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Utilisation of slow-release non-protein nitrogen produced from agro-industrial by-products: feed digestibility and ruminal parameters

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ABSTRACT. Over the past few years, air and surface water pollution with nitrogen from agro-industrial waste has become a global environmental concern. Generally, these discards have nutritional value and could be utilised inexpensively for various purposes. The study involved two different phases: 1) production of lactosylurea from agro-industrial by-products and 2) evaluation of the nutritive value of lactosylurea as a non-protein nitrogen source in the ruminant diet, including its effect on feed digestibility and ruminal parameters. The gas production test and *in vitro* disappearance method were used to describe the digestion kinetics of both dry matter (DM) and crude protein (CP) in the four experimental treatments. Protozoan count and total volatile fatty acid concentration were utilised to evaluate ruminal parameters. The treatments were as follows: 1) basal diet + urea (BDU), 2) basal diet + lactosylurea (BDL), 3) basal diet + concentrated lactosylurea (BDCL), 4) basal diet + slow-release non-protein nitrogen (Optigen) (BDO). According to our findings, gas produced, DM, and CP disappearance were significantly higher in the BDCL and BDO experimental treatments than in the other treatments ($P < 0.05$). Moreover, estimated levels of metabolisable energy, digestible organic matter and short chain fatty acids were significantly higher for the same treatments ($P < 0.05$). The number of protozoa (2.66 × 106 organism/ml) and total volatile fatty acid concentration (30.96 mmol/l) were significantly lower in urea treatment compared to others ($P < 0.05$). In conclusion, lactosylurea produced from agro-industrial by-products seems to be a good alternative for urea or Optigen that additionally reduces environmental contamination by agro-industrial waste.

Introduction

Recently, nitrogen utilisation efficiency has been one of the challenging environmental issues. The agricultural sector contributes to this problem by adding nitrogen to surface waters and the atmosphere (Pavlidis and Tsihrintzis, 2018; Sharifi et al., 2022). A ruminant production system should be

developed with the purpose to protect nature by reducing environmental pollution (Tan et al., 2021). The use of discards of food processing companies can play a major role in the sustainability of the livestock industry; however, fermentation properties of these materials must be carefully investigated for optimal performance (VandeHaar and St-Pierre, 2006). Of all processing discards, lactose and protein-rich whey are considered one of the main ones in cheese production (Bacenetti et al., 2018). Nonetheless, not only is whey powder production highly expensive, but the process can also pollute the environment. Whey can be used in livestock feeding as an accessible source of carbohydrates, of which lactose is the most abundant (Rocha and Guerra, 2020). Whey, is an easily fermentable excellent source of soluble carbohydrates for rumen microorganisms. In addition, it contains high-quality soluble protein, which can serve as a source of nitrogen for rumen microorganisms (Lee et al., 2019; Guedes et al., 2020). In the cheese production industry, approximately 88% of milk remains as whey, and only about 12% is converted into cheese. Utilisation of whey as animal feed can reduce the environmental pollution (Ahmadi et al., 2018; Rocha and Guerra, 2020). Therefore, providing low-cost feed is vital for a sustainable farming system, and whey can be a suitable inexpensive alternative. Feed protein is one of the most expensive and restrictive part of livestock feed (Kim et al., 2019). Non-protein nitrogen (NPN) sources are good alternatives to dietary protein due to their lower costs compared to true protein (Taylor-Edwards et al., 2009; Calsamiglia et al., 2010; Cherdthong and Wanapat, 2010). Ruminants can convert NPN into milk protein (Virtanen, 1966). Using urea as an NPN source could supply adequate amounts of nitrogen for rumen microbial growth and amino acid flow to the small intestine (Fessenden et al., 2019). The issue of rapid ammonia release, when rumen fluid urease reacts with urea consumed by animals, seems to be a significant limitation in the efficient utilisation of this compound. Generally, the hydrolysis rate of ammonia is higher than its use by ruminal bacteria, leading to molecule waste (Galo et al., 2003; Inostroza et al., 2010). However, a controlled rate of urea release simultaneously with carbohydrate breakdown could be a solution to this issue (Taylor-Edwards et al., 2009). Slow-release urea products are a good alternative to ruminant dietary protein (Kertz, 2010; Joysowal et al., 2019). Lactosylurea is a combination of urea and whey providing nitrogen and carbohydrates in a single substance (Álvarez-Cao et al., 2020). In this context, the main objective of this study was to investigate the potential use of whey-derived lactosylurea and urea as agro-industrial by-products on feed digestibility and ruminal parameters. The specific objective of our study was to reduce environmental contamination by secondary by-products of human food production.

Material and methods

Animals

This experiment did not require the approval of the ethics committee, as the animals were humanely slaughtered, and the cows were fed diets listed in Table 1 before slaughter. The experimental diets, except for the NPN source, had similar results of proximate analysis and were fed at a dose of 60 g per cow per day on the farm.

Sample preparation

Lactosylurea samples were prepared using two slightly different methods described by Merry et al. (1982) and Torkashvand and Nezamedost (2009). In the first method, 200 ml of whey (provided by Pegah Factory of Tabriz, Iran) was mixed with 0.11 ml of sulphuric acid and 0.125 g of urea and incubated at 55 °C for 72 h. Neutralization was performed with sodium hydroxide, and subsequently the solution was centrifuged for 10 min at 1300 rpm. The residue was separated, and the remaining liquid was refrigerated. After the formation of white crystals, they were separated from the initial liquor and incubated at 37 °C for 24 h in a vacuum drying oven (Memmert, Schwabach, Germany). In the second method, 200 ml of concentrated whey (provided by Pegah Factory of Tabriz, Iran) was mixed with 0.22 ml of sulphuric acid and 0.25 g of urea and incubated at 55 °C for 48 h. Then, the solution was neutralized with sodium hydroxide and centrifuged (10 min, 1300 rpm) (Hettich, Tuttlingen, Germany). The supernatant was transferred to the refrigerator. The formed crystals were washed twice with distilled water and incubated in a vacuum oven (Memmert, Schwabach, Germany) for 24 h at 37 °C.

Experimental treatments were prepared as four separate total mixed ration (TMR) diets with different NPN sources. The test diets were as follows: (1) basal diet + urea (BDU), (2) basal diet + lactosylurea (first method) (BDL), (3) basal diet + concentrated lactosylurea (second method) (BDCL), (4) basal diet + Optigen (commercial slow-release NPN source) (BDO).

Chemical analysis of diets

Proximate analysis of individual feedstuffs was carried out according to the methods proposed by As-

Table 1. Ingredients and chemical composition of the experimental diets (% DM)

Item	Diet			
	BDU	BDL	BDCL	BDO
Ingredients, % DM				
corn silage	20.07	20.07	20.07	20.07
alfalfa hay	18.24	18.24	18.24	18.24
wheat straw	0.73	0.73	0.73	0.73
cottonseed, whole	7.36	7.36	7.36	7.36
beet sugar pulp	1.87	1.87	1.87	1.87
barley grain, ground	15.04	15.04	15.04	15.04
corn grain, ground	14.63	14.63	14.63	14.63
corn gluten meal	4.12	4.12	4.12	4.12
soybean meal (solvent extracted)	6.17	6.17	6.17	6.17
soybean seeds, whole, heated	3.43	3.43	3.43	3.43
sunflower meal, solvent	0.91	0.91	0.91	0.91
meat and bone, rendered	2.33	2.33	2.33	2.33
canola meal, mesh. extract	1.42	1.42	1.42	1.42
corn germ	0.73	0.73	0.73	0.73
wheat bran	0.32	0.32	0.32	0.32
calcium soap of fatty acids	0.5	0.5	0.5	0.5
salt	0.18	0.18	0.18	0.18
calcium phosphate (di)	0.41	0.41	0.41	0.41
calcium carbonate	0.41	0.41	0.41	0.41
magnesium oxide	0.09	0.09	0.09	0.09
sodium bicarbonate	0.5	0.5	0.5	0.5
vitamin & mineral premix	0.5	0.5	0.5	0.5
urea, g/d/cow	60	–	–	–
lactosylurea, g/d/cow	–	60	–	–
concentrated lactosylurea, g/d/cow	–	–	60	–
Optigen, g/d/cow	–	–	–	60
Composition, % DM				
CP	17	17	17	17
RDP	10.7	10.7	10.7	10.7
RUP	6.3	6.3	6.3	6.3
NDF	31.1	31.1	31.1	31.1
ADF	23.1	23.1	23.1	23.1
NFC	41.9	41.9	41.9	41.9
Ca	1	1	1	1
P	0.6	0.6	0.6	0.6
NEL, Mcal/kg	1.66	1.66	1.66	1.66
Lys/Met, g/day	3.12	3.12	3.12	3.12

DM – dry matter, CP – crude protein, RDP – rumen degradable protein, RUP – rumen undegradable protein, NDF – neutral detergent fibre, ADF – acid detergent fibre, NFC – non fibre carbohydrate, NEL – net energy for lactation, Lys – lysine, Met – methionine; corn silage – 28.98% DM and (DM basis): 48.97% NDF and 6.84% CP; alfalfa hay (DM basis): 42.70% NDF and 14.17% CP; wheat straw (DM basis): 56.77% NDF and 2.9% CP; cottonseed, whole (DM basis): 74.5% NDF and 7.9% CP; beet sugar pulp (DM basis): 37.48% NDF and 7.97% CP; barley grain, ground (DM basis): 15.53% NDF and 11.51% CP; corn grain, ground (DM basis): 12.32% NDF and 8.57% CP; corn gluten meal (DM basis): 31.33% NDF and 58.85% CP; soybean meal (solvent extracted) (DM basis): 14.85% NDF and 44.88% CP; soybean seeds, whole, heated (DM basis): 21.83% NDF and 34.83% CP; sunflower meal, solvent (DM basis): 35.83% NDF and 29.72% CP; meat and bone, rendered (DM basis): 49.62% CP; canola meal, mash. extract (DM basis): 31.53% NDF and 30.11% CP; corn germ (DM basis): 53.07% NDF and 18.23% CP; wheat bran (DM basis): 31.86% NDF and 14.43% CP; BDU – basal diet + urea, BDL – basal diet + lactosylurea (first method), BDCL – basal diet + concentrated lactosylurea (second method), BDO – basal diet + Optigen (commercial slow release non-protein nitrogen source)

sociation of Official Analytical Chemists (AOAC International, 2005), and it included the following parameters: dry matter (method 930.15), crude protein (method 984.13), ether extract (method 920.39) and crude ash (method 942.05). Neutral detergent fibre and ADF were determined according to the previously described method of Van Soest et al. (1991). Test diets were formulated according to NRC (2001) requirements for cows with an average milk yield of 39 kg.

Gas production test and *in vitro* digestibility

We loaded 300 mg of ground test treatments (Wiley Mill, 2 mm) into four 50 ml glass vials. Buffer solution (synthetic saliva) was prepared as described by McDougall (1948). Ruminal fluid from at least three freshly slaughtered cows (Palangi et al., 2022) was percolated through a 4-layer cheesecloth to a flask warmed at 39 °C, and promptly transferred to the laboratory. The strained ruminal inocula were mixed thoroughly at 39 °C together with synthetic saliva (1:2 v/v) to obtain a homogeneous digestion medium. Homogeneous digestion medium (20 ml) was added to glass vials in six replicates for each treatment; blank vials contained only digestion medium. The vials were placed in a shaker (Jal Teb, Tehran, Iran) set to 39 °C and 120 rpm (Shirmohammadi et al., 2020). Gas production data were recorded at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h of incubation (Fedorah and Hrudey, 1983; Gallo et al., 2016).

In vitro disappearance method

In vitro digestibility of the experimental samples was determined according to the method of Khajehdizaj et al. (2014). Briefly, along with the gas production test, five vials with the samples in digestion medium were incubated for 2, 12, 24 and 48 h. To release the gas produced in the vials during incubation, syringe needles were fitted to the sealed vial caps. After each hour of incubation, the vials were kept at −20 °C until further analyses. Prior to analyses, the vials were thawed at 39 °C and the contents transferred to 50 ml Falcon tubes. Subsequently, the tubes were centrifuged (Hettich, Tuttlingen, Germany) (10 min, 3000 *g*), the supernatants were pipetted out, and the pellets were rinsed three times with phosphate buffer (pH 7.4) (Wang et al., 2012). Sample residues were oven dried (Memmert, Schwabach, Germany) at 60 °C, weighed and analysed for crude protein (CP) content. Subsequently, CP digestibility was calculated according to the following equation:

$$\textit{in vitro}\ \text{CP digestibility} = \frac{\{[(\text{sample DM}) \times (\text{sample \% CP})] + [(\text{residue DM}) \times (\text{\% CP residue})]\}}{[(\text{sample DM}) \times (\text{sample \% CP})]} \times 100$$

Protozoan count

Ruminal fluid samples, collected from the slaughterhouse, were added to vials containing the experimental diet (in triplicate) and incubated for 12 h. The samples were removed from the incubator and protozoa were fixed with a formaldehyde solution (1:4). The number of protozoa was counted according to the method of Dehority et al. (1989).

Measurements of total volatile fatty acids

Total volatile fatty acid content in the samples was measured according to the method previously described by Markham (1942). Briefly, total volatile fatty acids of rumen fluid incubated in triplicate for 12 h were measured in two distillation and titration steps. After collection, about 50 ml of solution in distillation step was titrated by adding a few drops of phenolphthalein reagent with 0.05 N NaOH solution (Merck, Darmstadt, Germany).

Calculations and statistical model

All documented data were analysed in a complete randomised design using SAS software (version 9.2, the ANOVA procedure, Duncan's multiple range test). Gas production kinetics was calculated with the following model:

$$Y = A\,(1 - e^{-c(t + lag)}),$$

where: A is gas production from the immediately soluble and insoluble fraction, c is the rate constant (%/h) of gas production from the insoluble fraction, t is the incubation time (h), lag is the lag time (h), and Y is the volume of gas produced in time t.

Metabolisable energy (ME) (MJ/kg DM) and net energy for lactation (NEL) (MJ/kg DM) were calculated using the equations described by Getachew et al. (2002); digestible organic matter (DOM) and short-chain fatty acid (SCFA) level were estimated according to the formula provided by Menke et al. (1979):

$$\text{ME (MJ/kg DM)} = 1.06 + 0.157\,\text{GP} + 0.084\,\text{CP} + 0.220\,\text{CF} - 0.081\,\text{CA},$$

$$\text{NEL (MJ/kg DM)} = -0.36 + 0.1149\,\text{GP} + 0.0054\,\text{CP} + 0.0139\,\text{CF} - 0.0054\text{CA},$$

$$\text{DOM (\% DM)} = 9.00 + 0.9991\,\text{GP} + 0.0595\,\text{CP} + 0.0181\,\text{CA},$$

$$\text{SCFA (mmol/200 mg DM)} = 0.0222\text{GP} - 0.00425,$$

where: GP is 24 h net gas production (ml/200 mg DM), CP, CF and CA are crude protein, crude fat and crude ash (% DM), respectively.

Results and discussion

Gas production

The obtained gas production (ml/g DM) data of the experimental diets are listed in Table 2. According to the results, BDCL had the highest gas production volume (290.91 ml/g DM) after 96 h of incubation, which was significantly different from the BDL diet. The lowest volume of gas produced was found for BDU. There were no significant differences between treatments in the initial hours of incubation, but significant differences were observed after 6 h of incubation ($P < 0.05$). BDL and BDCL did not differ significantly up to 48 h, which could be due to the same NPN source.

Table 2. Cumulative gas production of treatment samples (ml/g of DM)

Incubation hour	Experimental diets				SEM	*P*-value
	BDU	BDL	BDCL	BDO		
2	29.56	26.49	26.76	29.56	0.92	0.074
4	62.86	64.12	63.86	65.52	1.37	0.664
6	86.75^{b}	94.22^{a}	94.35^{a}	95.42^{a}	1.94	0.039
8	116.68^{b}	127.48^{a}	127.68^{a}	128.42^{a}	2.16	0.008
12	170.02^{b}	186.62^{a}	190.68^{a}	189.47^{a}	2.16	0.0001
24	206.10^{b}	240.43^{a}	246.43^{a}	246.96^{a}	2.08	0.0001
48	232.59^{c}	254.46^{b}	268.72^{a}	264.46^{a}	2.66	0.0001
72	242.64^{c}	266.18^{b}	284.44^{a}	276.84^{a}	2.97	0.0001
96	248.98^{c}	273.04^{b}	290.91^{a}	283.58^{a}	3.10	0.0001
A, ml/g DM	230.99^{c}	276.33^{b}	286.28^{a}	288.79^{a}	1.78	0.0001
c, /h	0.103^{a}	0.093^{b}	0.090^{b}	0.087^{b}	0.003	0.0707
lag, h	0.88^{b}	1.090^{a}	1.109^{a}	0.962^{b}	0.041	0.0202

DM – dry matter, A – asymptotic gas production, c – fractional rate of fermentation, lag – lag time, SEM – standard error of the mean; BDU – basal diet + urea, BDL – basal diet + lactosylurea (first method), BDCL – basal diet + concentrated lactosylurea (second method), BDO – basal diet + Optigen (commercial slow release non-protein nitrogen source); ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

BDU showed a significant difference in the treatments containing lactosylurea and Optigen, which could be due to the faster release of ammonia from urea compared to the other NPN sources after 4 h of incubation. BDU showed the lowest gas production at all incubation times except for the first 4 h ($P < 0.05$). The present study measured a higher fermentation rate (A, ml/g DM) for BDCL and BDO; however, BDCL had a lower rate (c, ml/h) of gas production ($P < 0.05$). Ruminal bacterial consortium is able to produce biogas from different feed sources

(Zhang et al., 2016). Cherdthong and Wanapat (2010) reported the highest gas production for urea calcium (slow-release urea product) together with cassava chips. The latter authors reported that an increase in the extent and rate of fermentation was related to the energy source (cassava chips). High gas production by BDCL was likely associated with the presence of lactose as an energy source in concentrated lactosylurea. High cumulative gas production indicated high levels of metabolic energy, fermentable nitrogen and other nutrients conducive to the activity of ruminal microorganisms (Menke et al., 1979). Gas production is positively correlated with dry matter digestibility, indicating that it is an integral part of feed fermentation. Typically, higher gas production is associated with the carbohydrate fraction of the feed compared to other nutrients. The amount of gas produced in the early hours was due to differences in the level of nonstructural carbohydrates, such as sugars, pectin, and starch, which rapidly ferment and produce gas (Menke and Steingass, 1988), however, we did not observe any significant differences in the initial hours of the experiment ($P < 0.05$).

Calculated gas production parameters, including ME, NEL, DOM and SCFA are presented in Table 3.

The highest values of the calculated parameters were recorded for BDCL and BDO ($P < 0.05$), while the lowest for BDU. BDL showed significant differences compared to the remaining treatments ($P < 0.05$). Recently, Besharati et al. (2019) conducted a study regarding the effect of adding whey and *L. buchneri* to alfalfa silage on *in vitro* gas production and degradability, and reported that fresh whey supplementation increased the analysed gas production parameters.

Table 3. Estimated gas production parameters

Item	Experimental diets				SEM	*P*-value
	BDU	BDL	BDCL	BDO		
ME, MJ/kg DM	10.54^{c}	11.61^{b}	11.80^{a}	11.82^{a}	0.025	0.0001
NEl, Mj/kg DM	4.56^{c}	5.35^{b}	55.49^{a}	5.50^{a}	0.019	0.0001
DOM, % DM	51.32^{c}	58.11^{b}	59.36^{a}	59.45^{a}	0.165	0.0001
SCFA, mmol/200 mg DM	0.91^{c}	1.06^{b}	1.08^{a}	1.09^{a}	0.0004	0.0001

ME – metabolisable energy, NEL – net energy for lactation, DOM – digestible organic matter, SCFA – short chain fatty acid, SEM – standard error of the mean; BDU – basal diet + urea, BDL – basal diet + lactosylurea (first method), BDCL – basal diet + concentrated lactosylurea (second method), BDO – basal diet + Optigen (commercial slow release non-protein nitrogen source); ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Apparent degradability of dry matter

The mean dry matter disappearance data are presented in Table 4. According to the results, BDCL and BDO showed the highest degradability after 12 h of incubation ($P < 0.05$). Below 12 h of incubation, no significant differences were recorded between the treatments ($P < 0.05$). After 24 h, significant differences were found not only for BDU, but also BDL. The time-delayed difference could be caused by the amount of nutrients present in whey as the basic substance in lactosylurea production.

Table 4. *In-vitro* disappearance of dry matter (DM) and crude protein

Item	Incubation time, h			
	2	12	24	48
In-vitro disappearance of dry matter, % DM				
BDU	21.6	34.6^{b}	44.9^{c}	53.0^{c}
BDL	21.5	38.5^{a}	46.5^{b}	55.6^{b}
BDCL	21.8	38.5^{a}	49.8^{a}	59.4^{a}
BDO	21.9	38.2^{a}	49.5^{a}	59.2^{a}
SEM	0.43	0.38	0.29	0.46
P-value	0.881	0.0001	0.0001	0.0001
In-vitro disappearance of crude protein, % DM				
BDU	13.26	30.33^{b}	38.09^{c}	45.85^{c}
BDL	13.50	34.83^{a}	43.02^{b}	51.97^{b}
BDCL	13.86	34.65^{a}	47.41^{a}	55.22^{a}
BDO	13.46	33.89^{a}	47.62^{a}	55.38^{a}
SEM	0.64	0.84	0.99	0.66
P-value	0.927	0.005	0.0001	0.0001

SEM – standard error of the mean; BDU – basal diet + urea, BDL – basal diet + lactosylurea (first method)], BDCL – basal diet + concentrated lactosylurea (second method), BDO – basal diet + Optigen (commercial slow release non-protein nitrogen source); ^{abc} – means within a column with different superscripts are significantly different at $P < 0.05$

Lactosylurea primarily contains CHO, N and minerals, which could affect digestibility. Chamebon et al. (2017) observed that dry matter degradability increased after urea addition to the treatments (orange pomace with 38.5% wheat straw and 1.5% urea, and orange pomace with 37% wheat straw and 3% urea) compared to non-urea treatment. Mahmoudi-Abyane et al. (2018) studied the effect of utilising different nitrogen sources on digestibility and nitrogen balance in Mehraban lambs. These authors reported the lowest and highest digestibility of NDF and ADF for diets containing soybean meal and slow-release urea, respectively. These results suggested that diets with a slow-release urea source of nitrogen can meet the requirements of cellulolytic bacteria, i.e. the main ammonia consumers in the rumen, consequently improving fibre digestion and

rumen microbial activity (Castro et al., 1999). Ruminal bacteria can receive 40–95% of nitrogen from ammonia depending on the diet, and using urea can create a balance of peptides and amino acids (Nolan et al., 1993). In a previous study, fibre digestibility increased when Optigen was replaced with soybean meal and oilseed rape meal, although ammonia nitrogen was high in both treatments (Sinclair et al., 2012). It was reported that adding 1.8 kg of slow-release urea supplementation to the beef diet containing sugarcane, cane molasses and maize significantly improved the digestibility of dry matter and NDF (Galina et al., 2003).

The apparent degradability of crude protein

The mean crude protein degradability data from the experimental treatments are presented in Table 4. According to the obtained results, the highest degradability was observed in the case of BDCL and BDO, while the lowest degradability was recorded for BDU. Treatments showed no significant deviations at 2 h, but differences became significant after 12 h ($P < 0.05$). This could be caused by the presence of lactose in BDCL and BDO, which was characterised by nitrogen release as opposed to other two treatments; Optigen, as a commercial slow-release urea product, showed highly similar kinetics to BDCL. A recent report by Sevim and Önol (2019) concerning the effect of slow-release urea supplementation on some feed digestibility and rumen parameters, showed that the use of slow release urea increased feed protein digestibility which was consistent with our findings.

Protozoa count

The results regarding the number of protozoa are presented in Table 5. The abundance of protozoa in the BDCL and BDO treatments was significantly higher compared to the other treatments ($P < 0.05$). The absence of protozoa reduces bacterial predation (Takahashi et al., 2005), resulting in decreased end product contents from ruminal bacterial degradation, while increasing the flow of microbial protein into the lower gastrointestinal tract (Hess et al., 2004). Protozoa can utilise starch granules, thereby creating a balance in the rumen environment and improving cellulose digestion (Orpin, 1984). Eugène et al. (2004) found that the use of high levels of concentrate could reduce the population of protozoa due to reduced ruminal pH. In the present study, the diets contained equal concentrate to forage ratios and the only source of variation was related to NPN origin; therefore, the difference in the number of protozoa cannot be related to the above fact. Protozoa use cellulose and starch as energy sources, and ruminal bacteria and insoluble proteins as a nitrogen sources (Coleman, 1986; Jouany, 1996). High-concentrate diets provide digestible energy sources for protozoa, stimulating their growth and allowing to more effectively compete with ruminal bacteria (Yuste et al., 2019). Based on the results of this study, it can be suggested that diets containing lactosylurea and Optigen provide easily available nitrogen sources for both ruminal bacteria and protozoa.

Table 5. Total volatile fatty acid and protozoan counts in rumen fluid

Item	Experimental diets				SEM	*P*-value
	BDU	BDL	BDCL	BDO		
VFA, mmol/l	30.96^{b}	101.29^{a}	104.44^{a}	104.60^{a}	1.18	0.0001
Protozoa, 10^6 organisms/ml	2.66^{c}	4.06^{b}	4.66^{a}	4.80^{a}	0.13	0.0001

VFA – volatile fatty acids, SEM – standard error of the mean; BDU – basal diet + urea, BDL – basal diet + lactosylurea (first method), BDCL – basal diet + concentrated lactosylurea (second method), BDO – basal diet + Optigen (commercial slow release non-protein nitrogen source); ^{abc} – means within a column with different superscripts are significantly different at $P < 0.05$

Ruminal total volatile fatty acids

The results of the determination of ruminal total VFA (mmol/ml rumen fluid) after 12 h of incubation are presented in Table 5. Lower concentration of volatile fatty acids was observed in BDU and higher levels were recorded for the other treatments ($P < 0.05$). It could be explained by the fact that the levels of ruminal VFA were significantly higher in the treatments containing slow-release urea source in comparison to the other NPN sources ($P < 0.05$). Which can lead to an increase in final products of ruminal microbial protein synthesis in the rumen fluid. Hence, higher microbial protein synthesis probably occurred in three treatments containing slow-release urea sources in comparison to the diet containing unprocessed urea (BDU). The total VFA concentration in the rumen can differ significantly depending on the diet variability and the time elapsed from the previous meal (Jeong et al., 2016). Taylor-Edwards et al. (2009) tested 1.6% urea supplementation in calves and reported VFA levels at 99.7 (mmol/ml), whereas in our study, the treatment containing 0.26% urea in the TMR diet resulted in 30 mmol/ml VFA. The reason for this difference could be related to various urea levels and incubation time. The same group estimated the VFA levels for the treatment containing 1.6% urea (103.2 mmol/ml), which was consistent with the results of our studies (BDCL and BDO).

Pinos-Rodríguez et al. (2010) estimated ruminal total VFAs in treatments containing 0.6% and 1.1% Optigen at 97.6 and 94.8 mmol/l, respectively. The higher percentage of Optigen reduced this parameter, which was in line with the data obtained for the diet containing Optigen (BDO). In an experiment with dairy cows, Xin et al. (2010) did not find any differences between urea and coated urea products in terms of total VFA levels, which contrasted with the results of the current study.

Conclusions

Lactosylurea can be synthesized using urea and whey (secondary by-products of the human food industry). Lactosylurea appears to be a suitable alternative for commercial NPN sources. Therefore, in addition to protecting the environment, it can be applied in sustainable dairy farming.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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