EFFECT OF REPLACING MAIZE GRAIN BY HYBRID RYE GRAIN IN THE TMR ON PERFORMANCE OF MID-LACTATING DAIRY COWS*

Patrycja Rajtar¹, Marek Sady², Paweł Górka¹, Sylvia Kehoe³, Piotr Micek^{1*}

¹Department of Animal Nutrition and Biotechnology, and Fisheries,
University of Agriculture in Krakow, Al. Mickiewicza 24/28, 30-059 Kraków, Poland

²Department of Animal Product Technology, University of Agriculture in Krakow,
Balicka 122, 30-149 Kraków, Poland

³Department of Animal and Food Science, University of Wisconsin – River Falls, 410 S. 3rd Street,
River Falls WI 54022, USA

*Corresponding author: rzmiczek@cyf-kr.edu.pl

Abstract

Grain from traditional varieties of rye is not commonly used in dairy cattle nutrition. However, new hybrid varieties of rye currently available are characterized by some nutritional and agro-technical benefits. This paper deals with the hypothesis that rye grain derived from a hybrid variety may be an alternative for maize grain in diets for dairy cattle. Sixteen lactating Polish Holstein-Friesian cows were divided into two groups according to their parity (8 primi- and 8 multiparous), stage of lactation (106 ± 30 days after calving) and milk yield (34 ± 4 kg/day). Cows were fed a total mixed ration (TMR) containing grass silage and whole crop maize silage and 29.2% of the concentrate (in dry matter). The latter contained approximately 48% (as fed) of either maize grain (M) or hybrid rye grain (HR) as a main source of cereal grain. Experimental diets were fed for 9 weeks. The use of HR as a substitute for M did not affect ($P>0.05$) dry matter intake and milk yield. There were no differences between treatments in the content of milk solids, amino acids, and fatty acid profile. However, substituting M by HR positively influenced composition of milk protein fractions by increasing the proportion of α -casein (37.0 vs 39.7%; $P<0.01$) and κ -casein (6.5 vs 7.3%; $P=0.02$) as well as decreasing the proportion of β -casein (28.8 vs 27.8%; $P=0.02$) and sensory characteristics of the milk (body and texture, and taste; $P<0.05$). In turn, the composition of the diet did not affect the technological suitability for processing of milk fat (acid and peroxide number, melting and solidification temperature), rennet coagulation time, heat stability or titratable acidity. This study has shown that hybrid rye grain may be an alternative for maize grain in a TMR based on grass and whole maize silage for mid-lactation dairy cows. Further studies are needed with higher proportion of hybrid rye grain in TMR or with other roughages used in a basal diet to fully determine efficiency of hybrid rye grain use in diets for dairy cows.

Key words: hybrid rye grain, dairy cows, feed intake, milk yield and composition

*This study was supported by funds from the National Centre for Research and Development (NCRD), ENERGYFEED grant, project No BIOSTRATEG2/297910/12/NCBR/2016.

Among cereal grains, maize grain is a preferable source of energy in diets for high yielding dairy cows. Particularly, maize grain contains starch that slowly degrades in the rumen which limits the probability of developing subacute rumen acidosis, commonly observed in high yielding dairy cows (Plaizier et al., 2009). Thus, feeding cows with maize grain and maize silage as a main source of dietary energy may cause an increase in milk yield while avoiding incidence of health issues. However, compared to other types of cereals, maize is more susceptible to contamination by various molds, especially *Fusarium* spp., which produce mycotoxins that have deleterious effects on animal health (Grenier and Oswald, 2011). Additionally, in some regions of the world, difficulties in its cultivation (e.g. low resistance to drought and difficulties in controlling pests) and increasing competition with biogas production, limit accessibility and increase the cost of maize for livestock production. Therefore, alternatives are needed that could replace maize in a total mixed ration (TMR) for dairy cows. Mid or late stage lactation cows are especially good candidates for such replacement due to a lower requirement for nutrients and energy, compared to cows in early lactation.

Grain from traditional varieties of rye (i.e. open-pollinated) is not commonly used in dairy nutrition. This is mostly due to rye grain's lower energy content and higher content of anti-nutritive substances, compared to other cereal grains (Sharma et al., 1981; Sauvant et al., 2004; Micek, 2008). There are also disadvantages of using rye grain over maize grain, such as higher ruminal degradation of starch (96.0% vs 48.1% respectively for rye and maize grain of flint-dent type; Micek, 2008). Also, rye grain's total non-starch polysaccharides (T-NSP) and soluble sugars as well as significant levels of phenolic compounds and water-soluble polysaccharides may negatively contribute to changes in rumen fermentation patterns (Rajtar et al., 2020). Specifically, their high susceptibility to rumen degradation in combination with rapidly fermented starch can lead to the risk of subacute ruminal acidosis (Krieg et al., 2017).

However, new hybrid varieties of rye are currently available on the market that could be attractive for dairy producers. Those varieties possess some nutritional and particularly agrotechnical benefits over traditional ones, such as higher yield and higher resistance to disease, frost and/or ergot and higher tolerance to low soil nitrogen availability and high plant population density (Petr and Mikšík, 2006). They are also characterized by lower content of anti-nutritional substances and mycotoxins compared to wheat or maize, and contain more minerals, lysine, and fiber (Jürgens et al., 2012; Rodehutschord et al., 2016). As a result, a use of hybrid rye varieties can translate into more efficient production of the crop as well as its utilization by dairy cows, similar to other cereal grains that are more commonly used. Furthermore, there are indications that using rye grain in lactating dairy cow diets can alter the proportion of unsaturated fatty acids in milk fat which can increase the health promoting properties of milk for humans (Pieszka et al., 2015).

In this study, we hypothesized that the substitution of maize grain with hybrid rye grain variety could not only positively modify the milking performance of Holstein cows but also the composition of the milk and its suitability for further processing. Therefore, the aim of this study was to determine the effect of substituting maize

grain with hybrid rye grain in the diet of mid-lactation dairy cows on feed intake, milk yield, milk composition, as well as the technological processing suitability characteristics for cheese or butter production, such as milk protein fractions or milk fat quality parameters.

Material and methods

The experimental procedures followed Polish Law on Animal Protection which is in accordance with EU Directive 2010/63/EU for animal experiments.

Animals, housing, husbandry and experimental design

The trial was conducted at a research dairy farm belonging to the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences in Jastrzębiec, located in the central part of Poland (Kosowo, Masovian Voivodeship, Poland). The research barn was a freestall barn equipped with electronic device (Roughage Intake Control System, Insentec B.V., The Netherlands), allowing for individual feed intake monitoring. The cows were fed TMR that was added to the feeders twice daily (at 6 a.m. and 6 p.m.) with refusals removed the next day prior to new feed allocation. Treatment TMR were prepared using a Super Data Ranger feeding wagon (SDR, American Calan, New Hampshire, USA), which was also used to remove refusals from individual feeders. The cows were milked twice daily (5 a.m. and 5 p.m.) in a herringbone milking parlor where milking performance of cows was recorded.

The experiment was conducted on 16 Polish Holstein-Friesian (8 primi- and 8 multiparous) mid-lactating dairy cows. The cows were divided into 2 groups, ensuring the same number of primi- and multiparous cows per treatment (parity 1.9 ± 1.1), similar stage of lactation (106 ± 30 DIM) and milk yield (34 ± 4 kg/day) at the beginning of the study. Cows were fed TMR based on grass silage and whole crop maize silage containing concentrate (29.2% of DM) with either maize grain (cultivar Podium; trilinear hybrid variety with flint-dent type of grain; KWS Lochow Poland; M) or hybrid rye grain (cultivar BONO, KWS Lochow Poland; HR). The concentrate mixture was delivered in meal (mash) form (Table 1).

The TMR were formulated using INRAration software (v. 4.0, INRA, France) based on INRA (2007) recommendations for a 650 kg cow producing 35 kg/day of milk containing 40 g/kg of fat and 34 g/kg of protein, and were fed free choice (10% refusals). Similar protein and energy content for M and HR were achieved by balancing the treatment cereal grain with canola cake proportions in the concentrate mixture (Table 1). In consequence, daily intake of maize grain or hybrid rye grain differed between treatments with estimated intakes of 3.65 and 3.80 kg/cow/day for maize grain and hybrid rye grain, respectively. The entire trial lasted 9 weeks (63 days) and consisted of 1 week of adaptation to the individual feeders, 4 weeks of adaptation to experimental diets, and 4 weeks of the data collection period.

Sampling and chemical analysis

Dry matter intake (DMI) was calculated daily based on individual feed intake for each cow and dry matter (DM) content in TMR and refusals. The samples of TMR for each treatment were collected daily and kept at 2°C, and then samples were pooled weekly and kept frozen (−18°C) for further analyses. The same procedure was used for individual refusals of each cow. For calculation of DMI, representative weekly samples of TMR and refusals were dried in a forced-air oven at 50°C for 48 hours. The content of DM, crude ash, crude protein (CP), ether extract (EE) and acid detergent fiber (ADF) were determined according to AOAC (2005) (procedure 934.01, 942.05, 954.01, 920.39 and 973.18, respectively) on dried and ground samples (1.0 mm sieve, Pulverisette 15 Laboratory Cutting Mill, Fritsh GmbH, Idar-Oberstein, Germany) of TMR and refusals. Neutral detergent fiber (aNDF) was analyzed with heat-stable amylase according to van Soest et al. (1991) using an Ankom²²⁰ Fiber Analyzer (ANKOM Technology, NY, USA). The starch content was determined by an enzymatic method (Faisant et al., 1995) and ADL according to Robertson and Van Soest (1981). Data on nutrient content in TMR and refusals were also used to calculate nutrient intake. Representative samples of ingredients used for diet formulation were taken and analyzed before beginning the experiment, and weekly, using the same procedures as described for TMR.

Milk analysis

Milk was collected from each cow once a week during the data collection period, always at the same day of the week (Monday evening/Tuesday morning), both during the evening and morning milkings. For milk composition analysis, samples collected during both milkings were subsampled proportionally to obtain about 1.5 liters and a 50 mL representative sample of daily milk for each cow was stored in polypropylene containers containing a preservative (Microtabs II, Bentley, Norwood, MA, USA). Additionally two 10 mL containers of daily milk were immediately frozen and stored at −18°C for analysis of amino acid (AA) content and fatty acid (FA) profile. Determination of the basic components of milk (protein, fat, lactose and urea) was performed using a MilkoScan FT2 (Foss Analytical, Hilleroed, Denmark). The somatic cell count (SCC) was measured using an IBCM device (Bentley Instruments, MN, USA).

The content of individual milk protein fractions (α -casein, β -casein, κ -casein, β -lactoglobulin, α -lactalbumin, immunoglobulins, serum albumin, lactoferrin and free peptides) were determined by SDS-PAGE gel electrophoresis performed according to the methodology described by Laemmli (1970). For this purpose, 0.3 ml of daily pooled fresh milk samples from each cow, or 20 μ l of whey, was dissolved in 1 ml or 40 μ l of a reducing buffer (0.5 M Tris-HCl, pH 6.8, glycerol, 10% SDS, 0.5% bromophenol blue, β -mercaptoethanol, and deionized water) and heated at 95°C for 4 minutes. Five μ l of prepared sample was added to each polyacrylamide gel. In the same volumes, milk protein standards were placed in the end wells of the gel. The separation was carried out at a voltage of 100 mV on a 12% SDS-PAGE polyacrylamide gel. The Mini Protean 3Cell device (Bio-Rad, Hercules, CA) was used for separation. The obtained gels were stained in R 250 Coomassie solution, and after

discoloration, scanned and quantified using a Gelscan v.2.0 software (Kucharczyk, Warsaw, Poland).

The percentage of fatty acids (FA) in milk fat was determined by gas chromatography using a Varian apparatus 450-GC with an FID detector and CP-SIL 88 (FAME, length 100 m, diameter 0.25 mm) column and lipid extraction from milk was carried out according to Folch and Lees (1957). The AA content in milk was determined by liquid chromatography using an INGOS AAA-400 amino acid analyzer (Prague, Czech Republic) equipped with an Ostion LG ANB (370 mm) column. Column temperature was 55°C, reactor temperature 120°C, detection wavelengths 440 and 570 nm. Fatty acid and AA content were performed on a previously daily pooled frozen milk sample, thawed to 18°C and further pooled per cow from 4 consecutive weeks of the data collection period.

The titratable acidity was determined by the Soxhlet–Henkel method (SH). Evaluation of heat stability of milk at 140°C was carried out using the method described by Crowley and Dowling (2016). Evaluation of the rennet coagulation time (RCT) of milk was carried out by Schern's method, using commercial rennet powder Fromase 2200TL (activity 2200 IMCU g⁻¹; DSM Specialities, Brøndby, Denmark). For this purpose, 100 mL of a daily pooled fresh milk sample from each cow was heated to 35°C in a water bath, after which 1 mL of rennet solution (6.7 g L⁻¹) was added. The RCT was the time, expressed in seconds, elapsed from rennet addition to the first appearance of casein flocs.

The procedure used for determination of acid number was in accordance with the Polish standard PN-EN ISO 660:2010 (2010), peroxide number in accordance with the PN-ISO 3976:2014-11 (2014), melting temperature in accordance with the PN-EN-ISO 6321:2004 (2004) and solidification temperature in accordance with the PN-A-86927 (1961). The melting point in the present research was assumed as the temperature at which the previously solidified fat became completely clear, whereas the solidification temperature was determined as the beginning of its solidification.

Sensory evaluation was carried out on fresh milk pooled per treatment group (in each of 4 consecutive weeks of the data collection period) and then pasteurized milk according to ISO 4121:2003 (2003). For this purpose, the milk was heated at 85°C for 15 sec then cooled and stored at 4°C until the next day in which the assessment was carried out. Directly before the analysis, the milk was heated to 18°C. Sensory evaluation was conducted according to a 5-point scale (5-excellent, 4-good, 3-fair, 2-poor, 1-unacceptable) by a panel of 5 assessors, all of whom fulfilled the basic requirements for sensory sensitivity according to PN-ISO 3972:2016-07 (2016). The following quality descriptors and corresponding importance factor were taken into account in this assessment: appearance and color – 0.15, body and texture – 0.10, odor – 0.35, and taste – 0.40. The descriptor of overall sensory quality for a product was evaluated by using the importance factor for individual quality descriptors. The overall score was established by dividing the total points (the product of the marks for particular characteristics and their importance factors) for a given product by the sum of the importance factors.

Statistical analysis and calculations

Data were analyzed as a completely randomized design. Data on dry matter and nutrient intake as well as milk yield and milk component yield were reduced to weekly means and then analyzed using the MIXED procedure of SAS (2002). The SCC were log-transformed prior to analysis to achieve normal distribution of the data. The statistical model included the random effect of cow and the fixed effects of time (week), diet, and the time by diet interaction, with time considered as a repeated measure in the model. Furthermore, effect of parity and its interaction with diet and time was tested in the model; however, it turned out to be not significant ($P>0.05$) and was removed from the model. Data on AA content, FA profile and indicators of milk quality were performed on pooled milk for each cow using the GLM procedure of SAS. Differences were considered significant at $P<0.05$ and tendencies between 0.05 and 0.10.

Results

The TMR used for M and HR groups (Table 1) had similar ash and CP content (in DM). The M diet contained slightly more EE and starch but less aNDF than HR, as an effect of differences shown in chemical composition of maize and rye grains (Table 2). The diets did not differ in net energy or absorbable true protein in the small intestine (PDI) content. No diet $\times$ time (week) interaction was shown for any of the intake (Table 3) or milk composition parameters apart from milk fat and milk urea content (Table 4). There were no significant differences ($P=0.29$) in DMI and CP intake ($P=0.26$) between treatment groups (Table 3). A higher intake of EE ($P<0.01$) and starch ($P=0.05$) was observed for the M, compared to HR group. Cows from the HR group consumed 9.12 kg/day aNDF, which tended to be 0.33 kg more than the M group ($P=0.07$). This was due to the slightly higher fiber content in HR compared to the M group. Average milk yield of cows was 27.26 and 28.61 kg/day for HR and M group, respectively, and did not differ between groups ($P\geq 0.14$; Table 4).

Table 1. Ingredients, chemical composition and nutritive value of treatment diets

Item	Diet ¹	
	M	HR
1	2	3
TMR ingredients (% of DM)		
whole-crop maize silage	18.8	18.8
grass silage	39.0	39.0
straw	5.6	5.6
high moisture corn grain silage	7.4	7.4
maize grain	13.7	—
rye grain	—	14.3
soybean meal	8.2	8.2
canola cake	5.0	4.4

Table 1 – contd.

1	2	3
limestone	0.7	0.7
NaHCO ₃	0.8	0.8
vitamix KW ²	0.8	0.8
Chemical composition and nutritive value of TMR		
dry matter (%)	52.4	51.6
in dry matter (g/kg of DM):		
crude protein	147	146
aNDF ³	377	397
ADF ⁴	230	232
starch	238	234
crude ash	94	97
ether extract	36	33
Ca	0.81	0.81
P	0.40	0.40
UFL ⁵ (in kg of DM)	0.90	0.90
PDIN ⁶ (g/kg of DM)	97.6	96.3
PDIE ⁷ (g/kg of DM)	91.2	90.2

¹Concentrates with M – maize grain or HR – hybrid rye grain.

²Mineral-vitamin additive (Polmass SA Poland). In 1 kg: Ca 190 g, P 40 g, Na 50 g, Zn 9000 mg, Mn 4000 mg, Cu 900 mg, Se 45 mg, I 90 mg, Co 25 mg, vit. A 1000000 iu, vit. D₃ 120000 iu, vit. E 4000 iu, vit. B₁ 150 mg, vit. B₂ 100 mg, vit. B₆ 50 mg, vit. B₁₂ 0.55 mg, niacinamide 2500 mg, biotin 100 mg, folic acid 100 mg, calcium pantothenate 300 mg.

³aNDF – neutral detergent fiber determined with heat-stable amylase.

⁴ADF – acid detergent fiber.

⁵UFL – net energy for lactation (INRA, 2007).

⁶PDIN – true protein absorbable in the small intestine when N is limiting in the rumen (INRA, 2007).

⁷PDIE – true protein absorbable in the small intestine when energy is limiting in the rumen (INRA, 2007).

Table 2. Chemical composition of maize and rye grain

Item	Maize	Rye
Dry matter (%)	89.4	88.3
In dry matter (g/kg of DM)		
crude protein	117	128
aNDF ¹	85	166
ADF ²	48	62
ADL ³	21	29
starch	781	653
crude ash	14	17
ether extract	48	15
UFL ⁴ (in kg of DM)	1.25	1.18
PDIN ⁵ (g/kg of DM)	83	88
PDIE ⁶ (g/kg of DM)	113	102

¹aNDF – neutral detergent fiber determined with heat-stable amylase.

²ADF – acid detergent fiber.

³ADL – acid detergent lignin.

⁴UFL – net energy for lactation (INRA, 2007).

⁵PDIN – true protein absorbable in the small intestine when N is limiting in the rumen (INRA, 2007).

⁶PDIE – true protein absorbable in the small intestine when energy is limiting in the rumen (INRA, 2007).

Table 3. Dry matter and nutrient intake by dairy cows (kg/d per cow)

Item	Diet ¹		Week ²				SEM ³	Effect, P-value		
	M	HR	1	2	3	4		Diet (D)	Week (W)	D × W
Dry matter	23.32	22.83	22.46	22.66	23.77	23.41	0.210	0.29	0.19	0.83
Crude protein	3.43	3.36	3.33	3.32	3.49	3.44	0.032	0.26	0.26	0.91
Ether extract	0.84	0.75	0.79	0.78	0.83	0.81	0.011	<0.01	0.30	0.93
Starch	5.55	5.34	5.30	5.85	5.61	5.52	0.053	0.05	0.15	0.79
aNDF ⁴	8.79	9.12	8.71	8.80	9.23	9.09	0.084	0.07	0.18	0.86

¹Concentrates with M – maize grain or HR – hybrid rye grain.²Consecutive weeks of the data collection period.³Standard error of the mean.⁴aNDF – neutral detergent fiber determined with heat-stable amylase.

Table 4. Daily milk and solids yield, proximate chemical composition, density and somatic cell count in milk

Item	Diet ¹		Week ²				SEM ³	Effect, P-value		
	M	HR	1	2	3	4		Diet (D)	Week (W)	D × W
Yield (kg/day per cow)										
milk	28.61	27.26	28.25	27.75	27.53	27.43	0.893	0.32	0.25	0.36
milk 3.5% fat (FCM ⁴)	32.64	31.26	32.61	32.50	31.86	30.82	1.160	0.53	0.07	0.09
total solids	3.85	3.70	3.81	3.80	3.78	3.72	0.134	0.96	0.25	0.84
protein	1.00	0.97	1.00	0.97	0.99	0.97	0.022	0.41	0.40	0.55
fat	1.22	1.21	1.22	1.23	1.19	1.14	0.053	0.63	0.06	0.07
lactose	1.40	1.31	1.37	1.35	1.34	1.34	0.051	0.32	0.44	0.40
Chemical composition										
total solids (%)	13.39	13.65	13.51	13.61	13.43	13.34	0.124	0.19	0.39	0.35
protein (%)	3.52	3.65	3.57	3.56	3.58	3.58	0.062	0.07	0.94	0.26
fat (%)	4.27	4.45	4.32 ab	4.47 a	4.26 ab	4.18 b	0.081	0.14	0.03	0.04
lactose (%)	4.89	4.86	4.87 ab	4.90 a	4.82 b	4.88 ab	0.043	0.65	0.23	0.96
urea (mg/dL)	22.12	22.01	22.71	22.43	21.23	21.88	0.910	0.94	0.40	0.01
somatic cell count (thousand cells/ml)	257.88	316.44	302.44	302.00	258.38	285.81	68.080	0.66	0.41	0.06
LSCC ⁵ (Log value)	2.08	2.34	2.18	2.29	2.16	2.23	0.133	0.31	0.12	0.07
Density (g/L)	1027.89	1027.92	1027.99	1028.01	1027.49	1028.11	0.384	0.95	0.64	0.74
SNF ⁶ (%)	9.24	9.26	9.25	9.28	9.24	9.24	0.058	0.77	0.93	0.32

a, b – P<0.05.

¹Concentrates with M – maize grain or HR – hybrid rye grain.²Consecutive weeks of the data collection period.³Standard error of the mean.⁴FCM – Fat-Corrected Milk (FCM (3.5%) = 0.35 M + 18.57 F where M = quantity of milk in kg, F = amount of fat in kg in 'M' quantity of milk).⁵Somatic cell count transformed to a logarithmic scale (LSCC = log_e (somatic cell count × 10³)).⁶Solids not fat.

Table 5. The fatty acid profile (g/100 g FA) of milk fat

Item	Diet ¹		SEM ²	P-value
	M	HR		
C4:0	2.34	2.68	0.689	0.59
C6:0	0.13	0.16	0.005	0.94
C8:0	0.25	0.24	0.004	0.70
C10:0	1.08	0.99	0.010	0.29
C10:1	0.08	0.06	0.003	0.11
C12:0	2.09	2.03	0.009	0.43
C13:0 c	0.02	0.02	0.002	1.00
C14:0	8.41	7.83	0.174	0.09
C14:1	0.28	0.27	0.003	0.36
C15:0	0.79	0.74	0.001	0.24
C16:0 c	0.19	0.18	0.004	0.39
C16:0	30.52	29.53	0.310	0.05
C16:1	2.52	2.76	0.041	0.12
C17:0	0.49	0.49	0.003	1.00
C18:0	9.15	8.71	0.140	0.14
C18:1 t	1.55	1.46	0.031	0.46
C18:1 c	28.61	29.50	0.549	0.14
C18:2	7.13	7.40	1.378	0.76
C20:0	0.09	0.09	0.002	0.56
C20:1	0.04	0.13	0.010	0.28
C18:3	0.31	0.33	0.004	0.56
CLA 9c 11t	1.11	1.29	0.03	0.20
CLA 10t 12c	0.33	0.39	0.028	0.61
Others	2.51	2.76	0.179	0.99
ΣUFA ³	41.95	43.58	1.271	0.09
ΣSFA ⁴	58.05	56.42	1.383	0.07
ΣMUFA ⁵	33.07	34.17	1.662	0.16
ΣPUFA ⁶	8.88	9.41	1.541	0.57
Omega3	0.31	0.33	0.004	0.56
Omega6	7.46	7.79	1.239	0.69
Omega6/omega3	24.06	23.61	30.009	0.90

¹Concentrates with M – maize grain or HR – hybrid rye grain.²Standard error of the mean.³Sum of unsaturated fatty acids.⁴Sum of saturated fatty acids.⁵Sum of monounsaturated fatty acids.⁶Sum of polyunsaturated fatty acids (C18:2, C18:3 and CLA).

No differences were shown between groups in 3.5% fat corrected milk yield (FCM), as well as total solids and milk component yield. Milk from HR and M groups did not differ in total solids and fat ($P>0.05$) but HR group tended to be higher for protein content ($P=0.07$). Effect of week was significant ($P=0.03$) only for fat content. The level of urea in the milk (22.1 vs. 22.0 mg/dL for M and HR, respectively; treatment $\times$ week interaction $P=0.01$) was within the standard range (Table 4).

The results for the FA profile of milk samples confirmed that the type of diet caused only a minor influence on FA composition of milk fat (Table 5). Milk fat from HR group tended to contain a slightly higher proportion of unsaturated fatty acids (UFA; $P=0.09$) and lower proportion of C14:0 ($P=0.09$) and C16:0 ($P=0.05$). In terms of AA content in milk, no differences or tendencies were found ($P\geq 0.16$; Table 6). Average content of Lys and Met in milk were 2.82 vs. 2.72 g/kg and 0.76 vs. 0.66 for HR and M groups, respectively.

Table 6. Amino acid content in milk (g/kg)

Item	Diet ¹		SEM ²	P-value
	M	HR		
Asp	2.49	2.64	0.059	0.221
Thr	1.40	1.43	0.027	0.658
Ser	1.74	1.74	0.030	0.969
Glu	7.08	7.27	0.152	0.540
Pro	3.21	3.23	0.071	0.891
Gly	0.64	0.64	0.024	0.942
Ala	1.02	1.05	0.027	0.703
Val	2.05	2.14	0.068	0.588
Ile	1.62	1.75	0.051	0.169
Leu	3.29	3.40	0.073	0.481
Tyr	1.54	1.60	0.033	0.422
Phe	1.49	1.56	0.040	0.431
His	0.99	1.07	0.021	0.160
Lys	2.70	2.82	0.036	0.174
Arg	1.11	1.15	0.020	0.237
Cys	0.29	0.32	0.022	0.502
Met	0.66	0.76	0.043	0.183

¹Concentrates with M – maize grain or HR – hybrid rye grain.

²Standard error of the mean.

The type of cereal grain significantly influenced the composition of milk protein fractions (Table 7). Milk protein from HR group had higher proportion of α - ($P<0.01$) and κ -casein ($P=0.02$; by 2.7 and 0.88 percentage points for α - and κ -casein, respectively), and a lower proportion of β -casein ($P=0.02$), immunoglobulins ($P<0.01$), and free peptides ($P<0.01$; by 1.1, 0.2, and 0.77 percentage points respectively). In turn,

the composition of the TMR did not affect the technical suitability for processing of milk fat (Table 7). The use of HR positively affected the sensory characteristics of milk, with significant differences in the body and texture ($P=0.03$) and taste ($P=0.01$) as well as the overall assessment ($P=0.02$).

Table 7. Indicators of milk quality and technological suitability for processing

Item	Diet ¹		SEM ²	P-value
	M	HR		
Milk protein fractions (% of total proteins)				
α-casein	37.01	39.71	0.350	<0.01
β-casein	28.83	27.76	0.212	0.02
κ-casein	6.46	7.34	0.161	0.02
β-lactoglobulin	12.01	11.72	0.274	0.47
α-lactalbumin	8.32	7.45	0.300	0.07
immunoglobulins	0.61	0.40	0.013	<0.01
serum albumin	2.27	2.22	0.267	0.89
lactoferrin	2.63	2.31	0.104	0.19
free peptides	1.86	1.09	0.021	<0.01
Milk fat quality parameters for processing				
acid number (mg KOH/100 g fat)	0.57	0.65	0.003	0.14
peroxide number (meq O ₂ /kg fat)	0.34	0.42	0.001	0.21
melting temperature (°C)	34.91	35.16	0.059	0.19
solidification temperature (°C)	20.07	20.08	0.012	0.89
Sensory evaluation of milk (points, 0–5)				
appearance and color	4.84	4.90	0.008	0.29
body and texture	4.93	5.00	0.010	0.03
odor	4.53	4.71	0.021	0.11
taste	4.69	4.90	0.007	0.01
overall score	4.68	4.84	0.009	0.02
Other indicators of milk				
titratable acidity (°SH)	7.13	7.25	0.190	0.70
rennet coagulation time (sec.)	247	230	16.234	0.56
heat stability (min)	6.64	7.30	0.452	0.21

¹Concentrates with M – maize grain or HR – hybrid rye grain.

²Standard error of the mean.

These last parameters were rated higher for HR milk by 0.21, 0.07, and 0.16 points, respectively. There were no treatment differences in RCT, heat stability or titratable acidity.

Discussion

In several Nordic countries, there is a long-standing tradition of rye consumption due to its relatively high concentration of bioactive compounds, such as alkylresorcinols, benzoxazinoids, lignans, phenolic acids, phytosterols and tocopherols, which may have independent or synergistic health effects (Andersson et al., 2014; Koistinen and Hanhineva, 2017). The use of rye grain in livestock nutrition is much less frequent (Pieszka et al., 2015). The main concern in feeding rye grain to animals is its high content of soluble non-starch polysaccharides (mainly arabinoxylans), which increase the viscosity of gastric contents, thus reducing the absorption and bioavailability of nutrients in pigs and poultry (Jürgens et al., 2012; Smit et al., 2019). Compared to wheat, barley or triticale grain, rye grain contains less CP and more dietary fiber (Micek, 2008; Rodehutsord et al., 2016; Krieg et al., 2017). For ruminants, rye has a high metabolizable energy value of 13.1 MJ/kg DM, which is similar to wheat, barley or triticale grain, and higher than oat grain (Denek and Deniz, 2004; Sauvant et al., 2004). In turn, the biological value of rye protein is quite low (Micek, 2008; Rodehutsord et al., 2016) and there is a risk of the appearance of harmful ergot alkaloids (Denek and Deniz, 2004). However, intensive work in rye genetics has focused on the development of new hybrid cultivars with reduced concentrations of antinutrient substances, higher winter hardiness, lower requirements for soil quality, and decreased susceptibility to ergot (Jürgens et al., 2012).

In the present study, feeding dairy cows by substituting maize grain with hybrid rye grain in the concentrate mixture did not cause any major differences in the chemical composition of diets as well as in DM, CP, and aNDF intake. Iso-energy and iso-protein diets were achieved by a 2 percentage point higher proportion of rye and lower proportion of canola cake in the concentrate mixture. Therefore, cows on the M diet had a higher intake of EE and starch due to a higher average DMI, slightly higher content of canola cake, and higher concentration of EE and starch in maize grain compared to rye grain. Compared with the starch in maize grain, rye grain starch is rapidly fermented in the rumen (Micek, 2008; Benninghoff and Paschke-Beese, 2015; Krieg et al., 2017; Rajtar et al., 2020) which may increase the risk of ruminal acidosis (Plaizier et al., 2009). On the other hand, when lactating dairy cows are fed grain containing slowly degradable starch, such as maize, substantial quantities of starch may escape from rumen fermentation thereby limiting microbial protein synthesis and the amount of protein available to the animal (Mills et al., 1999). The capacity of the rumen microorganisms to ferment starch at different rates from different grains (e.g. barley vs. maize) leads to scientific literature reporting varied DMI and milk yield responses (Micek, 2008; Kargar and Ghorbani, 2014). Casper and Schingoethe (1990) reported greater milk yields for cows fed maize grain as compared with cows fed barley grain. In contrast, Kargar and Ghorbani (2014) demonstrated that changing diet DM fermentability by replacing barley grain for maize grain did not affect feed intake or milk yield. However, milk fat yield tended to show a linear increase with increasing maize grain inclusion in the diet. Many other studies have also shown that milk yield is not affected by the type of grain or the amount of ruminally degradable starch (Oliveira et al., 1993; Slots et al.,

2009). In fact, the effects of wheat, barley or maize grain utilization in diets for dairy cows have been widely investigated, but in the scientific literature there are only a few studies on the utilization of rye grain (Sharma et al., 1981, 1983; Südekum and Brandt, 1994; Pieszkka et al., 2015), especially using hybrid rye grain, as a substitute for maize grain.

In our research, there was no difference in DMI between the HR and M group which is supported by other studies that concluded the palatability of rye is similar to other cereal grains for dairy cows (Sharma et al., 1983; Pieszkka et al., 2015). In contrast, some studies reported a negative impact of rye grain use in the diet for ruminants on DMI, but those studies used a higher inclusion of rye in the ration. For example, in the study of Sharma et al. (1981) twelve lactating Holstein cows were fed four diets containing 0, 25, 50, and 75% rolled rye within the grain mixture with grass silage in a switch back design. Replacement of barley with rye in the grain mixture reduced DMI, with little effect on average daily milk production. Moreover, our study, along with others (Sharma et al., 1983; Pieszkka et al., 2015), showed that replacement of crushed barley, wheat or maize grain by rye grain in dairy cow concentrate mixtures did not reduce feed intake, milk yield, and milk composition. Kirilov et al. (1994) found no reduction in milk yield compared to the control group fed crushed barley in diets containing 20, 40 or 50% rye grain in the concentrate mixture. However, although not significant, our research demonstrated numerically lower values of milk production for HR compared to M group by 1.35 kg/day as well as higher milk protein concentration by 0.13%. Part of the reason for this may be due to the fact that the M treatment cows had numerically higher DM intake and significantly higher starch and crude fat intake from HR. Also, based on previous research (Micek, 2008), we can speculate that the utilization of maize starch was better at providing energy for milk production when digested in the small intestine since it is a slower rumen degrading starch source which contains a higher proportion of bypass starch. On the other hand, the rye starch, having higher rumen degradability, may have provided more energy to rumen microbes for higher microbial synthesis which contributed to higher milk protein concentration. Of course, a dilution effect cannot be completely ignored when a higher content of protein in milk was observed with overall lower milk yield in the HR group. However, Kraszewski and Kozłowski (2000), in an experiment feeding dairy cows with concentrate mixtures containing 50% rye grain, showed a significant decrease in milk yield and milk fat quality due to a higher proportion of saturated fatty acids, compared to a control group fed barley grain.

The results obtained in our study did not indicate differences in milk fat indices when cows were fed HR. To the contrary, the trends of a lower proportion of myristic (C14:0) and palmitic (C16:0) acid in milk fat in this study may be considered beneficial. It should also be noted that the content of other fatty acids, which are considered as health promoting, such as linoleic (C18:2) and linolenic (C18:3) acid, were nearly identical in both groups. Pieszkka et al. (2015) reported higher levels of MUFA and PUFA in milk fat when cows were fed with hybrid rye grain but in our study only a tendency for higher UFA levels was observed. Although none of these numbers from the HR group were significant on their own, all of them taken together point

toward a milk product that is preferable from a consumer perspective, having lower saturated and higher unsaturated fatty acids.

Although the type of grain did not significantly influence the AA content in the milk, important variation in the composition of milk protein fractions from the HR group was observed. We assume that this situation is not unusual, because total content of AA in the milk (g/kg) comes from different milk protein fractions, expressed in this research as % of total proteins. Thus, the overall AA content in the milk may show no differences, despite the differences demonstrated in the composition of individual milk protein fractions. This effect may be related to modified microbial protein synthesis in the rumen as a result of fermentation processes modulated by different starch properties (Micek, 2008). Regardless of that, milk protein from the HR group was characterized by a higher proportion of α - and κ -casein fractions and a lower proportion of β -casein, immunoglobulins, and peptides. Casein, as the main component of the curd, has a major impact on cheese production efficiency. Therefore, it is worth mentioning the 2.5 percentage points higher ($P < 0.05$) total casein concentration in the milk protein from the HR group (data not shown). According to Cipolat-Gotet and Cecchinato (2018), the efficiency of cheese DM yield is positively associated with the amounts of α s1- and κ -casein, due to the strong influence of both fractions on the recovery rate of protein and fat from the curd. As a consequence, it can be assumed that the composition of protein fractions of milk from cows fed rye grain will probably have a beneficial effect on cheese production.

In turn, the type of grain did not affect technological suitability for processing of milk fat. The lack of differences between M and HR in the acid and peroxide numbers indicate no differences between lipolytic and oxidative changes of fat and its potential storage stability. As for the solidification and melting temperature of the milk, there were also no treatment differences, indicating similar processing capabilities. The solidification and melting temperature of milk fat are influenced mainly by the fatty acid composition of the triacylglycerols. As milk fat contains a large number of different types of triacylglycerols, which vary considerably in molecular weight and degree of unsaturation, its melting point shows wide temperature ranges from -35 to $+40^{\circ}\text{C}$ (Taylor and MacGibbon, 2003). The values of both parameters for milk fat vary between 19 – 24°C and 31 – 40°C , respectively (Ziajka, 2008), and obtained results are consistent with the above data. Although there were tendencies for different degrees of unsaturation between the two treatments, this had no effect on the solidification and melting temperature of the milk fat. Thus, small differences in FA composition between treatments (only 1.63 percentage points for UFA) did not translate into changes in milk fat quality parameters for processing.

The composition of the ration fed to cows is one of the main factors that can significantly affect the sensory properties of milk. This is mainly due to the fact that some chemical compounds, especially volatile ones, contained in the feeds may enter the milk, affecting its smell and flavor (Schiano et al., 2017). Feeding cows with rye grain positively affected the sensory characteristics of milk, with significant differences in the individual parameters as well as in the overall assessment, although this effect is not clear and is difficult to explain. The rennet coagulation time and milk heat stability are the parameters of the processing suitability of milk for the

production of cheeses and high-temperature milk products (such as UHT milk), respectively. The main impact on these characteristics of milk comes from protein and mineral content and their composition, as well as from acidity. Despite the differences in the composition of protein fractions between the tested milk types, they did not differ significantly in either rennet coagulation time, heat stability or titratable acidity. It can, however, be assumed that the tendency of longer heat stability time and shorter rennet coagulation time for HR was associated with a higher proportion of casein, including its κ fraction. Due to its strong hydrophilic properties, the κ fraction of casein has a major influence on the stability of the colloidal system of milk proteins (Horne and Banks, 2004).

Future research will need to evaluate any differences in actual technological suitability of milk for processing from cows fed rye or maize grain. Although the current study indicates no, or only slight, differences in this respect, more studies are needed. Also, the use of alternative grain source, such as rye, should be evaluated not only for mid-lactation cows, but also for other lactation stages. With a numerical decrease in milk yield for cows fed concentrate containing hybrid rye grain, the potential for feeding high producing dairy cows will have to be carefully evaluated. And lastly, using different combinations of rye grain with other grain species or other roughages in the basal diet should also be researched.

In conclusions, the use of HR as a substitute for M in the diet of mid-lactating dairy cows did not cause a dramatic decrease in DMI or significant reduction in quality of milk or milk products. To the contrary, milk from cows fed rye grain tended to have higher milk protein concentrations and a higher proportion of unsaturated fatty acids. Additionally, milk from the HR group was characterized by higher total casein proportion in milk protein by 2.5 percentage points and higher scores in milk sensory indices, without any negative effect on the quality and technological suitability for processing of milk fat. However, compared to maize grain, concentrates containing rye grain have a much lower potential of milk synthesis, which could significantly limit milk yield in high producing dairy cows.

In summary, this study has shown that hybrid rye grain could be an alternative for maize grain in a diet based on grass silage and whole crop maize silage for mid-lactation dairy cows, with the potential of decreasing milk yield but improving some milk quality characteristics. Further research is needed to determine the effect of hybrid rye grain concentrate mixtures fed to dairy cows in combination with other cereal grain species, as a higher proportion in the diet or with other roughages used in a basal diet.

Acknowledgements

The authors acknowledge the staff of Kosowo Farm of the Institute of Genetics and Animal Breeding Polish Academy of Sciences, Jastrzębiec, for their help in conducting the study and collecting the data.

References

- Andersson A.A.M., Dimberg L., Åman P., Landberg R. (2014). Recent findings on certain bioactive components in whole grain wheat and rye. *J. Cereal Sci.*, 59: 294–311.
- AOAC (2005). Association of Official Analytical Chemists. Official methods of analysis. 18th Ed. AOAC International, Washington, DC.
- Benninghoff J., Paschke-Beese M. (2015). *In situ* and *in vitro* ruminal degradation of maize grain and untreated or xylose-treated wheat, barley and rye grains. *Anim. Feed Sci. Tech.*, 210: 86–93.
- Casper P., Schingoethe D.J. (1990). Response of early lactation dairy cows fed diets varying in source of nonstructural carbohydrate and crude protein. *J. Dairy Sci.*, 73: 1039–1050.
- Cipolat-Gotet C., Cecchinato A. (2018). Variations in milk protein fractions affect the efficiency of the cheese-making process. *J. Dairy Sci.*, 101: 8788–8804.
- Crowley S.V., Dowling A.P. (2016). Impact of α -lactalbumin: β -lactoglobulin ratio on the heat stability of model infant milk formula protein systems. *Food Chem.*, 194: 184–190.
- Denek N., Deniz S. (2004). The determination of energy levels in some cereal grains used in ruminant diets by *in vivo* and *in vitro* methods. *Turk. J. Vet. Anim. Sci.*, 28: 185–193.
- Faisant N., Planchot V., Kozłowski F., Pacourent M.P., Colonna P., Champ M. (1995). Resistant starch determination adapted to products containing high level of resistant starch. *Sci. Alim.*, 15: 83–89.
- Folch J., Lees M. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497–509.
- Grenier B., Oswald I.P. (2011). Mycotoxin co-contamination of food and feed: Meta-analysis of publications describing toxicological interactions. *World Mycotoxin J.*, 11: 285–313.
- Horne D.S., Banks J.M. (2004). Rennet-induced coagulation of milk. In: *Cheese Chemistry, Physics and Microbiology*, Fox P.F., McSweeney P.L.H., Cogan T.M.C., Guinee T.P. (eds). Elsevier Academic Press, London, UK, pp. 47–70.
- INRA (2007). Alimentation des bovins, ovins et caprins: Besoins des animaux – Valeurs des aliments. Editions Quae, Versailles, France, 2007.
- ISO 4121:2003 (2003). Sensory analysis – guidelines for the use of quantitative response scales.
- Jürgens H.U., Jansen G., Wegener C.B. (2012). Characterisation of several rye cultivars with respect to arabinoxylans and extract viscosity. *J. Agric. Sci.*, 5: 1–12.
- Kargar S., Ghorbani G.R. (2014). Reciprocal combinations of barley and corn grains in oil-supplemented diets: Feeding behavior and milk yield of lactating cows. *J. Dairy Sci.*, 97: 7001–7011.
- Kirilov M.P., Kumarin S.V., Ilyukhina L.A., Golovin A.V., Udalova E.V. (1994). Rye in feed mixtures. *Životnovodstvo*, 33: 16–18.
- Koistinen V.M., Hanhineva K. (2017). Microbial and endogenous metabolic conversions of rye phytochemicals. *Molec. Nutr. Food Res.*, 61: 7.
- Kraszewski J., Kozłowski J. (2000). Effect of feeding ground rye in feed rations for cows on the nutritional value of milk and butter (in Polish). *Rocz. Nauk. Zoot.*, Supp. 6: 62–72.
- Krieg J., Siegfried N., Steingass H., Rodehutsord M. (2017). *In situ* and *in vitro* ruminal starch degradation of grains from different rye, triticale and barley genotypes. *Animal*, 11: 1745–1753.
- Laemmli U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680–685.
- Micek P. (2008). Nutritional usefulness to ruminants of grain of Polish cereal species and cultivars (in Polish). *Zesz. Nauk. UR Kraków*, 449: 1–127.
- Mills J.A.N., France J., Dijkstra J. (1999). A review of starch digestion in the lactating dairy cow and proposals for a mechanistic model. 1. Dietary starch characterisation and ruminal starch digestion. *J. Anim. Feed Sci.*, 8: 291–340.
- Oliveira J.S., Huber J.T., Ben-Ghedalia D., Swingle R.S., Theurer C.B., Pessarakli M. (1993). Influence of sorghum grain processing on performance of lactating dairy cows. *J. Dairy Sci.*, 76: 575–581.
- Petr J., Mikšík V. (2006). Rye quality of hybrid and population varieties from intensive and ecological conditions. *Sci. Agric. Bohem.*, 37: 1–8.

- Pieszka M., Kamyczek M., Rudzki B., Łopuszańska-Rusek M., Pieszka M. (2015). Evaluation of the usefulness of hybrid rye in feeding Polish Holstein-Friesian dairy cows in early lactation. *Ann. Anim. Sci.*, 15: 929–943.
- Plaizier J.C., Krause D.O., Gozho G.N., McBride B.W. (2009). Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *Vet. J.*, 176: 21–31.
- PN-A-86927 (1961). Edible vegetable fats – Determination of crystallization temperature. Polish Official Standard (in Polish).
- PN-EN ISO 6321:2004 (2004). Animal and vegetable fats and oils – Determination of melting point in open capillary tubes (slip point). Polish Official Standard (in Polish).
- PN-EN ISO 660:2010 (2010). Animal and vegetable fats and oils – Determination of acid value and acidity. Polish Official Standard (in Polish).
- PN-ISO 3976:2014-11 (2014). Milk fat – Determination of peroxide value. Polish Official Standard (in Polish).
- PN-ISO 3972:2016-07 (2016). Sensory analysis – Methodology – Method of investigating sensitivity of taste. Polish Normalization Committee (in Polish).
- Rajtar P., Górka P., Schwarz T., Micek P. (2020). Effect of hybrid rye and maize grain processing on ruminal and postruminal digestibility parameters. *Ann. Anim. Sci.*, 20: 1065–1083.
- Robertson J.B., Van Soest P.J. (1981). The detergent system analysis and its application to human foods. In: *The Analysis of Dietary Fiber in Food*, Theander J. (ed.). Dekker INC, pp. 123–157.
- Rodehutscord M., Rückert C., Maurer H.P., Schenkel H., Schipprack W., Bach Knudsen K.E., Schollenberger M., Laux M., Eklund M., Siegert W. (2016). Variation in chemical composition and physical characteristics of cereal grains from different genotypes. *Arch. Anim. Nutr.*, 70: 87–107.
- Sauvant D., Perez J.M., Tran G. (2004). *Tables INRA-AFZ de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage: 2ème édition*. INRA Editions, Versailles France.
- Schiano A.N., Harwood W.S., Drake M.A. (2017). A 100-year review: Sensory analysis of milk. *J. Dairy Sci.*, 100: 9966–9986.
- Sharma H.R., Ingalls J.H., McKirdy J.A., Sanford L.M. (1981). Evaluation of rye grain in the diets of young Holstein calves and lactating dairy cows. *J. Dairy Sci.*, 64: 441–448.
- Sharma H.R., Ingalls J.R., McKirdy J.A. (1983). Feeding value of alkali-treated whole rye (*Secale cereale* L.) grain for lactating cows and its digestibility for sheep. *Anim. Feed Sci. Tech.*, 10: 77–82.
- Slots T., Butler G., Leifert C., Kristensen T., Skibsted L.H., Nielsen J.H. (2009). Potentials to differentiate milk composition by different feeding strategies. *J. Dairy Sci.*, 92: 2057–2066.
- Smit M.N., Zhou X., Landero J.L., Young M.G., Beltranena E. (2019). Increasing hybrid rye level substituting wheat grain with or without enzyme on growth performance and carcass traits of growing-finishing barrows and gilts. *Tran. Anim. Sci.*, 3: 1561–1574.
- Südekum K.H., Brandt M. (1994). Site and extent of cell-wall neutral monosaccharide digestion in dairy cows receiving diets with rolled cereal grains. *Anim. Feed Sci. Tech.*, 46: 307–320.
- Taylor M.W., MacGibbon A.K.H. (2003). Lipids. General characteristics. In: *Encyclopedia of Dairy Sciences*, Roginski H., Fuquay J.W., Fox P.F. (eds). Academic Press, London, UK, pp. 1544–1550.
- Van Soest P.J., Robertson J.B., Lewis B.A. (1991). Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 74: 3583–3597.
- Ziajka S. (2008). *Dairying* (in Polish). UWM, Olsztyn, Poland.

Received: 21 II 2020

Accepted: 20 I 2021