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Effects of 00-rapeseed meal inclusion in Parmigiano Reggiano hay-based ration on dairy cows' production, reticular pH and fibre digestibility

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ABSTRACT

Hay-based diets are typically used in Parmigiano Reggiano cheese production. Parmigiano Reggiano feeding regulation prohibits 00-rapeseed dietary inclusion. The objectives of this study were to investigate the effects of substituting soybean-meal with different levels of 00-rapeseed-meal in dairy cows' diets, to evaluate the possibility to include it in Parmigiano Reggiano regulation. The study had a Latin square design with 8 tie stall dairy cows. The isoenergetic and isoproteic dietary treatments differed in protein source (% of DM): S (0.0% 00-rapeseed-meal, 9.3% of soybean-meal), LR (3.8% 00-rapeseed-meal, 6.8% of soybean-meal), MR (8.5% 00-rapeseed-meal, 3.4% of soybean-meal), and HR (13.2% 00-rapeseed-meal, 0.0% of soybean-meal). DMI, milk production and composition, rumination and reticular-pH were recorded daily. Dietary fibre digestibility was evaluated by *in vitro* fermentation and milk goitrin content was quantified by liquid chromatography–tandem mass spectrometry. Statistical analysis was performed by mixed model. Milk yield, protein and casein content increased for incremental 00-rapeseed-meal dietary levels. MR treatment compared to the others resulted in higher daily reticular-pH (5.92), and fewer minutes with pH below 5.8 (467) and 5.5 (72). Goitrin was detectable in milk when 00-rapeseed-meal was included in the diet. Overall, the inclusion of 00-rapeseed-meal in the Parmigiano Reggiano type ratio did not compromise the performances of cows pointing to it as a reliable substitute for soybean-meal. Our results suggest that 00-rapeseed-meal should be tested in feeding studies to determine its effects on milk organoleptic characteristics and cheese production and quality to see if it can be included in the Italian PDO cheese regulation.

HIGHLIGHTS

- 00-rapeseed inclusion on hay-based diet improve DMI, ECM and rumen health
- 00-rapeseed-meal introduction in Italian PDO cheese regulation is suggested

Abbreviations: TMR: Total mixed ration; S: Soy diet; LR: Low rapeseed meal inclusion diet; MR: Medium rapeseed meal inclusion diet; HR: High rapeseed meal inclusion diet; DM: Dry matter; DMI: Dry matter intake; ECM: Energy corrected milk; PDO: Protected designation of origin; PR: Parmigiano Reggiano; GP: Grana Padano; BW: Body weight; SD: Standard deviation; CP: Crude protein; aNDFom: amylase- and sodium sulfite-treated neutral detergent fibre with ash correction; ADF: Acid detergent fibre; PEF: Physically effectiveness factor; peNDF: Physically effective neutral detergent fibre; IVNDFD: *In vitro* neutral detergent fibre digestibility; ADL: Acid detergent lignin; uNDF: Unavailable neutral detergent fibre; SCS: Somatic cell score; FCM: Fat corrected milk; r-pH: Reticular pH; AUC: Area under the curve; pdNDF: Potentially digestible neutral detergent fibre; TTD: Total tract digestibility; NDIP: Neutral detergent insoluble protein; ADIP: Acid detergent insoluble protein

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Introduction

Parmigiano Reggiano (PR) is one of the best-known and most exported Italian cheeses all over the world (Mordenti et al. 2017). It is an extra-hard, long-ripened

cheese produced from partially skimmed raw cow milk and it is registered as a Protected Designation of Origin (PDO) cheese (Council Regulation (EC) No. 510, 2006). To obtain the PDO status, a product must be

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entirely manufactured within a delimited geographic area and follow specific processing techniques and feeding rules for cows (Ministero Politiche Agricole Ambientale e Forestali (MIPAAF) 2016; Mordenti et al. 2017; Disciplinary di Produzione del Formaggio Parmigiano Reggiano 2019).

In PR production, dietary inclusion of silages, non-protein nitrogen sources (i.e. urea), added fat and many byproducts (like distillers) are not permitted. Dry hays must be included at least of 45–50% of dietary dry matter (DM) and alfalfa hay represent one of the main protein source in the rations. It is well known that the forage quality impacts cow performance improving dry matter intake and productivity, as well as rumen fermentations (Ferraretto et al. 2015; Fustini et al. 2017; Miller et al. 2021). Hays of good quality can be obtained harvesting plants at the proper stage of maturity and well preserved over the year (Palmonari et al. 2014, 2016). However, other protein sources are usually included in the diets to cover specific cow's requirements. Major protein sources used are represented by different by-products, especially soybean or sunflower meal, corn gluten feed, wheat bran and other protein sources like peas and lupins (Mordenti et al. 2007).

In Europe, rapeseed with low levels of erucic acid (<2%) and glucosinolates (<30 $\mu\text{mol/g}$) are called "double-zero" or "double-low" rapeseeds or 00-rapeseeds, while in North America, such varieties are identified as canola (Shahidi 1990; Newkirk 2009). 00-rapeseed is commonly cultivated in Europe, and represent the second most traded protein source in the global animal feed market (Carré and Pouzet 2014). Actually, 00-rapeseed meal cannot be fed to the cows producing milk for PR and Grana Padano cheese (Disciplinary Grana Padano DOP 2019). The use of 00-rapeseed is not allowed because of the concern that some compounds like glucosinolates, among which goitrin is the most concentrated, could be transferred to the milk and cheese. These molecules have several effects on animal and human health, and can also compromise milk and cheese sensorial characteristics (EFSA: Scientific Panel on Contaminants in the Food Chain 2008). Bertuzzi et al. (2016) showed the possibility to detect those compounds in milk by liquid chromatography-tandem mass spectrometry.

Previous studies indicated that replacing soybean with canola meal leads to an increased dry matter intake (DMI) and milk composition, due to its better amino acid profile (Huhtanen et al. 2011; Martineau et al. 2013). Broderick et al. (2015) reported that feeding canola meal instead of soybean meal increased

the feed intake, milk yield, energy-corrected milk (ECM), and true protein. However, PR cow diets, based on dry hays, have important differences compared to diets commonly fed to dairy cows in others part of the world commonly based on silages (Puhakka et al. 2016; Sánchez-Duarte et al. 2019; Paula et al. 2020) or hay-silage mixture (Martineau et al. 2014, 2019). Thus, the replacement of soybean with 00-rapeseed meal in these conditions could have different effects on cow performances, rumen health and fibre digestibility.

Therefore, the objective of this study was to test the effect of 00-rapeseed meal inclusion in a typical Italian PR diet. In particular, we tested these effects in high producing dairy cows, fed hay-based diets with different protein sources, soybean or 00-rapeseed meal at different levels, and, at the same time, we verified the possibility to detect goitrin in milk produced. Our hypothesis is based on the possibility to include 00-rapeseed meal on PR type diets without any negative effects on production and rumen health.

Materials and methods

The study was conducted at the experimental dairy farm of the Department of Veterinary Medical Science of University of Bologna (Italy). All experimental procedures involving animals were approved by the University of Bologna Animal Care and Use Committee.

Experimental design and data collection

Eight multiparous Holstein cows (avg. BW 699 ± 55 kg, mean \pm SD) were blocked by parity (3.13 ± 1.09 d, mean \pm SD), milk production (38.53 ± 7.59 kg/d, mean \pm SD), and days in milk (87 ± 28 d, mean \pm SD), and used in a replicated 4×4 Latin square design study with 21d periods, with 14 d of adaptation and 7 d of collection.

Animals were housed in a naturally ventilated tie-stall barn and milked twice a day (0800 am, and 0730 pm) in a double-five herring-bone milking parlour. Meadow hay-based diets differed for major protein sources (soybean or 00-rapeseed meal), while other components were the same in all diets. Rations were formulated (DinaMilk5, Fabermatica, Ostiano, Italy) to mimic common diets used in the PR cheese production area of Italy, consisting of all dry and non-fermented components given as TMR, replacing soybean meal with increasing levels of 00-rapeseed meal. The objective was to formulate isonitrogenous and isoenergetic diets, and these results were achieved

with different inclusion of steam flaked corn and grass hay to balance the relative content in protein and fibre of soybean and 00-rapeseed meal. Treatments were identified as control (S; 0% DM basis of 00-rapeseed meal and 9.3% of soybean-meal), low 00-rapeseed meal inclusion (LR; 3.8% 00-rapeseed meal, 6.8% of soybean-meal), medium inclusion (MR; 8.5% 00-rapeseed meal, 3.4% of soybean-meal) and high 00-rapeseed meal (HR; 13.2% 00-rapeseed meal, 0.0% of soybean-meal). Diets were offered in individual feed bunks as a TMR for ad libitum intake (approximately 1.10× expected intake) once a day at 0800 am (Zago Mixer; Padova, IT).

Individual DMI was determined daily by recording feed offered and refused. Water intake was determined daily by individual water metre. Then, water intake per unit DMI was calculated. Individual feed ingredients were collected weekly and dried in a forced-air oven at 65 °C for 48 h for DM. Samples of diets and orts were collected daily and dried in a forced-air oven at 65 °C for 48 h for DM determination. Feedstuff and TMR were analysed for chemical composition according to the following methods: neutral detergent fibre (aNDFom) and acid detergent fibre (ADF), and crude protein (CP) according to Mertens et al. (2002), and AOAC 973.18, respectively. Starch was determined according to AOAC official method (AOAC 996.11) and ether extract according to AOAC 920.390020. Composite samples of each diet were used to evaluate particle size distribution on as-fed basis to determine the physical effectiveness factor (PEF) using a RoTap Separator (W.S. Tyler, Mentor, OH). Physically effective neutral detergent fibre (peNDF) of diets was calculated as:

$$\text{peNDF, \% of DM} = \text{aNDFom} * \text{PEF}$$

where *PEF* is the physical effectiveness factor, as described by Heinrichs (2013).

In vitro aNDFom digestibility (24, and 240 h) were determined using *in vitro* fermentation in buffered media containing ruminal fluid (Palmonari et al. 2016). The four donor animals were fed respectively two the S and two the HR diet. Rumen fluid used for the inoculation was a mix of the four collected. Digestibility was performed on forage, TMR and faeces according to the procedure described by Palmonari et al. (2017a, 2017b). Briefly, *in vitro* aNDFom digestibility at 24 h and 240 h (IVNDFD_{24h} and IVNDFD_{240h}) was performed using the Tilley and Terry modified technique (Palmonari et al. 2017a, 2017b). Faeces were analysed for aNDFom, ADF, acid detergent lignin (ADL), undigestible NDF (uNDF₂₄₀) and potentially digestible NDFom (pdNDFom), in order to calculate

total tract digestibility of pdNDFom (TTPdNDFomD) according to the procedure described by Fustini et al. (2017).

In each collection period, multiple time points faecal samples were collected: on day 16 at 0800 am and 1100 pm, on day 17 at 0200 pm, on day 18 at 0500 am and 0800 pm, on day 19 at 1100 am and 0500 pm, and on day 20 at 0200 am.

TMR composition is reported in Table 1.

Chemical composition of the diets was similar among treatments, and the CP amount was 14.6% on average. Theoretical metabolisable energy (Mcal/kg/DM) content resulted similar among treatments (S = 2.34, LR = 2.33, MR = 2.34, HR = 2.35) as well as theoretical metabolisable protein content expressed as g/kg/DM (S = 102.04, LR = 101.14, MR = 101.70, HR = 102.17). Physically effective fibre average value resulted 15.55% of DM. The amount of peNDF of TMR decreased as 00-rapeseed meal inclusion level increased (S = 16.01, LR = 16.01, MR = 15.41, HR = 14.76, % of DM).

Cows were milked twice a day and individual milk yield was recorded daily (Afimilk Information Management System; Afikim, Israel) during d 15–21 of each experimental period. Milk samples from two consecutive milkings for each cow were collected on d 17 and 18 of each period, preserved (Bronolab-W II Liquid Preservative; D & F Control Systems, Inc., Dublin, CA) and analysed by a qualified laboratory (Artest SpA, Modena, Italy) for fat, total protein, lactose, casein, somatic cell score (SCS), expressed as Log 10 base (Lacy-Hulbert et al. 1999), urea, titratable acidity, and pH, as described in Mammi et al. (2018a), and goitrin as described in Bertuzzi et al. (2016). Energy-corrected milk yield (ECM) and 3.5% fat-corrected milk (FCM) were calculated according to Davidson et al. (2008). Feed efficiency as milk/DMI (kg/kg), 3.5% fat-corrected milk/dry matter intake, and solids-corrected milk/dry matter intake were calculated during the experimental weeks.

Cows were monitored for rumination activity from day 15 to 21 of each period. Rumination time was measured using the Hi-Tag rumination monitoring system (SCR Engineers Ltd., Netanya, Israel). Data flow software analysed the rumination time with a resolution of 2 h (Schirmann et al. 2009) and calculated the rumination time during the last 24 h.

To continuously monitor pH, all cows received an indwelled wireless pH-transmitting unit (SmaXtec Animal Care Sales GmbH, Graz, Austria), which has been validated with rumen-cannulated dairy cows (Klevenhusen et al. 2014). These units (3.5 cm i.d. 12 cm long, and weighing 210 g) were calibrated following the manufacturer's instruction protocol and

Table 1. Experimental diet and chemical composition (mean \pm SD).

Item	00-rapeseed levels, % dry matter (DM)			
	0.0 ^a	3.8 ^b	8.5 ^c	13.2 ^d
Ingredient, g/kg DM				
Grass hay	426	395	368	331
Steam flaked corn	137	151	165	188
Soybean meal	92	68	34	–
Rapeseed meal	–	38	85	132
Cane-beet molasses blend	35	36	36	36
Grain mix ^e	309	313	313	313
Chemical composition				
DM, g/kg	889 \pm 10	891 \pm 14	890 \pm 13	894 \pm 13
Ether extract, g/kg DM	28 \pm 3	29 \pm 3	30 \pm 4	32 \pm 3
Ash, g/kg DM	80 \pm 4	80 \pm 4	79 \pm 4	79 \pm 4
aNDFom ^f , g/kg DM	323 \pm 27	313 \pm 26	313 \pm 25	304 \pm 20
ADF, g/kg DM	211 \pm 19	201 \pm 18	197 \pm 18	187 \pm 15
ADL, g/kg DM	28 \pm 3	28 \pm 3	29 \pm 3	29 \pm 3
IVNDFD ^g 24 h, g/kg aNDFom	450 \pm 21	448 \pm 24	435 \pm 24	423 \pm 21
IVNDFD ^g 240 h, g/kg aNDFom	729 \pm 19	736 \pm 25	732 \pm 20	734 \pm 21
uNDF 240h ^h , g/kg DM	87 \pm 10	85 \pm 18	84 \pm 10	81 \pm 9
peNDF ⁱ , g/kg DM	160 \pm 16	160 \pm 16	154 \pm 14	148 \pm 15
Starch, g/kg DM	248 \pm 18	252 \pm 17	254 \pm 19	265 \pm 20
Sugar, g/kg DM	74 \pm 6	76 \pm 7	76 \pm 7	75 \pm 7
CP, g/kg DM	144 \pm 11	148 \pm 11	147 \pm 11	145 \pm 8
Soluble protein, g/kg DM	42 \pm 4	44 \pm 4	44 \pm 5	44 \pm 4
NDIP ^j , g/kg DM	22 \pm 4	23 \pm 3	22 \pm 4	21 \pm 4
ADIP ^k , g/kg DM	10 \pm 1	10 \pm 1	10 \pm 1	11 \pm 1

^aControl diet (S).^bLow rapeseed meal inclusion diet (LR).^cMedium rapeseed meal inclusion diet (MR).^dHigh rapeseed meal inclusion diet (HR).^eGrain mix ingredients: 418 g/kg wheat bran, 209 g/kg sorghum grain, 209 g/kg maize fine, 105 g/kg flaked fullfat soybean, 21 g/kg calcium carbonate, 21 g/kg cane molasses, 11 g/kg sodium chloride, 3 g/kg magnesium oxide and 3 g/kg vitamin and mineral premix (provided per kg of mix: 40,000 IU/kg vitamin A, 4,000 IU/kg vitamin D3, 300 mg/kg vitamin E 920 g/kg α -tocopherol, 50 mg/kg vitamin B1, 30 mg/kg vitamin B2, 15 mg/kg vitamin B6, 0.6 mg/kg vitamin B12, 50 mg/kg vitamin K, 50 mg/kg vitamin H1 (para-aminobenzoic acid), 1.5 g/kg vitamin PP (niacin), 500 mg/kg choline chloride, 1 g/kg Fe, 1 mg Co, 50 mg/kg I, 1.2 g/kg Mn, 100 mg/kg Cu, and 1.3 g/kg Zn).^fAmylase- and sodium sulfite-treated NDF with ash correction.^g*In vitro* NDF digestibility, g/kg aNDFom.^hUnavailable NDF estimated via 240-h *in vitro* fermentation.ⁱPhysically effective NDF (aNDFom*pef), calculated using the Ro-Tap system (Heinrichs 2013).^jNeutral detergent insoluble protein (Neutral detergent insoluble nitrogen*6.25).^kAcid detergent insoluble protein (acid detergent insoluble nitrogen*6.25).

CP: crude protein.

then manually inserted into the reticulorumen via the oesophagus, on day 14 of the first period. The units measured pH and temperature every 10 min and transmitted the data in real-time to a dock station using the ISM band (433 MHz). Both data of pH and temperature were collected using an analog-to-digital converter and stored in an external memory chip. Data of pH from d 15 to d 21 of each period were analysed as daily mean pH and time (min/d) below specific cut-off points (5.5 and 5.8). Because wireless sensors were located in the ventral reticulorumen and little differences were observed between pH measured by wireless units and in the ventral rumen sac (Klevenhusen et al. 2014), the term “reticular pH” (r-pH) will be used.

Statistical analysis

The Latin square is designed to evaluate any possible statistically significant inferences even with a small number of animals involved in a study (Kononoff and Hanford 2006).

As reported in a similar study (Cavallini et al. 2018), data for DMI, milk yield and composition, feed efficiency, fibre digestibility and body weight were analysed as a replicated Latin square design with model effects for diet using the RELM procedure of JMP (version 12.0 pro, Statistical Analysis Systems Institute Inc., Cary, NC). Cow within replicate and period was considered as a random effect. Least square means were separated using the Tukey's procedure when a significant *F* test ($p < .05$) was detected. Moreover, sums of squares were further separated using orthogonal contrasts into single degree of freedom comparisons to test the significance of linear, quadratic and cubic effects of 00-rapeseed meal inclusion.

Results

Results of DMI, water intake, body weight and rumination time are reported in Table 2

The inclusion of rapeseed tended to increase DMI ($p = .04$): in particular, $a + 0.30, +0.43, +0.53$ kg of DMI

Table 2. Effect of 00-rapeseed meal on DMI, water intake, BW and rumination in dairy cow fed PR type ration.

Item	00-rapeseed levels, % DM				SEM	p value			
	0.01 ¹	3.82 ²	8.53 ³	13.24 ⁴		TRT	Linear	Quadratic	Cubic
DMI ⁵ , kg/d	23.35 ^b	23.65 ^{ab}	23.88 ^a	23.78 ^{ab}	1.23	0.04	0.272	0.522	0.850
Water intake, L/d	166.86	183.98	154.53	173.86	2.25	0.18	0.386	0.613	<.001
Water intake/DMI ⁵ , L/kg	7.22 ^{AB}	8.09 ^A	6.51 ^B	7.40 ^{AB}	0.89	<.01	0.090	0.949	<.001
BW ⁶ , kg	670.95	687.29	679.18	679.93	21.02	0.25	0.077	0.001	0.002
Rumination, min/d	347.31	320.51	327.69	308.94	41.78	0.61	0.010	0.664	0.149

^{a,b,c}Values within rows with different superscripts differ ($p \leq .05$).

^{A,B,C}Values within rows with different superscripts differ ($p \leq .01$).

¹Control diet (S)

²Low rapeseed meal inclusion diet (LR)

³Medium rapeseed meal inclusion diet (MR)

⁴High rapeseed meal inclusion diet (HR)

⁵DMI: dry matter intake

⁶BW: body weight.

PR: Parmigiano Reggiano.

Table 3. Effect of rapeseed meal on ruminal pH in dairy cow fed PR type ration.

Item	00-rapeseed levels, % DM				SEM	p value			
	0.0	3.8 ²	8.5 ³	13.2 ⁴		TRT	Linear	Quadratic	Cubic
Daily average r-pH	5.88 ^B	5.84 ^B	5.92 ^A	5.71 ^C	0.09	<.01	<.001	<.001	<.001
Time r-pH < 5.8, min/d	550.8	689.6	467.2	758.4	181.12	0.13	<.001	0.002	<.001
Time r-pH < 5.5, min/d	127.1	239.0	72.1	336.1	113.40	0.10	<.001	<.001	<.001
AUC ⁵ r-pH < 5.8	107.2 ^b	165.0 ^b	72.1 ^c	255.0 ^a	7.35	0.04	<.001	<.001	<.001
AUC ⁵ r-pH < 5.5	15.0 ^b	23.4 ^b	6.0 ^c	97.1 ^a	3.53	0.02	<.001	<.001	<.001

^{a,b,c}Values within rows with different superscripts differ ($p \leq .05$).

^{A,B,C}Values within rows with different superscripts differ ($p \leq .01$).

¹Control diet (S)

²Low rapeseed meal inclusion diet (LR)

³Medium rapeseed meal inclusion diet (MR)

⁴High rapeseed meal inclusion diet (HR)

⁵AUC: area under curve, calculate like area under pH 5.8 and 5.5.

PR: Parmigiano Reggiano.

were observed in LR, HR, MR respectively, compared to control treatment (S diet). Moreover, the different 00-rapeseed levels influenced the water intake ($p < .001$) and the water intake/DMI ($p < .001$) in a cubic manner. Medium inclusion level (8.5% DM) resulted in the lowest water intake per unit DMI ($p < .01$). BW resulted quadratically influenced by the treatment ($p = .001$). Daily rumination time was not influenced by the treatments, however, it linearly decreased ($p = .01$) according to the incremental 00-rapeseed and corn flakes levels, and the reduction of grass hay in the ration.

Data of daily reticular pH, time and AUC under values of 5.8 and 5.5 are reported in Table 3.

Inclusion of rapeseed led to a significant variation in daily average r-pH ($p < .01$), and the major difference was observed between S and HR (5.88 vs. 5.71, respectively). Diet LR showed intermediate values (5.84) between S and HR, while MR registered the highest pH value (5.92) and lower minutes and area ($p = .04$) under the curve (AUC). In particular, AUC for pH < 5.8 resulted as: 72.1, 107.2, 165.0, and 255.0 in

MR, S, LR and HR, respectively and 6.0, 15.0, 23.4, and 97.1 in MR, S, LR, and HR treatment, respectively, for pH < 5.5. Finally, inclusion of 00-rapeseed led to a cubic variation in r-pH parameters ($p < .001$).

Data of milk yield and components are reported in Table 4.

Milk production, ECM and FCM resulted significantly higher in diet MR (8.5% DM). Specifically, raw milk production was 32.30, 33.28, 34.44 and 33.46 kg/d in S, LR, MR and HR treatment, respectively ($p = .01$); ECM was 32.47, 33.73, 34.63 and 33.64 kg in S, LR, MR and HR treatment, respectively ($p = .05$); while FCM was 30.04, 30.84, 31.97 and 30.61 kg in S, LR, MR and HR treatment, respectively ($p = .04$). Moreover, incremental levels of 00-rapeseed had a significant impact on milk yield, both linear ($p = .012$) and quadratic.

Milk fat concentration (g/kg) linearly decreased ($p = .016$), while daily milk fat production (g/d) resulted higher in MR diet ($p = .01$).

Milk protein concentration (g/kg) tended to be higher in rapeseed diets ($p = .08$), as well as casein content (+0.14 on avg, $P = .07$), and when the daily

Table 4. Effect of rapeseed meal on milk production and quality in dairy cow fed PR type ration.

Item	00-rapeseed levels, % DM				SEM	p value			
	0.0	3.8 ²	8.5 ³	13.2 ⁴		TRT	Linear	Quadratic	Cubic
Milk, kg/d	32.30 ^b	33.28 ^b	34.44 ^a	33.46 ^b	3.42	0.01	0.012	0.017	0.201
ECM ⁵ , kg/d	32.47 ^b	33.73 ^b	34.63 ^a	33.64 ^b	3.39	0.05	0.425	0.365	0.782
FCM ⁶ 4%, kg/d	30.04 ^b	30.84 ^b	31.97 ^a	30.61 ^b	3.02	0.04	0.558	0.317	0.560
Milk kg/DMI kg	1.39	1.42	1.45	1.42	0.09	0.37	0.557	0.457	0.726
Fat, g/kg	35.5	34.1	34.3	32.8	0.91	0.10	0.016	0.959	0.322
g/d	1139 ^{AB}	1118 ^{AB}	1184 ^A	1061 ^B	33.8	0.02	0.333	0.187	0.110
Protein									
g/kg	29.5	31.6	31.0	31.6	0.41	0.08	0.001	0.041	0.021
g/d	957 ^b	1045 ^a	1066 ^a	1043 ^{ab}	83.7	<.01	0.082	0.121	0.880
Casein									
g/kg	22.9	24.4	24.0	24.4	0.68	0.07	0.001	0.033	0.020
g/d	744 ^b	809 ^{ab}	825 ^a	810 ^{ab}	23.0	<.01	0.099	0.163	0.892
Lactose									
g/kg	48.5	47.9	48.3	47.6	0.86	0.12	0.075	0.973	0.116
g/d	1577	1605	1668	1613	88.0	0.49	0.550	0.515	0.595
SCS, Log	2.11	2.38	2.37	3.03	0.25	0.21	0.004	0.362	0.331
Urea, mg/dL	24.88	23.70	23.53	20.67	0.87	0.08	0.004	0.008	0.001
SH°50, degrees	3.04	3.14	3.15	3.10	0.16	0.16	0.454	0.185	0.896
Milk pH	6.71	6.73	6.71	6.73	0.02	0.59	0.161	0.809	0.122
Goitrin, µmol/g	0.00 ^c	0.32 ^{bc}	0.61 ^{AB}	0.73 ^A	0.07	<.01	<.001	<.001	<.001

^{a,b,c}Values within rows with different superscripts differ ($p \leq .05$).

^{A,B,C}Values within rows with different superscripts differ ($p \leq .01$).

¹Control diet (S)

²Low rapeseed meal inclusion diet (LR)

³Medium rapeseed meal inclusion diet (MR)

⁴High rapeseed meal inclusion diet (HR)

⁵ECM: energy corrected milk.

⁶FCM: fat corrected milk.

PR: Parmigiano Reggiano.

Table 5. Effect of rapeseed meal on faecal fibre content and total tract fibre digestibility in dairy cow fed PR type ration.

Item	00-rapeseed levels, % DM				SEM	p Value			
	0.0	3.8 ²	8.5 ³	13.2 ⁴		TRT	Linear	Quadratic	Cubic
Faecal aNDFom ⁵ , g/kg DM	587.1	586.6	586.2	585.7	0.86	0.69	0.512	0.470	0.031
Faecal uNDFom ⁶ , g/kg DM	280.5 ^A	272.7 ^B	264.8 ^C	257.0 ^D	0.26	<.01	<.001	<.001	0.294
Faecal pdNDF ⁷ , g/kg DM	306.5 ^d	313.1 ^c	319.6 ^b	326.1 ^a	0.65	0.01	0.015	0.001	0.112
aNDFom TTD ⁸ , %	45.15 ^A	43.40 ^B	41.64 ^C	39.88 ^D	1.00	<.01	<.001	0.500	0.056
pdNDF TTD ⁸ , %	61.25 ^A	58.94 ^B	56.63 ^C	54.32 ^D	0.99	<.01	<.001	0.084	0.225

^{a,b,c,d}Values within rows with different superscripts differ ($p \leq .05$).

^{A,B,C}Values within rows with different superscripts differ ($p \leq .01$).

¹Control diet (S)

²Low rapeseed meal inclusion diet (LR)

³Medium rapeseed meal inclusion diet (MR)

⁴High rapeseed meal inclusion diet (HR)

⁵aNDFom: Amylase- and sodium sulfite-treated NDF with ash correction.

⁶uNDF: Unavailable NDF estimated via 240-h *in vitro* fermentation.

⁷pdNDF: Potentially digestible NDF.

⁸TTD: Total tract digestibility.

PR: Parmigiano Reggiano.

yield was calculated these differences resulted significant ($p = .01$). Finally, lactose concentration (g/kg) slightly decreased ($p = .075$) linearly. Urea values, on the contrary, tended to be lower (-4.21 mg/dl in HR compared with S diet, $p = .08$), and negatively influenced by incremental levels of 00-rapeseed dietary inclusion ($p = .004$, $.008$ and $.001$ for linear, quadratic and cubic contrast, respectively). Somatic Cell Score (SCS), showed no differences between diets. No differences were observed also in titrable milk acidity expressed in SH°50 degrees and pH. Analysis of goitrin

content pointed out detectable values in milk produced by cows fed with 00-rapeseed meal and it increased according to incremental dietary levels ($p < .001$).

Data of faecal fibre fractions and fibre digestibility are reported in Table 5.

The level of 00-rapeseed meal inclusion had no effect on faecal aNDFom (g/kg DM), however, affected the other faecal parameters evaluated. Estimated faecal uNDF and TTPdNDFomD declined linearly ($p < .001$) from control diet S (0.0% inclusion) to the

HR diet (13.2% inclusion). Accordingly, faecal pdNDFom content was higher in rapeseed diets ($p = .015$ and 0.01 for linear and quadratic contrast respectively).

Discussion

TMRs were adequate in terms of nutrient composition (NRC, 2001). Results of crude protein content and other major nutrients show the isonitrogenous and isoenergetic diet formulation, similar to those observed in other commercial farms of PR area (Mammi et al. 2018a, 2018b). The dietary CP content resulted adequate (145 g/kg DM on average) to sustain the productions and pointed out the possibility to maintain relatively low CP levels in dairy rations when the diets are well managed (Formigoni and Piva 1996) and diet is well balanced in terms of energy (Formigoni and Biagi 2007). The experimental diets were balanced thanks to an adjustment of the corn flakes and grass hay proportions among the treatments, thus no substantial changes were observed in chemical composition and theoretical supply of ME and MP. TMRs were lower in particle size than usually recommended (Heinrichs 2013), but in accordance with previous trial (Fustini et al. 2017, Cavallini et al. 2018) and similar to those observed in several commercial farms of PR area (Fustini et al. 2016). This level of dietary particle size minimised the risk of cows sorting (Fustini et al. 2016). Feed and water intake were similar to those observed in previous studies conducted under the same experimental conditions (Cavallini et al. 2018).

In all diets, rumination time was lower than the threshold value (390 min/d.) proposed by other authors (Zebeli et al. 2010). In particular, 00-rapeseed diets led to lower rumination times, this result could be explained by the lower aNDFom and peNDF content of these rations (Table 1). No differences were observed on DMI of rapeseed diets, confirming that the inclusion of this feed did not compromise the diet palatability. This result is in line with those obtained by Broderick et al. (2015), where intakes and yield were increased by feeding canola meal.

All the treatments showed an average r-pH lower than 6, which could be explained by the low forage inclusion in the diets, especially in treatment HR (33.1% DM), in which also the starch level was the highest (26.5% DM). Consistently with the higher daily r-pH, pH dynamics, minutes and AUC were lower in MR compared to other treatments. Since experimental diets were balanced for theoretical metabolisable

energy and protein content, dietary starch and hay content differed among treatments, due to the higher fibre amount of 00-rapeseed meal. This condition could be responsible for the differences in rumination time and r-pH values, being the lowest in the HR diet (Table 1).

We observed an increase of milk production, especially in MR diet. Similar results are presented in other trials, in which authors related the higher milk production to a more balanced amino acid profile when canola was included in the diets in place of soy (Huhtanen et al. 2011; Martineau et al. 2013; Paula et al. 2020). For the same reason, according to these authors, it could be possible to correlate canola meal inclusion and increased content of milk protein and casein (Huhtanen et al. 2011; Martineau et al. 2019), as reported in the current study, where 00-rapeseed was used. It should be noted that in our experiment the lowest urea values were observed in the HR compared to S milk, suggesting a major nitrogen efficiency due to a better aminoacid profile and/or to a different level of starch in the diet. No differences were also observed in milk acidity expressed in SH°50 degrees and pH, suggesting that milk obtained from treated animals could be considered absolutely suitable for cheese making.

Analysis of goitrin content pointed out detectable values in milk produced by cows fed with 00-rapeseed meal. This fact is interesting since it could be considered as a marker to identify milk produced by cows fed with 00-rapeseed meal. The importance of this detection is even higher when the use of 00-rapeseed meal is prohibited, like in PDO production (Ministero Politiche Agricole Ambientale e Forestali (MIPAAF) 2016). Additionally, detected levels of goitrin in milk were lower than those considered potentially harmful for human health as set by EFSA: Scientific Panel on Contaminants in the Food Chain (2008). In this report, thresholds of $0.7 \mu\text{mol/g}$ of goitrin and $0.18 \mu\text{mol/g}$ of thiocyanate were considered safe in milk used for human consumption. Results of fibre digestibility appeared lower than values obtained in other experiments in similar conditions (Fustini et al. 2017) and in other trials (Cotanch et al. 2014). Faecal uNDFom content was lower in 00-rapeseed diets. The decrease of the observed digestibility in 00-rapeseed-based diets could be explained considering the higher passage rate of concentrates, such as rapeseed meal, instead of dry forages and the higher fibre content of rapeseed meal instead of soybean meal (Krizsan et al. 2010).

Conclusions

The results obtained in this study confirm that 00-rapeseed meal could be used in PR type diets without negatively influence palatability and feed intake. Replacing soybean with medium levels of 00-rapeseed meal (8.5% DM) led to an improved milk production and milk protein content, with similar or higher results compared to PR type TMR with soybean meal. At this level of inclusion, we can also confirm the safe use of rapeseed meal on rumen health considering the more stable ruminal pH and the lower time spent below subacidosis threshold. We confirm the possibility to detect 00-rapeseed meal used in diets by milk goitrin analysis as a process control check in PDO cheese chain production. In conclusion, this study showed that 00-rapeseed meal could be used in diets without negative effects on performances and milk quality in PR type rations.

Disclosure statement


The authors do not have any conflicts of interest to declare.

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