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Effects of replacing rye silage with mixed rye-vetch-straw silage on feed intake, milk production, digestion processes, and blood metabolites in dairy cows

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ABSTRACT

Mixed silage of whole catch crops—crops grown between 2 main crops—and straw might be an alternative forage source for dairy cattle in times of forage shortage but is still understudied. This research sought to evaluate the effects of feeding mixed rye-vetch-straw silage (RVSS) in replacement of whole crop rye silage on feed intake, ECM yield, milk composition, nutrient digestibility, and metabolic variables of dairy cows. The study was a crossover trial with 10 Holstein and 4 Simmental cows (average of 593 kg BW and 53 DIM at the start of the trial) tested in 2 experimental runs of 4 wk each, whereby the last 2 wk were used for the measurements. The cows were randomly allocated to one of the 2 diets differing only in the major forage source, either RVSS (RVSS treatment) or a pure rye silage (RS treatment) as the control. The diets were fed as partial mixed rations consisting on a DM basis of 43.8% RVSS or RS, 14.6% corn silage, 14.6% meadow hay, and 27.0% concentrate mixture. The cows were additionally supplemented with 5 kg of concentrate per day. Data showed that feeding the RVSS diet tended to decrease the DMI without affecting performance (mean ECM yield: 28.4 kg/d) or the mean BW during the sampling period. Feeding the RVSS diet tended to increase dairy feed efficiency (kg ECM/kg DMI), likely due to the higher apparent total-tract OM digestibility of the RVSS diet compared with the RS diet. Furthermore, cows fed the RVSS diet spent significantly more time on rumination per kilogram of intake of physically effective NDF. Diet did not affect the concentration of glucose, BHB, urea, total protein, albumin, triglycerides, cholesterol, liver enzymes, or macro minerals in blood. Neither of the diets affected the concentration or proportion of short-chain fatty acids in the rumen fluid and feces. In conclusion, the mixed silage of catch crop and straw holds potential to replace traditional rye silage in the feeding of dairy cows yielding up to 30 kg of ECM per day. Feeding the RVSS diet enhanced the apparent total-tract OM digestibility and rumination index, without any negative effects on ECM yield, milk composition, BW, ruminal and fecal VFA concentration, or key blood variables. Future research may evaluate the potential of mixed silages of catch crops and straw in the feeding of high-producing dairy cows as a viable option to overcome shortages in forage production.

Key words: alternative forage, catch crop, digestibility, feed efficiency, performance

INTRODUCTION

The high temperatures and extensive droughts during the last few years in Europe have jeopardized the production and quality of forages for ruminants. Estimates suggest that the yearly mean temperature in Europe's alpine regions may rise by up to 4°C until the end of the 21st century compared with the period from 1981 to 2010 (Kotlarski et al., 2023). The summer months seem to be particularly affected in terms of both increased temperature and decreased precipitation (Kotlarski et al., 2023). In Austria, forages from permanent grassland represent most of the forage resources in dairy cattle feeding (62%–84%; Ledinek et al., 2019). Permanent grassland is very sensitive to drought events, as recently indicated by a simulation study. Accordingly, a 3°C increase in air temperature and 0.3 mL/L greater atmospheric CO_2 concentration combined with long dry periods led to a 50% decreased grassland yield (Schaumberger et al., 2022). For dairy cattle, forages are an essential part of their diet, supplying energy, protein, minerals, and most importantly physically effective NDF to maintain rumen health. Therefore, forage shortages cannot be compensated for by grains or nonforage fiber sources.

In many countries, including Austria, catch crops could be produced as a forage source using precipitation during

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

winter and spring months. Catch crops are grown between 2 main crops to help retain nutrients and water and prevent their leaching or evaporation from the soil. Earlier studies have suggested the use of silages from winter cereals harvested at boot stage (Collar and Aksland, 2001) due to a higher nutritional value than silage harvested at a later stage of vegetation (Acosta et al., 1991; Jacobs et al., 2009; Zhao et al., 2021). Silages from winter whole crop cereals can be ensiled alone or as mixed silages with other feedstuffs of lesser quality, such as cereal straw. Wheat or barley straw is an abundant forage resource in summer but has a poor feed value due to high lignocellulose content. Because of this, straw is only marginally fed to dairy cattle in Central Europe. The mixed ensiling of straw with winter crops rich in fermentable substrates may aid the cleavage of straw's lignocellulosic bonds during ensiling (Abo-Donia et al., 2022). This would ameliorate the digestibility and the feed value of the ensiled straw in the mixed silage, being both an alternative forage resource and making use of winter catch crops and straw in the feeding of dairy cows. Earlier studies have shown that mixed silages of straw and sugar-rich feeds (e.g., molasses or sugar beet pulp) can be substituted for common diets without negative effects on feed intake, metabolic status (Abo-Donia et al., 2022; Wang et al., 2022), or milk yield of ruminants (Wang et al., 2022). Therefore, it is feasible to assume that mixed silages of sugar-rich whole crop cereals and straw could also be a good alternative feed for ruminants.

This study aimed to evaluate the effects of feeding mixed rye-vetch-straw silage (**RVSS**) or whole crop rye silage (**RS**), both harvested at boot stage, on feed intake; milk production; ruminal and fecal fermentation; apparent total-tract digestibility (**ATTD**) of nutrients; glucose, BHB, urea, total protein, albumin, triglyceride, cholesterol, liver enzyme, and macro mineral concentrations in blood; and the chewing and ruminating behavior of dairy cows. We hypothesized that mixed silage of whole crop rye, vetch, and straw could replace whole crop rye silage in the diet of lactating dairy cows without negative effects on feed intake, milk production, digestion processes, or key blood variables.

MATERIALS AND METHODS

Production of Experimental Silages

For this experiment, the rye (2.0 ha) and the rye-vetch mixture (2.5 ha) were cultivated on an experimental field at AREC Raumberg-Gumpenstein (47.51° N, 14.09° E) on September 28, 2022, 2 wk after the corn silage harvest. The rye-vetch mixture consisted of 60% rye (*Secale cereale*), 20% hairy vetch (*Vicia villosa*), and 20% Hungarian vetch (*Vicia pannonica*). This mixture was used for RVSS to achieve similar protein contents in both silages. The sowing densities were 160 kg/ha for rye and 120 kg/ha for the rye-vetch mixture. No manure was brought to the field before seeding or during growth of the catch crops. Both crops were harvested at the boot stage on May 4, 2023, and the rye-vetch mixture was tedded once, whereas the rye was tedded twice due to a higher amount of biomass at harvest compared with the rye-vetch mixture. Before windrowing, 1,000 kg/ha chopped barley straw was spread on the rye-vetch mixture with a cleaned manure spreader. The proportion of straw in the RVSS was ~20% on a DM basis. The silage was made with a round baler (Comprima 150XC, Bernhard Krone GmbH & Co., KG, Spelle, Germany), with the RVSS being produced on the same day of mowing and the RS on the day after. All bales were wrapped with 6 layers of stretch film with a bale wrapper (UN7517, Kverneland group, Klepp, Norway). Afterward, the bales were stored at the farm of AREC Raumberg-Gumpenstein until the beginning of the feeding experiment. Two months after harvest, samples were drawn from 4 bales of each silage and chemically analyzed (Table 1).

Animals, Housing, and Dietary Treatments

The animal experimental protocols and the use of dairy cows were approved by the national authority according to §26 of the Law for Animal Experiments, Tierversuchsgesetz 2012-TVG (Act No.: 2023–0.556.467 and Act No.: BMBWF-66.019/0017-V/3b/2019). The feeding experiment was carried out at the dairy research barn (cubicle housing system with straw) at AREC Raumberg-Gumpenstein from October to December 2023. The trial was designed as a crossover experiment with 2 treatments and 2 experimental runs. The cows were offered 2 different diets based on either the RS or RVSS as the main forage. Each experimental run lasted 28 d; the first 14 d were used for adaptation followed by 14 d for measurements and samplings. Between the 2 runs, there was a 1-wk washout period. Before starting the trial, the DMI and ECM yield of cows (means ± SD: 19.8 ± 1.9 kg DMI and 26.8 ± 5.5 kg ECM) were recorded for 7 d to assess covariates for the data analysis.

A total of 14 dairy cows (10 Holstein Friesian and 4 Simmental) were used. The cows were matched for breed, lactation number, stage of lactation, and milk yield (on average before the start of the trial: 2.7 lactations, 593 ± 83 kg BW, and 53 ± 47 DIM) and randomly allocated to 1 of the 2 groups. All cows were fed a partial mixed ration (**PMR**) ad libitum. Except for the catch crop silage (43.8% of either RS or RVSS), the PMR had the same composition in both groups (Table 2). During the covariance week, cows were fed the same PMR, but the RS or RVSS was replaced with grass si-

Table 1. Measured chemical composition, apparent total-tract nutrient digestibility, energy concentration, and fermentation products of catch crop rye silage (RS) and rye-vetch-straw silage (RVSS; mean ± SD; n = 4)

Item	RS	RVSS
Nutrient composition, g/kg DM unless otherwise stated		
DM, g/kg FM	228 ± 21	232 ± 11
Ash	94.3 ± 12.1	89.3 ± 9.5
CP	134 ± 6	122 ± 4
Ether extract	23.8 ± 1.7	23.3 ± 1.3
NDF	523 ± 9	568 ± 23
ADF	334 ± 9	366 ± 15
ADL	35.9 ± 6.6	41.2 ± 3.8
Hemicellulose[1]	189 ± 13	201 ± 11
Cellulose[2]	298 ± 14	325 ± 14
NFC[3]	225 ± 24	197 ± 16
Apparent total-tract digestibility of nutrients,[4] %		
OM	71.8 ± 2.0	69.5 ± 3.9
CP	65.2 ± 2.1	66.7 ± 3.0
NDF	69.8 ± 4.1	67.2 ± 5.3
ADF	67.0 ± 4.7	62.3 ± 5.2
NFC	81.4 ± 1.6	78.0 ± 3.1
Energy concentration,[4] MJ/kg DM		
ME	9.94 ± 0.24	9.65 ± 0.51
NEL	5.91 ± 0.17	5.71 ± 0.37
Fermentation products, g/kg DM		
Lactic acid	6.62 ± 3.01	23.5 ± 8.5
Acetic acid	9.27 ± 5.58	12.9 ± 0.7
Propionic acid	4.49 ± 2.09	3.53 ± 0.40
Butyric acid	47.2 ± 7.7	48.8 ± 5.8
Ethanol	17.9 ± 3.3	29.5 ± 1.5
pH	4.94 ± 0.16	4.59 ± 0.08

[1]Calculated as NDF − ADF.

[2]Calculated as ADF − ADL.

[3]Nonfiber carbohydrates, calculated as 1,000 − ash − CP − ether extract − NDF.

[4]Apparent total-tract digestibility and energy concentration were examined in an in vivo experiment with wethers according to GfE (1991).

Table 2. Ingredients and chemical composition of the partial mixed rations (PMR) containing either catch crop rye silage (RS) or rye-vetch-straw silage (RVSS; mean ± SD; n = 2)

Item, g/kg DM unless otherwise stated	RS PMR	RVSS PMR
Ingredients of PMR		
RS	438	0
RVSS	0	438
Corn silage	146	146
Meadow hay	146	146
Corn (ground)	62	62
Barley (ground)	62	62
Wheat (ground)	30	30
Rapeseed meal	103	103
Mineral-vitamin premix[1]	5.2	5.2
Limestone	5.2	5.2
Sodium chloride	2.6	2.6
Composition		
DM, g/kg FM	359 ± 9	335 ± 16
CP	131 ± 2	133 ± 4
Ether extract	22.0 ± 0.8	22.7 ± 2.9
Ash	82.4 ± 1.9	75.4 ± 1.5
NDF	484 ± 39	486 ± 3
ADF	306 ± 24	309 ± 1
ADL	44.5 ± 3.0	43.5 ± 4.0
Hemicellulose[2]	179 ± 15	177 ± 3
Cellulose[3]	261 ± 21	265 ± 5
$peNDF_{>8mm}$[4]	386 ± 16	371 ± 11
NFC[5]	280 ± 35	283 ± 1
Starch	88.7 ± 3.1	105 ± 8
ME,[6] MJ/kg DM	9.89 ± 0.02	10.03 ± 0.43
NEL,[6] MJ/kg DM	5.88 ± 0.01	5.97 ± 0.31

[1]Rindamin GM-T (H. Wilhelm Schaumann GmbH & Co. KG, Brunn am Gebirge, Austria); composition per kg: 150 g Ca, 45 g P, 50 g Mg, 120 g Na, 1.2 g Cu, 6 g Zn, 4 g Mn, 50 mg Se, 50 mg Co, 350 mg I, 800,000 IU vitamin A, 80,000 IU vitamin D_3, and 2,500 IU vitamin E.

[2]Calculated as NDF − ADF.

[3]Calculated as ADF − ADL.

[4]$peNDF_{>8mm}$ = physically effective NDF greater than 8 mm.

[5]Nonfiber carbohydrates, calculated as 1,000 − ash − CP − ether extract − NDF.

[6]Apparent total-tract digestibility of PMR was analyzed in vitro using the cellulase method according to GfE (2009).

lage. The PMR was mixed on the day of feeding and cut to ~4 cm theoretical particle length using a feed mixer (TrailedLine Premium 12, Siloking Mayer Maschinenbau GmbH, Tittmoning, Germany). Silage bales were put into the feed mixer immediately after removing the stretch film. Each cow also received 2 kg/d of a pelleted commercial concentrate (Kuhkorn PLUS Energie, Garant Tiernahrung GmbH, Pöchlarn, Austria) via the automatic milking system. In addition, the cows had access to 2 kg/d of a concentrate mixture (25% barley, 25% corn, 10% wheat, 25% sugar beet pulp, and 15% wheat bran) and 1 kg/d rapeseed meal via a computer-controlled concentrate feeder (Supplemental Table S1, see Notes). All concentrates were provided during the whole feeding trial, including the covariate period and washout, adaptation, and sampling periods. Fresh feed was provided at 0530 and 1430 h, and daily feed intake was recorded using the Calan Gate System (American Calan, Northwood, NH). Cows were milked in an automatic milking system (Dairy Robot R9500, GEA, Düsseldorf, Germany), which recorded milk yield and BW at each milking. On d 3 and 10 during each sampling period, 2 milk samples were taken from each cow per day and sent to a laboratory for milk analysis (Qualitätslabor Österreich, St. Michael, Austria). Based on milk yield as well as fat and protein content of the milk, ECM yield was calculated according to GfE (2001).

Sampling and Analyses

Feed. Samples of pure RS, pure RVSS, PMR, and PMR residues were collected daily, and samples of concentrates were taken weekly. The DM content of all samples was determined by drying for 24 h at 103°C. Pooled samples of feeds and PMR per sampling period were analyzed for nutrient contents using official meth-

Table 3. Effects of replacing rye silage (RS) with rye-vetch-straw silage (RVSS) in the diet and feeding week[1] on BW, nutrient and energy intake, and dairy performance and efficiency of dairy cows

Item	RS W1	RS W2	RVSS W1	RVSS W2	SEM	P-value Diet	P-value Week	P-value Diet × Week
BW, kg	602	605	599	604	18	0.94	0.04	0.92
Nutrient and energy intake								
DMI PMR, kg/d	17.0	17.3	15.3[b]	16.5[a]	0.5	0.08	<0.01	0.06
DMI concentrate,[2] kg/d	4.32	4.28	4.36	4.39	0.05	0.33	0.67	0.10
Total DMI, kg/d	21.3	21.5	19.6[b]	20.9[a]	0.5	0.10	<0.01	0.04
CP intake, kg/d	3.09	3.11	2.91[b]	3.08[a]	0.06	0.24	<0.01	0.05
Ash intake, kg/d	1.63	1.66	1.37[b]	1.48[a]	0.04	<0.01	<0.01	0.06
NDF intake, kg/d	9.28	9.39	8.48[b]	9.11[a]	0.24	0.13	<0.01	0.04
$peNDF_{>8mm}$ intake,[3] kg/d	6.51	6.60	5.67[b]	6.08[a]	0.16	<0.01	<0.01	0.05
ADF intake, kg/d	5.72	5.79	5.25[b]	5.65[a]	0.15	0.17	<0.01	0.04
ADL intake, kg/d	0.91	0.92	0.81[b]	0.87[a]	0.03	0.04	<0.01	0.04
NFC intake, kg/d	6.79	6.87	6.36[b]	6.72[a]	0.15	0.20	<0.01	0.06
Starch intake, kg/d	2.54	2.87	2.93[b]	3.07[a]	0.05	0.06	<0.01	0.01
ME intake, MJ/d	224	226	210[b]	222[a]	5	0.20	<0.01	0.05
NEL intake, MJ/d	135	136	127[b]	134[a]	3	0.21	<0.01	0.05
uCP intake,[4] kg/d	3.13	3.16	2.94[b]	3.11[a]	0.06	0.22	<0.01	0.05
RNB,[5] g/d	−6.79	−6.67	−5.32	−5.36	1.55	0.53	0.92	0.84
Milk yield and composition								
Milk yield, kg/d	27.2	26.1	26.5	26.2	0.6	0.66	0.02	0.22
ECM yield,[6] kg/d	29.0	27.8	28.5	28.3	0.5	0.99	0.19	0.34
Milk fat, %	4.59	4.61	4.64	4.72	0.17	0.75	0.61	0.74
Milk protein, %	3.43	3.42	3.52	3.49	0.07	0.43	0.58	0.71
Lactose, %	4.82	4.78	4.80	4.82	0.06	0.94	0.85	0.31
Milk urea, mg/100 mL	16.9	14.6	17.6[a]	14.6[b]	1.0	0.81	<0.01	0.67
SCS	2.27	1.98	1.63	1.66	0.37	0.37	0.61	0.50
Energy and protein balance								
NEL balance,[7] %	104.8	108.0	100.7	105.6	3.2	0.48	0.04	0.64
uCP balance,[8] %	113.5	117.5	109.6	115.3	2.0	0.28	<0.01	0.60
Dairy efficiency								
kg ECM/kg DMI	1.37	1.30	1.46[a]	1.36[b]	0.03	0.09	<0.01	0.60
kg ECM/kg NEL intake	0.216	0.204	0.226	0.211	0.005	0.19	<0.01	0.67
kg milk protein/kg uCP intake	0.298	0.282	0.317[a]	0.291[b]	0.006	0.09	<0.01	0.39

[a,b]Different superscript letters indicate a significant difference between weeks within a diet type.

[1]W1 = feeding wk 1; W2 = feeding wk 2.

[2]Each cow had access to 2 kg/d of AMS concentrate (Kuhkorn PLUS Energie, Garant Tiernahrung GmbH, Pöchlarn, Austria) in the automatic milking system, 2 kg/d of an energy concentrate (25% barley, 25% corn, 10% wheat, 25% sugar beet pulp, and 15% wheat bran), and 1 kg/d rapeseed meal at a concentrate feeder.

[3]$peNDF_{>8mm}$ = physically effective NDF greater than 8 mm.

[4]uCP = estimated utilizable crude protein at the duodenum (GfE, 2001).

[5]RNB = ruminal nitrogen balance, calculated as (CP intake − uCP intake)/6.25.

[6]ECM yield was calculated according to GfE (2001) as [(0.38 × % milk fat + 0.21 × % milk protein + 0.95)/3.2] × kg milk/d.

[7]NEL balance was calculated as (NEL intake/NEL demand) × 100.

[8]uCP balance was calculated as (uCP intake/uCP demand) × 100.

ods (VDLUFA, 2012): DM, method 3.1; CP, 4.1.2; ether extract, 5.1.1; ash, 8.1; NDF assayed with heat stable amylase and expressed exclusive of residual ash, 6.5.1; ADF expressed exclusive of residual ash, 6.5.2; ADL, 6.5.3; enzyme-soluble organic matter, 6.6.1.; and HCl-insoluble ash, 8.2. The total starch content was determined using a commercially available kit (Megazyme, Wicklow, Ireland) in sample aliquots that were ground through a 0.5-mm screen in an ultracentrifugal mill (ZM 200, Retsch, Haan, Germany). The content of physically effective NDF > 8 mm (**$peNDF_{>8mm}$**) of PMR was determined by sieving the PMR using a Penn State Particle Separator (model C24682N, Nasco) and calculated using the equations published by GfE (2023). The DM and nutrient contents of the PMR and the residues were corrected for unavoidable DM losses using the method described by Weißbach and Kuhla (1995).

The contents of ME and NEL in PMR and concentrates were calculated using equations published in GfE (2009), and the concentration of utilizable crude protein at the duodenum (**uCP**) was estimated based on equations of GfE (2001). Energy concentrations of pure RS and RVSS

Table 4. Effects of replacing rye silage (RS) with rye-vetch-straw silage (RVSS) in the diet on eating and ruminating behavior of dairy cows

Item	RS	RVSS	SEM	*P*-value
Eating				
Min/d	291	249	23	0.19
Min/kg DMI	13.6	13.2	1.2	0.79
Min/kg NDF intake	31.5	30.6	2.8	0.82
Min/kg $peNDF_{>8mm}$[1] intake	44.6	45.9	4.1	0.83
Ruminating				
Min/d	522	520	15	0.91
Min/kg DMI	24.8	27.5	1.0	0.05
Min/kg NDF intake	57.2	63.8	2.5	0.06
Min/kg $peNDF_{>8mm}$ intake	81.0	95.4	3.6	<0.01
Ruminating boli, no./d	587	597	15	0.65
Ruminating chews/bolus	55.5	53.2	2.3	0.47
Eating + ruminating				
Min/d	813	775	30	0.37
Min/kg DMI	38.2	40.7	1.8	0.30
Min/kg NDF intake	88.1	94.7	4.5	0.30
Min/kg $peNDF_{>8mm}$ intake	125	142	7	0.08

[1]$peNDF_{>8mm}$ = physically effective NDF greater than 8 mm.

were analyzed in a digestibility trial with adult wethers according to GfE (1991). The digestibility trial lasted for 19 d, with the first 14 d used for adaptation and the last 5 d for sampling. A total of 8 wethers (4 wethers per feed, average BW of 80 kg) were used in this trial and fed 1 kg of DM of RS or RVSS as well as 20 g of mineral mixture and 4 g of salt (as fed) per day at maintenance level. The DMI was recorded daily, and total feces were collected and weighed after each morning and evening feeding. Feces were put into a cooling chamber (at 4°C) and thoroughly mixed at the end of the sampling period. Pooled samples of feces per wether were analyzed using the same methods as described above, except for the N content of feces, which was determined in fresh material to prevent N losses during the drying process (method 4.1.1; VDLUFA, 2012). The ME and NEL contents of both silages were calculated using the equations recommended by GfE (2001). The content of volatile organic compounds (lactic acid, acetic acid, propionic acid, butyric acid, and ethanol) in fresh silage was analyzed according to VDLUFA (2012) using a gas chromatograph (3900, Varian Inc., Palo Alto, CA). Furthermore, the pH value was assessed with a pH meter (WTW Multi 3620, Xylem Inc., Washington, DC) in a batch of silage and distilled water (sample-to-water ratio of 1:10) according to methods 18.1 and 18.2 in VDLUFA (2012).

Eating and Rumination. The chewing and eating activity of each cow was measured on d 2 to 5 during each sampling period using noseband sensor halters (RumiWatch System, ITIN + Hoch GmbH, Liestal, Switzerland), as outlined in detail by Rivera-Chacon et al. (2022). The duration of eating, ruminating, and total chewing time (eating + ruminating times; min/d), number of ruminating boli (no./d), and chews per bolus (no./bolus) were measured. Additionally, the chewing indices for each chewing category were related to feed intake data of the same day (min/kg of DMI, NDF, and $peNDF_{>8mm}$ intake).

Rumen Fluid. Rumen fluid was collected before the afternoon feeding from each cow on d 10 of each sampling period using a stomach tube with the method described by Steiner et al. (2015). After discarding the first 100 mL, ~300 mL of rumen fluid was collected and filtered through 4 layers of gauze compresses (Wilhelm Weisweiler GmbH & Co. KG, Münster, Germany). Afterward, the pH was measured, and aliquots were stored at −20°C until analysis of VFA and ammonia-N. The VFA concentrations were determined by GC using a GC apparatus (Shimadzu GC Plus with FID detector, Shimadzu, Kyoto, Japan) that was equipped with a 30 m × 0.53 mm inner diameter × 0.53 μm capillary column (Trace TR Wax, Thermo Fisher Scientific, Waltham, MA). The injector and detector had temperatures of 170°C and 220°C, respectively, and helium was used as the carrier gas with a flow rate of 1 mL/min. The ammonia-N was analyzed colorimetrically based on the Berthelot reaction (Hinds and Lowe, 1980), and lactic acid was analyzed by HPLC (UltiMate 3000 HPLC system, Thermo Fisher Scientific, Vienna, Austria) following the method of Weiß and Kaiser (1995).

Feces. Fecal samples were collected from the rectum of the cows on d 3 and 8 of each sampling period in the afternoon right after feeding for analyzing pH and VFA. The pH was measured immediately with a portable pH

Table 5. Effects of replacing rye silage (RS) with rye-vetch-straw silage (RVSS) in the diet of dairy cows on the ruminal and fecal pH, ammonia-N (only rumen fluid), VFA, and apparent total-tract digestibility of the diet

Item	RS	RVSS	SEM	*P*-value
Rumen fluid				
pH	6.81	6.80	0.05	0.84
Ammonia-N, mmol/L	5.96	5.65	0.34	0.52
Total VFA, mmol/L	90.9	86.4	3.0	0.30
Acetic acid, % of VFA	61.6	61.4	0.8	0.80
Propionic acid, % of VFA	18.2	18.1	0.5	0.81
Isobutyric acid, % of VFA	0.77	0.76	0.05	0.90
Butyric acid, % of VFA	16.0	16.4	0.7	0.57
Isovaleric acid, % of VFA	1.47	1.40	0.11	0.56
Valeric acid, % of VFA	1.09	1.05	0.05	0.45
Caproic acid, % of VFA	0.88	0.92	0.08	0.66
Feces				
pH	6.31	6.37	0.02	0.21
Total VFA, mmol/L	38.8	38.6	1.9	0.95
Acetic acid, % of VFA	73.8	74.0	0.6	0.78
Propionic acid, % of VFA	16.3	15.9	0.3	0.27
Isobutyric acid, % of VFA	1.14	1.25	0.08	0.16
Butyric acid, % of VFA	6.88	6.88	0.43	0.99
Isovaleric acid, % of VFA	0.71	0.82	0.09	0.26
Valeric acid, % of VFA	1.23	1.20	0.07	0.72
Apparent total-tract digestibility,[1] %				
OM	77.6	80.0	0.6	0.01
CP	71.2	76.5	0.9	<0.01
NDF	66.8	69.2	1.2	0.16
ADF	64.5	67.4	1.2	0.11
NFC	96.1	96.6	0.2	0.07
Starch	98.6	98.9	0.1	0.02

[1]Apparent total-tract digestibility of diets in dairy cows was tested using the marker method (marker: HCl-insoluble ash) as described by Hafez et al. (1988).

meter (Mettler-Toledo AG Analytical, Schwerzenbach, Switzerland). For determining VFA, aliquots were filled in 8-mL tubes (Sarstedt, AG) and stored at −20°C until analysis. The VFA concentrations were analyzed as described above for rumen fluid. Furthermore, samples of feces from each cow were taken during the morning and afternoon feedings on d 8 to 10 of each sampling period for measuring ATTD. The fecal samples were analyzed with the same methods as described for feed, and N content was also analyzed in fresh feces. The HCl-insoluble ash and nutrient concentrations in feed and feces were used to calculate the ATTD of nutrients according to Hafez et al. (1988).

Blood. Blood samples were collected on d 2 and 9 of each sampling period, right after the afternoon feeding. Samples were taken from the coccygeal vein using 9-mL serum evacuated tubes and 6-mL evacuated tubes with sodium fluoride-potassium oxalate (Vacuette, Greiner Bio One International GmbH, Kremsmünster, Austria). Serum tubes were stored for 2 h at room temperature, whereas plasma tubes were stored at 4°C. Samples were centrifuged at 2,000 × *g* for 15 min at 20°C (Centrifuge 5702, Eppendorf AG), and serum and plasma aliquots were stored in 2-mL tubes (Sarstedt, AG, Wiener Neudorf, Austria) at −20°C until analysis. Concentrations of the following blood parameters were analyzed using standard enzymatic colorimetric assays with a fully automated analyzer for clinical chemistry (Cobas 6000/c501, Roche Diagnostics GmbH, Vienna, Austria) at the laboratory of the Department of Biological Sciences and Pathobiology (University of Veterinary Medicine, Vienna, Austria): glucose in plasma, BHB, urea, total protein, albumin, triglycerides, cholesterol, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, glutamate dehydrogenase, γ-glutamyl transferase, calcium, phosphate, magnesium, sodium, and potassium in serum.

Statistical Analysis

The statistical analysis was carried out using SAS 9.4 (SAS Institute Inc., Cary, NC) and the MIXED procedure. The fixed effects included diet, experimental run, feeding week (within run), and the diet × week interaction. Other interactions were not considered in the statistical model as they were not significant. The covariates ECM yield and total DMI before the start of the trial were used in the analysis of BW, feed and nutrient intake, milk yield,

Table 6. Effects of replacing rye silage (RS) with rye-vetch-straw silage (RVSS) in the diet on blood metabolites of dairy cows

Item	RS	RVSS	SEM	*P*-value
Glucose, mg/100 mL	61.3	64.7	1.4	0.10
Urea, mg/100 mL	19.7	18.4	0.7	0.23
Total protein, g/100 mL	7.20	7.17	0.08	0.81
Triglycerides, mg/100 mL	17.0	16.3	0.5	0.30
Cholesterol, mg/100 mL	198	199	8	0.92
Albumin, mg/100 mL	4.18	4.16	0.06	0.84
Alkaline phosphatase, U/L	71.0	72.1	9.8	0.94
Aspartate aminotransferase, U/L	79.4	78.9	2.9	0.91
Alanine aminotransferase, U/L	30.4	30.7	2.9	0.96
Glutamate dehydrogenase, U/L	11.6	11.3	0.8	0.79
γ-Glutamyl transferase, U/L	26.1	25.5	1.5	0.77
BHB, mmol/L	1.45	1.36	0.16	0.69
Calcium, mmol/L	2.50	2.52	0.02	0.59
Phosphorus, mmol/L	2.07	1.96	0.05	0.11
Magnesium, mmol/L	1.07	1.08	0.02	0.87
Sodium, mmol/L	143	143	0.5	0.27
Potassium, mmol/L	4.84	4.78	0.07	0.53

milk components, and efficiency parameters. Repeated measurements in individual animals in consecutive weeks within a run were considered in the model, and the autoregressive covariance structure was chosen based on the Akaike information criterion (**AIC**; smaller is better). In the analysis of eating and ruminating behavior, ATTD of nutrients, VFA concentrations, and blood metabolites, diet and run were used as fixed effects. Furthermore, measurements of eating and ruminating behavior taken from the same cow on different days within a run were considered as repeated measures in the ANOVA using the autoregressive covariance structure (chosen based on the AIC). The results are presented as LSM and SEM. Significant differences were assumed if $P < 0.05$, and a trend for a difference was interpreted if $0.05 \leq P < 0.10$. The Tukey-Kramer post hoc test was used for multiple comparisons of LSM.

RESULTS

Nutrient Composition of Silages and Diets

The nutrient composition, the ATTD of the nutrients, the energy content, and the fermentation characteristics of both silages used in this experiment are presented in Table 1. In both silages, the DM content was quite low, and the butyric acid content was high (47.2 g/kg DM in RS and 48.8 g/kg DM in RVSS). Furthermore, an intense smell of butyric acid was observed in both silages. The pH was below 5.0 in both silages, with a numerically lower pH in the RVSS due to a markedly higher lactic acid content. The RVSS was numerically lower in CP and NFC but higher in NDF, ADF, and ADL concentration than the RS. Moreover, the ATTD of OM, NDF, ADF, and NFC was also numerically lower in the RVSS, resulting in a lower energy content than the RS. In contrast, the nutrient composition and energy content of PMR (Table 2) made with RS and RVSS were very similar, except for an ~10% higher ash content in RS-based PMR.

Feed, Nutrient, and Energy Intake

The results on DMI, nutrient intake, and energy intake are presented in Table 3. Feeding of the RVSS diet tended to decrease the DMI of PMR (P = 0.081) and total DMI (P = 0.098). However, the type of catch crop silage did not affect nutrient or energy intake except for ash, $peNDF_{>8mm}$, and ADL intake, which were lower in the RVSS diet. Except for DMI from concentrate, there was a significant effect of feeding week on DMI, nutrient intake, and energy intake, as well as a significant or tendential diet × week interaction. No difference was observed in DMI, nutrient intake, or energy intake between feeding weeks in cows fed the RS diet. In contrast, cows fed the RVSS had a significantly higher intake of DM, nutrients, and energy in the second experimental week.

BW, Milk Yield, and Milk Components

Results on BW, milk yield, and milk components are presented in Table 3. The mean BW and the BW change of cows during the sampling period were not affected by dietary treatment. The BW change from the beginning to the end of the sampling period (2 wk) was −0.01 kg and 5.08 kg in the RS and RVSS groups, respectively (P = 0.409). The ECM yield was in the range of 28 and 29 kg/d and did not differ between treatments. Furthermore, contents of milk components were not affected by diet type.

A significant week effect was found for milk yield (P = 0.02) and milk urea concentration (P < 0.01), with lower levels in the second week for both mentioned parameters. The treatment × week interaction was not significant for any parameter of milk yield or milk composition.

Energy and Protein Balance and Efficiency of Milk Production

The diet type did not influence the NEL or uCP balance of cows (Table 3). Both NEL and uCP intake were beyond the NEL and uCP demand in both experimental groups. However, in both groups, the NEL and uCP balance was higher in the second feeding week, with no interaction of diet type and week. A trend was observed for a difference in ECM production per kilogram of DMI (P = 0.09) and in milk protein production per kilogram of uCP intake (P = 0.09), with higher values in the RVSS group. In contrast, ECM production per kilogram of NEL intake did not differ between treatments. All 3 parameters of dairy efficiency were higher in the first compared with the second feeding week.

Eating and Ruminating Behavior

Results on eating and ruminating behavior are presented in Table 4. Treatment did not influence time spent on eating, ruminating, or the sum of eating and ruminating per day. In contrast, cows fed the RVSS diet spent significantly more time on ruminating per kilogram of $peNDF_{>8mm}$ intake (P < 0.01) and tended to ruminate more per kilogram of DMI (P = 0.05) and NDF intake (P = 0.06) than cows fed the RS diet. As a result, a trend was observed for more time spent on eating and ruminating per kilogram of $peNDF_{>8mm}$ intake (P = 0.08) in the RVSS group, whereas eating and ruminating per kilogram of DMI and NDF intake did not differ between treatments. Furthermore, the number of ruminating boli per day and the number of ruminating chews per bolus were also approximately the same in RS and RVSS diets.

Digestibility Data and Blood Metabolites

Diet did not affect ruminal and fecal pH, total VFA concentration and composition, ruminal ammonia-N concentration, or the ATTD of NDF and ADF (Table 5). In contrast, the ATTD of OM (P = 0.01), CP (P < 0.01), and starch (P = 0.02) was significantly higher in the diet containing RVSS than RS. Furthermore, a trend was observed for a higher ATTD of NFC (P = 0.07) in the RVSS treatment. Analysis of blood metabolites did not show any diet effects, except for a trend for higher glucose concentration (P = 0.10) in cows fed the RVSS diet than in cows fed the RS diet (Table 6).

DISCUSSION

This study evaluated whether feeding a mixed silage of catch crop (rye-vetch mixture) and straw leads to similar performance in dairy cows as feeding a diet including pure rye silage. Results of this study showed that feeding the RVSS diet tended to decrease the DMI of dairy cows, especially due to a significantly lower DMI in the first compared with the second feeding week. The DMI of dairy cows is influenced by several factors. Gruber et al. (2005) suggested that forage quality is one of the factors influencing DMI of the total diet. Accordingly, the DMI increases with rising NEL concentration and OM digestibility or decreasing crude fiber content of forage. Indeed, the mixed ensiling of ~20% straw and 80% rye-vetch mixture led to a numerically lower ATTD of OM and NEL concentration (measured in vivo with wethers), as well as higher NDF and ADF factions compared with pure rye silage, which might explain the lower DMI in the RVSS group. In contrast, Mertens (1994) described that the NDF content of the diet regulates the physical rumen fill and therefore the feed intake capacity of cows when low-quality diets are fed, which applies to both diets in the present experiment (NEL concentration <6.0 MJ/kg DM). However, the NDF and $peNDF_{>8mm}$ concentration in the PMR were very similar in both diets. Therefore, the different DMI in the RS and RVSS groups cannot be explained by varying NDF or $peNDF_{>8mm}$ concentrations in diets as proposed by Mertens (1994).

Another factor influencing the DMI of cows is the high butyric acid concentration of silages. In our study, the butyric acid concentration was similarly high in the RS and RVSS diets (47.2 and 48.8 g/kg DM, respectively), likely due to the low DM concentrations of both silages (Kung et al., 2018). Thus, this may not explain differences in DMI between groups either. In a study by Senel and Owen (1967), the DMI of lactating dairy cows decreased when feeding a diet containing 20 g/kg DM butyric acid compared with a control diet consisting of alfalfa hay and concentrates. When related to the complete diet, butyric acid concentrations were similar, as in the study by Senel and Owen (1967). It is possible that mixing RS and RVSS with other ingredients of proper sensory quality, which accounted for 56.2% of the PMR DM, might have positively affected the palatability of these PMR. The DMI of cows fed the experimental diets was similar or even slightly higher than in the covariance period, when cows were fed the same diet, except for grass silage (with low butyric acid concentration) replacing RS or RVSS in the PMR. Further research is needed to study the long-term effects of feeding PMR high in butyric acid on diet palatability and cow performance. Furthermore, research efforts are needed to improve the quality of catch crop silages.

The DMI of cows in the RVSS treatment was especially low in the first week, whereas it significantly increased in the second feeding week. In an earlier study, Kleefisch et al. (2018) found a slower increase in DMI in cows fed fiber-rich hay than in cows fed high-quality hay with low fiber content in the first weeks after calving. The difference in DMI was ~2 kg in the first week after calving, but DMI was approximately the same 4 wk after calving. This indicates that higher NDF or $peNDF_{>8mm}$ concentrations in diets might extend the adaptation period of cows to a new diet. Although we had a 2-wk adaptation period before starting data recording and sampling in this trial, this period seemed to be too short to allow the full adaptation of cows to a new, fiber-rich diet. Another reason for the longer adaptation period could be the high butyric acid concentration and the low palatability of the RVSS. However, this assumed prolonged adaptation to the new diet was not found in cows fed the RS diet, although the NDF concentrations of both PMR and the butyric acid concentrations of both catch crop silages were on a similar level. A possible reason for the differing effects of week on DMI in the RS and RVSS groups could be the higher NDF concentration and the lower NDF digestibility of RVSS, indicating a higher concentration of indigestible NDF in forage of the RVSS compared with the RS diet. Future research projects should clarify whether longer adaptation periods and longer periods of data recording change the effects on DMI when feeding mixed silage of catch crops and straw or silages high in butyric acid concentration to dairy cows.

In contrast to DMI, feeding the RVSS diet did not affect the nutrient or energy intake of dairy cows compared with the RS diet. The lack of difference in nutrient and energy intake was likely due to slightly higher starch and energy concentrations in PMR containing RVSS. The higher ATTD of OM and the numerically higher ATTD of NDF found in the digestibility measurements of dairy cows explain the higher energy concentration of the RVSS diet. Associative effects of feeds in mixed rations, as described by Metzler-Zebeli et al. (2012), could have led to the slightly higher energy concentration in the RVSS compared with the RS diet. However, it remains unclear why such associative effects did not appear in the RS diet. Another explanation for similar nutrient intake could be fermentation processes in the silage bales. Results of nutrient analysis show that the hemicellulose content of RVSS was higher than that of RS but numerically lower than a weighted average of the hemicellulose contents of rye silage and straw (LfL Bayern, 2023). Furthermore, lactic acid, acetic acid, and ethanol concentrations were numerically higher in RVSS than in RS. These results indicate that mixed ensiling of rye-vetch mixture and straw promoted activity of heterofermentative lactic acid bacteria in RVSS. These microbes are known to degrade pentosanes, such as xylose, which are a major fraction of hemicellulose in straw (Sun et al., 1996; Pessione, 2012). This assumption is supported by the ME concentration of RVSS, which was roughly 0.4 MJ/kg DM higher than the expected value (weighted average of ME concentration in rye silage and straw). This indicates that mixed ensiling of straw and catch crops improved the expected nutritive value of straw, providing a higher level of energy than mixing catch crop silage and straw just before feeding.

Diet type did not affect the milk production or milk composition of dairy cows, showing that straw inclusion in the diet did not impair animal performance and thus confirming our hypothesis. Similar to DMI, an effect of feeding week was also found for milk yield, which decreased from the first to second feeding week in both groups. Energy and protein balance were continuously above 100% in both groups, indicating surplus supply of energy and protein to cows. This energy and protein surplus was higher in the second week due to the lower milk yield and, in the case of the RVSS group, due to the higher DMI. These results could be an indicator that cows adapted their metabolism to the changed diet in the 4 wk of the trial. The forage proportion and the forage quality may influence energy partitioning in cows. Studies have shown that dairy cows fed high-forage diets use a higher share of energy for milk production and a lower share for BW gain (Moe, 1981; Allen and Piantoni, 2014). However, in the study by Gordon et al. (1995), feeding low-digestibility grass silages decreased the utilization of ME for milk production by 3 percentage units compared with cows fed high-digestibility grass silages. The quality of catch crop silages used in our study was medium to low compared with that of typical grass silages (DLG, 1997). The results of our study indicate that cows adapted to the low-quality forage by decreasing milk production from wk 1 to wk 2 and using the energy for other purposes, such as maintenance or BW gain.

We found a trend for higher feed efficiency in cows fed the RVSS diet than cows receiving the RS diet. Furthermore, the ATTD of OM and CP was significantly higher, and NFC digestibility tended to be higher in cows fed RVSS than those fed the RS diet. The reason for the higher ATTD of OM in the RVSS group could be the tendentially higher starch intake of cows fed the RVSS diet due to numerically higher starch concentration in PMR and numerically higher concentrate intake. Another reason could be the lower DMI in the first week. As samples for analyzing ATTD were collected at the beginning of the second feeding week, the higher ATTD of RVSS treatment could be related to the lower feed intake of cows in the first week, which may also apply for the higher feed efficiency of cows fed the RVSS diet. A lower feed intake reduces the passage rate of feed in the digestive tract and therefore increases time available for feed digestion (Mertens, 1994). This is also supported by the longer time of rumination per kilogram of DMI, NDF, or $peNDF_{>8mm}$ intake in cows fed the RVSS diet, whereas treatment did not affect the daily time cows spent eating or ruminating. As both high feed intake and high feed digestibility are important prerequisites for supplying adequate energy and nutrients to high-yielding dairy cows, the tendentially lower DMI may limit the use of PMR containing RVSS in the feeding of high-yielding dairy cows, especially in early lactation. However, RVSS could indeed be a good alternative forage for dairy cows with a medium to low milk yield potential, including late-lactation or dry cows as well as young cattle.

In terms of blood metabolites, no significant differences were observed between the treatment groups. The BHB concentration was slightly elevated in both groups, at 1.45 and 1.36 mmol/L in the RS and RVSS groups respectively, which apparently indicated subclinical ketosis. However, both RS and RVSS showed elevated contents of butyric acid (i.e., 47.2 and 48.8 g/kg DM in RS and RVSS, respectively), and the ruminal VFA profile showed higher butyric acid proportions than in cows fed a common TMR consisting of grass silage, corn silage, and concentrates (Castillo-Lopez et al., 2021). It is more likely that the slightly elevated serum BHB concentrations originated from high ruminal absorption of butyric acid, which is then metabolized into BHB (Bergman, 1990), and from blood sampling taking place right after the afternoon feeding. The BHB concentrations in blood are higher right after feeding due to elevated endogenic BHB synthesis (Ametaj et al., 2009). These assumptions are also supported by the positive energy balance of all cows as well as the glucose concentrations that were within the range typically found for lactating cows (e.g., Pawliński et al., 2023). Regarding the other blood variables, such as liver enzymes, no differences or negative effects of the treatment diets were observed. Likewise, serum mineral concentrations indicated an appropriate provision of micronutrients by both diets. Hence, feeding the RVSS diet did not affect the metabolism of cows compared with the provision of the RS diet. The concentration of total VFA and individual VFA proportions in rumen fluid and feces did not differ between treatments. The high proportions of lipogenic VFA along with low proportions of glucogenic VFA in both gut locations clearly indicated a microbial fermentation pattern typical when feeding forage-rich diets to ruminants (Wang et al., 2020).

CONCLUSIONS

Including mixed silages of rye, vetch, and straw instead of pure rye silage as the main forage source in dairy cow diets tended to decrease DMI but did not impair the nutrient supply or milk performance of dairy cows at a production level of ~25 to 30 kg ECM/d. Moreover, feeding RVSS did not adversely affect efficiency parameters or digestive and metabolic processes, but increased rumination per kilogram of $peNDF_{>8mm}$ intake. Therefore, silages of catch crops and straw hold potential to replace common cereal silages in the feeding of dairy cows yielding up to 30 kg ECM/d and could help to cope with the scarcity of forage production. Further research should evaluate the potential of silages from catch crops and straw in the feeding of high-yielding dairy cows during a complete lactation period and in other ruminant categories and effects of fermentation characteristics of catch crop silages on DMI and performance in dairy cows.

NOTES

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Nonstandard abbreviations used: AIC = Akaike information criterion; ATTD = apparent total-tract digestibility; $peNDF_{>8mm}$ = physically effective NDF greater than 8 mm; PMR = partial mixed ration; RS = whole crop rye silage; RVSS = rye-vetch-straw silage; uCP = utilizable CP at the duodenum.

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