rumen fluid was collected 2 h after the animals had consumed the feed. Rumen fluid was placed into a thermos, and then filtered using muslin with a pore diameter of 1–5 µm to obtain the inoculum. The buffer mixture contained bi-distilled water, macro-mineral solution (5.7 g Na_2HPO_4, 6.2 g KH_2PO_4 and 0.6 g $MgSO_4$ in 1 l of bi-distilled water), buffer solution (35 g $NaHCO_3$ and 4 g NH_4HCO_3 in 1 l of bi-distilled water), trace mineral solution (13.2 g $CaCl_2{\cdot}2H_2O$, 10 g $MnCl_2{\cdot}4H_2O$, 1 g CoCl2·$6H_2O$ and 0.8 g $FeCl_3{\cdot}6H_2O$ in 100 ml of bi-distilled water), resazurin solution (0.1 g resazurin in 100 ml of bi-distilled water) and reducing solution (285 mg $Na_2S{\cdot}7H_2O$ and 4 ml of 1 N NaOH in 96 ml bi-distilled water). For the *in vitro* gas production technique, by-products samples (10 ml) and buffer mixture (20 ml) in an aerobic glass fermenter (100 ml volume; Model Fortuna, Haberle Labortechnik, Germany) at 39 °C for up to 24 h. Additionally, three blank glass fermenters (no sample, only rumen fluid + buffer mixture) were incubated as correction values (Menke et al., 1979 and 1988).

Determination of total gas and methane production

The total volume of gas and produced substrates were read from the volume lines on the glass fermenter after 6, 12, 18 and 24 h (n = 15). The volume of methane gas in the total gas produced after 6, 12, 18 and 24 h was determined using an infrared methane measurement device (Sensor, Europe GmbH, Erkrath, Germany) according to Kara et al. (2015).

Estimated metabolisable energy (ME), net energy lactation (NE_L), and organic matter digestibility (OMdgas) calculated from total gas production

ME, NE_L, OMdgas and short chain fatty acids (SCFA) levels of the studied by-products and dairy cattle TMR, including PP and PPs, were calculated using the equations of Menke and Steingass (1988) (Table 2).

Table 2. Estimated fermentation parameters of potato starch by-products

	ME, MJ/kg DM	NE_L, MJ/kg DM	OMdgas, % DM	SCFA, mmol	Methane, ml/0.2 g OMD
PP	13.24[a]	8.56[a]	86.83[a]	1.79[a]	24.24[a]
PPp	9.37[b]	5.72[b]	62.45[b]	1.19[b]	16.63[b]
Pp	4.90[c]	2.44[c]	33.69[c]	0.51[c]	10.44[c]
SD	3.57	2.62	22.76	0.54	6.18
SEM	0.84	0.61	5.36	0.12	1.50
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

PP – potato pulp, PPp – potato pulp with peel, Pp – potato peel; ME – metabolisable energy, NE_L – net energy for lactation, OMdgas – organic matter digestibility levels calculated from total gas production, SCFA – short chain fatty acids calculated from total gas production, SEM – standard error of the mean, SD – standard deviation; [abc] – average values with different superscripts in the same column are significantly different at $P < 0.05$

$$\text{ME, MJ/kg DM} = 0.157 \times GP + 0.0084 \times CP + 0.022 \times EE - 0.0081 \times ash + 1.06$$

$$NE_L\text{, MJ/kg DM} = 0.115 \times GP + 0.0054 \times CP + 0.014 \times EE - 0.0054 \times ash - 0.36$$

$$\text{OMdgas, \%} = 0.9991 \times GP + 0.0595 \times CP + 0.0181 \times ash + 9$$

$$\text{SCFA, mmol} = 0.0239 \times GP - 0.0601,$$

where: GP is 24-h net gas production (ml/200 mg DM), and CP, EE, ash and OMdgas are crude protein, ether extract, crude ash (% DM) and organic matter digestibility, respectively.

Total mixed rations for dairy cattle

PP and PPp, which are among the by-products of the potato industry and are characterised by a beneficial nutrient content and *in vitro* fermentation parameters, were added to the dairy cattle total mixed ration (TMR) cows and their effect on *in vitro* ration fermentation were determined (Tables 3 and 4).

Dried and ground potato starch by-products (PP and PPp) were added in different proportions (0 – control TMR, and 5, 7.5 and 10%, DM) to dairy cattle TMR, which was prepared for 620 kg live weight, 9th week of lactation, body condition score: 3.25, milk yield: 28 l/day and a 50-month-old Holstein cows. The control TMR included approx. 14% crude protein (CP), 38% neutral detergent fibre (NDF) and 36% non-fibre carbohydrate (NFC) on a dry matter (DM) basis (NRC, 2001).

In vitro fermentation values of TMRs with PP and PPs

In vitro gas generation and predicted rumen fermentation product levels of the tested dairy cattle TMRs were determined according to the aforementioned *in vitro* gas production technique. Analyses were carried out in six replicates.

Determination of *in vitro* true dry matter disappearance and *in vitro* true organic matter disappearance

Three fermentation syringes were stopped after 24 h and *in vitro* true dry matter disappearance (tDMd) and *in vitro* true organic matter disappearance (tOMd) were analysed. *In vitro* tDMd and tOMd were determined by filtering the fermentation residue using a vacuum unit (Velp Dietary Fibre Analyzer, Velp Scientifica, Usmate, Italy) on pre-weighed glass crucibles (porosity #2, Velp Scientifica, Usmate, Italy), drying at 105 °C and burning at 550 °C. *In vitro* tDMd was calculated as: tDMd = 1 − [(DM residue − DM blank) / initial DM)] × 100. *In vitro* tOMd was calculated as: tOMd = 1 − [(OM residue − OM blank) / initial OM)] × 100.

Statistical analysis

One-way analysis of variance was performed for homogeneous variances using general linear model procedures to test treatment differences. Data were analysed based on the following model: $Y_{ij} = \mu_{ij} + S_i + e_i$, where: Y_{ij} – overall mean common for each parameter studied. S_i – the effect (*i*) of potato starch by-products on the variables studied, e_i – standard

Table 3. Supplementation of potato starch by-products to total mixed ration (TMR)

Feeds	Supplementation of TMR with potato pulp											
	Control TMR			5% PP supp. to TMR			7.5% PP supp. to TMR			10% PP supp. to TMR		
	Wet basis, kg	DM basis, kg	DM basis, %	Wet basis, kg	DM basis, kg	DM basis, %	Wet basis, kg	DM basis, kg	DM basis, %	Wet basis, kg	DM basis, kg	DM basis, %
Maize silage	20.00	6.00	28.58	20.00	6.00	28.58	20.00	6.00	28.58	20.00	6.00	28.58
Wheat straw	2.50	2.25	10.72	2.50	2.25	10.72	2.50	2.25	10.72	2.50	2.25	10.72
Lucerne herbage*	3.00	2.76	13.15	3.00	2.76	13.15	3.00	2.76	13.15	3.00	2.76	13.15
Barley grain	3.00	2.76	13.15	2.40	2.21	10.53	2.10	1.93	9.22	1.80	1.66	7.90
Maize flake	2.00	1.84	8.76	1.50	1.38	6.58	1.25	1.15	5.49	1.00	0.92	4.39
Sunflower meal, 28% CP	2.00	1.82	8.67	2.00	1.82	8.67	2.00	1.82	8.67	2.00	1.82	8.67
Cottonseed meal, 28% CP	1.50	1.37	6.50	1.50	1.37	6.50	1.50	1.37	6.50	1.50	1.37	6.50
Soybean meal, 44% CP	1.40	1.27	6.07	1.40	1.27	6.07	1.40	1.27	6.07	1.40	1.27	6.07
Wheat bran	1.00	0.93	4.41	1.00	0.93	4.41	1.00	0.93	4.41	1.00	0.93	4.41
Potato pulp	–	–	–	4.50	0.99	4.72	6.75	1.49	7.08	9.00	1.98	9.45
Total	36.40	21.00	100.00	39.80	21.00	100.00		21.00	100.00		21.00	100.00
ME, Mcal/kg DM		2.32		2.32			2.31			2.31		
NE_L, Mcal/kg DM		1.44		1.45			1.45			1.46		
Analyses values												
CP, % DM	14.14			13.53			13.35			13.25		
ash, % DM	6.78			6.83			6.93			6.96		
EE, % DM	3.30			3.29			3.28			3.28		
NFC, % DM	36.86			38.08			38.50			38.89		
NDF, % DM	38.92			38.08			38.50			38.89		

* – lucerne herbage contained 17–19% CP and 40–44 % NDF; DM – dry matter, CP – crude protein, EE – diethyl ether extract, ME – metabolisable energy, NE_L – net energy lactation, NDF – neutral detergent fibre, NFC – non-fibrous carbohydrate, PP – potato pulp

Table 4. Supplementation of potato pulp with peel to total mixed ration (TMR)

Supplementation of TMR with potato pulp with peel												
	Control TMR			5% PPp supp. to TMR			7.5% PPp supp. to TMR			10% PPp supp. to TMR		
Feeds	Wet basis, kg	DM basis, kg	DM basis, %	Wet basis, kg	DM basis, kg	DM basis, %	Wet basis, kg	DM basis, kg	DM basis, %	Wet basis, kg	DM basis, kg	DM basis, %
Maize silage	20.00	6.00	28.58	20.00	6.00	28.61	20.00	6.00	28.62	20.00	6.00	28.64
Wheat straw	2.5	2.25	10.72	2.50	2.25	10.73	2.50	2.25	10.73	2.50	2.25	10.74
Lucerne herbage*	3.00	2.76	13.15	3.00	2.76	13.16	3.00	2.76	13.17	3.00	2.76	13.17
Barley grain	3.00	2.76	13.15	2.40	2.21	10.53	2.10	1.93	9.22	1.80	1.66	7.90
Maize flake	2.00	1.84	8.76	1.50	1.38	6.58	1.25	1.15	5.49	1.00	0.92	4.39
Sunflower meal, 28% CP	2.00	1.82	8.67	2.00	1.82	8.68	2.00	1.82	8.68	2.00	1.82	8.69
Cottonseed meal, 28% CP	1.50	1.37	6.50	1.50	1.37	6.51	1.50	1.37	6.51	1.50	1.37	6.52
Soybean meal, 44% CP	1.40	1.27	6.07	1.40	1.27	6.07	1.40	1.27	6.08	1.40	1.27	6.08
Wheat bran	1.00	0.93	4.41	1.00	0.93	4.42	1.00	0.93	4.42	1.00	0.93	4.42
Potato pulp with peel	–	–	–	4.50	0.99	4.72	6.75	1.49	7.08	9.00	1.98	9.45
Total	36.40	21.00	100.00		21.00	100.00		21.00	100.00		21.00	100.00
ME, Mcal/kg DM		2.32			2.31			2.31			2.31	
NE_L, Mcal/kg DM		1.44			1.43			1.42			1.41	
Analysis results												
CP, % DM		14.14			14.00			14.10			14.52	
ash, % DM		6.78			7.20			7.39			7.54	
EE, % DM		3.30			3.29			3.29			3.28	
NFC, % DM		36.86			37.20			37.23			36.97	
NDF, % DM		38.92			38.31			37.99			37.69	

* – lucerne herbage contained 17–19% CP and 40–44 % NDF; DM – dry matter, CP – crude protein, EE – diethyl ether extract, ME – metabolisableenergy, NE_L – net energy lactation, NDF – neutral detergent fibre, NFC – non-fibrous carbohydrate, PPp – potato pulp with peel, PP – potato pulp

error. Means were separated by Tukey's multiple range test at $P < 0.05$. (SPSS 17.0 software, IBM Corp., Armonk, NY, USA).

Results

Chemical composition of potato starch by-products

The average dry matter contents were 22.07% in PP, 15.63% in PPp, and 16.51% in Pp. The crude protein percentages of PP, PPp and Pp were 6.04, 13.97 and 10.79%, respectively. The ether extract values of samples ranged from 0.42 to 0.83%. The highest amount of inorganic substance was found in Pp. The highest content of fibrous substances (aNDFom, ADFom) was determined in the PPp samples. The total starch content of potato starch by-products amounted to approx. 38.3% in PP, 16.7% in PPp and 0.5% in Pp (Table 1).

In vitro ruminal fermentation values of potato starch by-products

Cumulative *in vitro* gas production from 6 up to 24 h was statistically different between individual potato starch by-products ($P < 0.001$) (Figure 1). After 6 h of incubation, the cumulative *in vitro* gas production from 0.2 g DM was about 60 ml for PP, 30 ml for PPp and 10 ml for Pp ($P < 0.001$). Cumulative *in vitro* gas production after 12, 18 and 24 h was approx. 65, 70 and 75 ml for PP, 40, 45 and 50 ml for PPp, and 15, 20 and 25 ml for Pp, respectively ($P < 0.05$) (Figure 1).

The volume of cumulative *in vitro* gas production of potato starch by-products reached approx. 78 ml/0.2 g DM for potato pulp, 52 ml/0.2 g DM for potato pulp with peel and 24 ml/0.2 g DM for potato peel after 24 h of incubation. The volume of the cumulative *in vitro* methane production of potato starch by-products reached about 21 ml/0.2 g

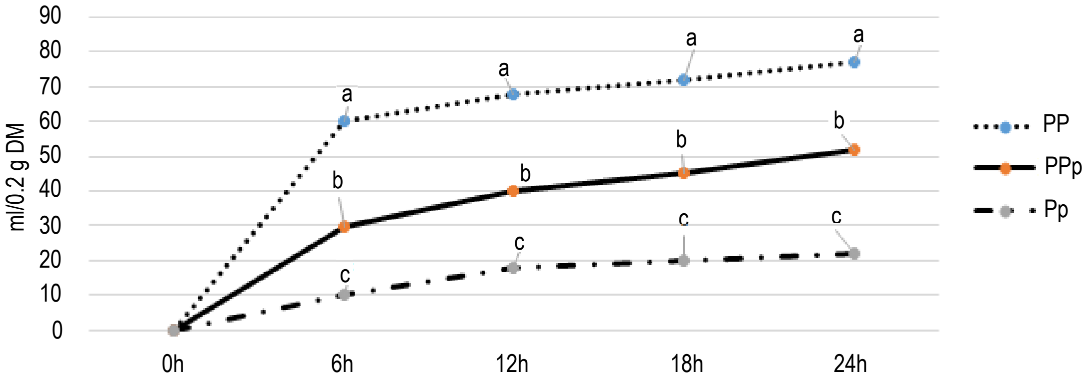

Figure 1. *In vitro* cumulative total gas production of potato starch by-products

PP – potato pulp, PPp – potato pulp with peel, Pp – potato peel, DM – dry matter; abc – means on a curve with different superscripts are significantly different at $P < 0.05$

DM for potato pulp, 10 ml/0.2 g DM potato pulp with peel and 4 ml/0.2 g DM for potato peel up to 24 h of incubation. Cumulative *in vitro* methane production of potato starch by-products differed statistically from each other from 6 h up to 24 h ($P < 0.001$) (Figure 2).

The ME values of potato starch by-products were 13.24 MJ/kg DM for potato pulp, 9.37 MJ/kg DM for potato pulp with peel and 4.90 MJ/kg DM for potato peel ($P < 0.001$). The NE_L values of potato starch by-products were 8.56 MJ/kg DM for potato pulp, 5.72 MJ/kg DM for potato pulp with peel and 2.44 MJ/kg DM for potato peel ($P < 0.001$). The OMD values of potato starch by-products were 86.33% for potato pulp, 62.45% for potato pulp with peel and 33.69% DM for potato peel ($P < 0.001$). The SCFA levels of potato starch by-products were 1.79 mmol for potato pulp, 1.19 mmol for potato pulp with peel and 0.51 mmol for potato peel ($P < 0.001$). Methane production by 0.2 g of digested OM of potato starch by-products was 24.24 ml for potato pulp, 16.63 ml for potato pulp with peel and 6.18 ml for potato peel ($P < 0.001$) (Table 2). These results showed that the addition of potato peel reduced the fermentation parameters in the studied samples in comparison to potato pulp.

Cumulative *in vitro* gas production of dairy cattle TMRs with potato starch by-products

Supplementation ratios of PP and PPp from potato starch by-products to TMR are given in Tables 3 and 4. The use of up to 5% PP by-product instead of maize and barley in dairy cattle ration did not change *in vitro* cumulative gas production up to 48 h ($P > 0.05$) compared to the group that received 0% PP. The application of PP at 7.5 and 10% in TMR reduced the cumulative *in vitro* gas production up to 24 h ($P < 0.05$); however *in vitro* gas production after 48 h at these supplementation levels was similar to the control TMR ($P > 0.05$). The cumulative *in vitro* gas production values after PPp supplementation at 5, 7.5 and 10% TMR at 6, 12, 18 and 24 h were similar to those of the control TMR ($P > 0.05$) (Table 5).

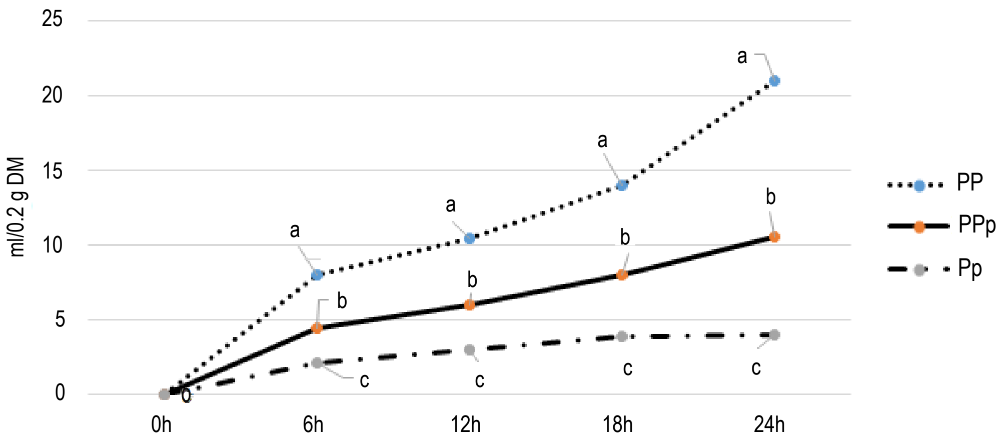

Figure 2. *In vitro* cumulative methane production of potato starch by-products

PP – potato pulp, PPp – potato pulp with peel, Pp – potato peel, DM – dry matter; abc – means within a curve with different superscripts are significantly different at $P < 0.05$

Table 5. *In vitro* cumulative total gas productions of potato pulp and potato pulp with peel, %

By-products	Supp. level to TMR	Gas6h	Gas12h	Gas18h	Gas24h	Gas48h
PP	0%	23.33^{a}	35.30^{a}	41.38^{a}	47.05^{a}	49.55^{a}
	5%	20.56^{ab}	32.97^{ab}	41.15^{a}	45.52^{a}	49.05^{a}
	7.5%	17.32^{b}	30.03^{b}	36.73^{b}	40.90^{b}	46.82^{ab}
	10%	17.32^{b}	29.87^{b}	36.65^{b}	40.22^{b}	43.39^{a}
	SD	3.49	3.02	3.37	3.56	3.02
	SEM	0.72	0.63	0.70	0.74	0.91
	P-value (linear contrast)	0.001	0.001	0.005	<0.001	0.020
PPp	0%	23.33^{a}	35.30^{a}	41.38^{a}	47.05^{a}	49.55^{b}
	7.5%	24.50^{a}	37.06^{a}	44.55^{a}	47.87^{a}	56.56^{a}
	10%	24.50^{a}	37.06^{a}	44.55^{a}	48.62^{a}	56.56^{a}
	SD	17.82^{ab}	29.98^{ab}	36.92^{ab}	42.36^{ab}	42.98^{c}
	SEM	3.75	3.61	4.01	3.09	6.23
	P-value (linear contrast)	0.76	0.73	0.82	0.63	1.79
		0.001	<0.001	<0.001	<0.001	<0.001
P-value	By-products	<0.001	0.001	0.004	<0.001	<0.001
	Supplementation level to TMR	0.500	0.388	0.606	0.054	0.005
	Interaction	<0.001	0.001	0.001	<0.001	<0.001

PPp – potato pulp with peel, PP – potato pulp; Gas6h – total gas produced after 6 h of incubation, Gas12h – total gas produced after 12 h of incubation, Gas18h – total gas produced after 18 h of incubation, Gas24h – total gas produced after 24 h of incubation, Gas48h – total gas produced after 48 h of incubation, TMR – total mixed ration, SEM – standard error of the mean, SD – standard deviation; ^{abc} – average values with different superscripts in the same column are significantly different at $P < 0.05$

In vitro ruminal fermentation values of dairy cattle TMRs with potato starch by-products

ME, NEL, ruminal SCFA molarities and OMdgas levels calculated from gas production of TMRs with PP and PPp increased with incrementing gas production values of TMRs (Table 6).

The tDMd (about 74%) and tOMd (about 79%) values and estimated *in vitro* ruminal fermentation values (ME, NEL, OMdgas and SFCA) of PP applied at 5% concentration to TMR were similar to those (about 75 and 79%, respectively) of the control TMR ($P > 0.05$). The values of tDMd, tOMd, ME, NEL, OMdgas and SFCA for ruminal fermentation of TMRs with 7.5 and 10% PP linearly decreased with increasing supplementation doses compared to the control TMR ($P < 0.001$). The tDMd and tOMd values of PP supplemented at 7.5 and 10% in TMR

Table 6. *In vitro* ruminal fermentation values of dairy cattle TMRs with potato starch by-products, %

By-products	Supp. level to TMR	tDMd	tOMd	ME	NE_L	OMdgas	SCFA
PP	0%	75.43^{a}	79.19^{a}	9.81^{a}	5.49^{a}	65.64^{a}	1.04^{a}
	5%	74.03^{a}	78.80^{a}	9.51^{a}	5.28^{a}	63.76^{a}	1.00^{a}
	7.5%	40.67^{b}	53.80^{b}	8.76^{b}	4.73^{b}	59.06^{b}	0.90^{b}
	10%	32.63^{b}	49.30^{b}	8.64^{b}	4.65^{b}	58.33^{b}	0.88^{b}
	SD	20.74	14.88	0.58	0.42	3.71	0.07
	SEM	1.72	1.52	0.06	0.05	0.43	0.01
One-way ANOVA, Tukey	*P*-value (linear contrast)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PPp	0%	75.43	79.19	9.81^{a}	5.49^{a}	65.64^{a}	1.04^{a}
	5%	70.24	76.67	9.88^{a}	5.55^{a}	66.56^{a}	1.05^{a}
	7.5%	71.89	77.42	10.02^{a}	5.65^{a}	67.58^{a}	1.07^{a}
	10%	69.74	72.31	9.03^{ab}	4.93^{ab}	60.96^{ab}	0.93^{ab}
	SD	5.63	5.83	0.64	0.46	4.12	0.09
	SEM	1.73	1.53	0.06	0.04	0.42	0.01
One-way ANOVA, Tukey	*P*-value (linear contrast)	0.663	0.583	<0.001	<0.001	<0.001	<0.001
Two-way ANOVA	By-products	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Supplementation level to TMR	<0.001	<0.001	<0.001	0.001	0.001	0.002
	Interaction	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

PP – potato pulp, PPp – potato pulp with peel; tDMd – *in vitro* true dry matter disappearance, tOMd – *in vitro* true organic matter disappearance, ME – metabolisable energy, NE_L – net energy lactation, OMdgas – organic matter digestibility calculated from the total gas produced, SCFA – short chain fatty acids, TMR – total mixed ration, SEM – standard error of the mean, SD – standard deviation; ^{abc} – average values with different superscripts in the same column are significantly different at $P < 0.05$

were lower than in the control TMR ($P < 0.05$). The tDMd, tOMd, ME, NEL, OMdgas and SCFA values of PPp added at a concentration of up to 10% to TMRs were similar to the corresponding results obtained for the control TMR ($P > 0.05$) (Table 6).

Discussion

The level of total *in vitro* gas production depends on factors such as the level of substrate fermentation, dietary supplements, readily soluble/fermentable carbohydrates, bacterial and protozoan populations in the donor rumen fluid and the quality of fermentation (Makkar and Becker, 1996; Kara, 2015). In the current study, the high cumulative total gas production and estimated digestibility parameters (OMdgas, ME, and NE_L) of PP could be particularly related to the high starch content as well as the high pectin and soluble carbohydrate contents (Mayer, 1998). The high levels of gas production and ME in potato pulp indicated that it could serve as an important energy sources in cattle diet. These levels of gas production in PP during 6–24 h incubation were higher than the reference values of grain feeds (Abolfazl et al., 2020). In addition, the fermentation values of PPp, given that it was an agricultural by-product, were at very satisfactory levels. On the other hand, PP showed lower fermentability than nutritional capacity. Consequently, the use of potato starch by-products in ruminant diet can be suggested due to their high and fast fermentation potential, especially of potato pulp without peel. Besides, substances contained in potato peel may be added as a fibre source to the diets of dairy and beef cattle. Each of these potato starch side products is formed in separate steps of the process, and thus they are independent of each other (Mayer, 1998).

There are conflicting values in the literature regarding the nutrient content of potato starch by-products. The main reason for this may be the use of different ingredients, i.e. potato pulp and whether potato peel was used or not, and at what proportions. NRC (2001) reported that potato pulp by-products contained 35% DM and 10.5% CP, 22% NDF, 16.5% ADF, 12.8% ash and 2.3% lignin in DM, and 2.84 Mcal/kg DM for ME-3x. However, a study conducted in Turkey (Şenyüz and Karslı, 2020) reported that potato pulp contained 15.5–16.05% DM and 5.25–5.00% CP, 33.40–35.0% NDF, 19.94–17.33% ADF, 2.60–2.20% ADL, 13.77–13.00% crude cellulose and 3.40–3.20% ash in DM. In the present study, the ash value of PPp was similar to those reported by NRC (2001). The crude protein content was approximately 14% in PPp, which was higher than those determined by NRC (2001) and Şenyüz and Karslı (2020). The CP value of PP in the current study was higher than the results of Şenyüz and Karslı (2020). Potato proteins are fractions of albumin and globulin, soluble in water and salt solutions, respectively. Approximately 30–50% of soluble potato proteins contain numerous protease inhibitor fractions, they are often heat-resistant, and show a wide spectrum of health-promoting effects. The nutritional value of proteins is related to their amino acid content, their mutual proportions and digestibility (Pęksa, and Miedzianka, 2021). Potato protein has a particularly high lysine (essential amino acid) content, which differs significantly between plant proteins, while the limited amino acids generally include methionine, tryptophan and cysteine (Van den Broek et al., 2004). The most abundant in potato tubers are aspartic and glutamic acids and their amides (Kowalczewski et al., 2019). The higher CP content of PPp compared to the others by-products (PP and Pp) also indicated that it would be a good source of essential amino acids.

The NDF, ADF and ADL values obtained in the current study differed from findings of previous works (NRC, 2001; Şenyüz and Karslı, 2020). The possible reasons for these differences could involve the characteristics of the soil where the potato was grown, potato type, processing technique (obtaining flour, starch or protein), sampling method and time of receipt of potato by-products from the factory. However, the results have indicated that the chemical content of peels and peeled potato pulp has important values that can be utilised in animal nutrition and needs to be studied.

The volume of total *in vitro* gas production depends on variables such as inoculum collection time, inoculum to buffer ratio, donor animal condition, population of rumen bacteria and protozoa of donor animal, substrate fermentation level, feed additives, easily soluble/fermentable carbohydrates and phenological stage of plants (Menke and Steingass, 1988; Makkar and Becker, 1996; Pandey, 2012; Kara, 2015). Further, the *in vitro* fermentation values of the peeled potato pulp were also found to be excellent. On the other hand, the *in vitro* fermentation capacity of potato peel was low, similarly to its nutrient content. Therefore, the present study did not include potato peels in the analysis, but focused on PP and PPp supplementation to a dairy cow TMR, as they showed beneficial nutrient contents and fermentation parameters.

In this study, the high cumulative total gas production and predicted digestibility parameters (OMD, ME and NE_L) of PP could be associated with its high pectin and soluble carbohydrate contents. Cumulative levels of total *in vitro* gas production by potato pulp after 6–24 h of incubation were higher than the reference values reported for cereals (Abolfazl et al., 2020). According to the results of the present study, the high *in vitro* total gas production and ME levels of PPp and PP indicated that these materials have proven to be important industrial by-products and can be used as an energy source in rations for cows. Based on the chemical content of the pure by-products and the results of *in vitro* fermentation in the present study, PP is recommended for use in the diets of ruminants due to its high and rapid fermentation potential. However, cumulative gas production, ruminal fermentation, *in vitro* tDMd and *in vitro* tOMd values in the experimental TMR did not change with the 5% supplementation level of PP, but negatively affected the 7.5% and 10% supplementation level of PP. It was determined that the addition of skinless potato pulp up to 5% to a TMR of dairy cows did not change the *in vitro* tDMd and tOMd values, but the addition 7.5% and 10% of the by-product affected these values negatively. An interesting result was that *in vitro* gas production and fermentation of PP were not observed when it was added to the TMR. The dose-dependent negative effect of the addition of PP (5, 7.5 and 10% DM) instead of barley grain and corn flake to the TMR was likely related to an overall increase in the proportion of resistant starch or pectin in the TMR; however, this result was not expected. Furthermore, the fact that the addition of up to 10% PPp to the TMR did not change the *in vitro* tDMd and tOMd values has indicated that PP is a good, highly digestible source of both protein and carbohydrates.

Conclusions

Based on our results, it can be concluded that potato peel can be used up to 5% and potato pulp with peel can be used up to 10% in dairy cattle TMR (DM) cattle due to their nutrient content and *in vitro* ruminal fermentation values. The high content and composition of inorganic matter in potato peel is noteworthy, but it cannot be added to the TMR for dairy cattle like other pulps due to the low *in vitro* fermentation parameters. Due to the high content of easily soluble carbohydrates and water, it may be recommended to silage or dry the potato pulp, especially the pulp without peel, as it may cause problems in stocking when used in TMR in the dairy cattle farm. Further research should focus on the field trials involving *in vivo* feeding, taking into account the high pectin and water absorption capacity of the pulp.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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