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Effects of feeding early-harvested orchardgrass–perennial ryegrass mixed silage instead of heading stage harvested timothy silage on digestion and milk production in dairy cows

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Abstract
We evaluated the effects of replacement of heading stage harvested timothy silage with early-harvested orchardgrass–perennial ryegrass mixed (OP) silage while maintaining or reducing concentrate input on dry matter intake (DMI), milk production, nutrient digestibility, and N balance in dairy cows. Nine multiparous Holstein cows were used in a replicated 3 × 3 Latin square design with three dietary treatments: TYL, a diet containing timothy silage where forage-to-concentrate ratio (FC) was 50:50; OPL, a diet containing OP silage where FC ratio was 50:50; and OPH, a diet containing OP silage where FC ratio was 60:40. We observed that an equal replacement of timothy with OP silage increased DMI, milk yield, milk protein production, and nutrient digestibility but decreased milk fat content (TYL versus OPL). We observed that replacing timothy with OP silage while reducing concentrate input increased milk fat and protein yield, nutrient digestibility, and feed efficiency and reduced urinary N loss with no effect on DMI or milk fat content (TYL versus OPH). These results show that replacing timothy with OP silage can be a good approach to improve milk production, feed efficiency, and N utilization and reduce concentrate input. However, milk fat depression should be considered when an equal substitution is performed.

KEYWORDS
concentrate input, digestibility, feed intake, milk production, orchardgrass–perennial ryegrass mixture grass

1 | INTRODUCTION

High-quality forages are essential diets for high-yielding dairy cows. Grass silage is the main dietary source for dairy cow in Japan. In northern regions of Japan, mainly timothy (*Phleum pratense* L.) is cultivated as the material for grass silage to optimize the dry matter (DM) yield. However, timothy is less competitive than other grass species, and the DM yield and forage quality are thus reduced due to invasion of rhizomatous grass weeds such as quackgrass and reed canarygrass to the timothy-based grassland in those regions (Deguchi, 2016;Kitamura, 2016). Compared to timothy, orchardgrass (*Dactylis glomerate* L.) is more competitive and has greater regrowth ability, and perennial ryegrass (*Lolium perenne* L.) has greater water-soluble carbohydrate content (Masuko, Ono, Furukawa, & Otani, 1994). In addition, these grass species are suitable for grazing and early and short interval harvesting, whereas timothy is not suitable for early harvesting to maintain grassland persistency and nutrient yield (Ishiguri, 1975;Mowat, Fulkerson, Tossell, & Winch, 1965). Thus, timothy is commonly harvested twice a year and orchardgrass and perennial ryegrass are harvested three or four times a year for early and short interval harvesting. Using orchardgrass–perennial ryegrass mixture grass (OP) harvested early and short interval as a substitute for timothy harvested twice a year increases production cost and labor but becomes a good approach to reduce weed invasions in grassland, improve forage quality, and increase annual nutrient yield (Miyaji, Yajima, Sudo, & Aoki, 2020).

Miyaji et al. (2020) reported that the annual DM yield of timothy harvested twice a year is similar to that of OP harvested four times a year, but the DM yield of 1st-cut timothy harvested at the heading stage (FTY) is higher than that of 1st-cut OP harvested at the late vegetative stage (FOP). And the DM yield of FTY is nearly equal to the sum of the FOP plus 2nd-cut OP (42 days regrowth; SOP). It is thus desirable to feed FOP and SOP silages mixture as a replacement for FTY silage for matching the DM yield.

Compared to FTY silage, the neutral detergent fiber (NDF) content of both FOP and SOP silages was lower and the NDF digestibility was higher (Yajima, Sudo, Tada, Miyaji, & Aoki, 2020). The dry matter intake (DMI) depends on the NDF content and digestibility (Huhtanen, Rinne, & Nousiainen, 2007;NRC, 2001). It is thus possible that increasing the NDF digestibility by feeding FOP and SOP silages mixture instead of FTY silage could increase cow's DMI and then improve their milk production. However, some researchers have reported that feeding a lower-forage NDF diet decreased milk fat production (Beauchemin, 1991;Yan et al., 2011;Yang et al., 2018). An equal replacement of timothy silage with OP silage as a forage source decreases the dietary forage NDF content and could thus cause milk fat depression.

Improving forage quality can allow the concentrate supplementation to be reduced without compromising the animal performance or milk production (Cabezas-Garcia, Krizsan, Shingfield, & Huhtanen, 2017;Huhtanen, Jaakkola, & Nousiainen, 2013). Both FOP and SOP silages have a lower NDF content and higher total digestible nutrients (TDN) and CP contents compared to FTY silage (Yajima et al., 2020). Thus, replacing FTY silage with FOP and SOP silages mixture with increasing forage input, that is, maintaining the dietary forage NDF content, could not only maintain the milk production level with a reduced concentrate input but also decrease the risk of depressing the milk fat. However, the replacement of FTY silage with FOP and SOP silages mixture while reducing the concentrate (starch sources) input reduces dietary starch content. Some researchers have reported that feeding a lower starch diet increases the excess N not used for microbial protein synthesis, and thus it increases the urinary N loss and/or reduce the milk protein content (Miyaji, Matsuyama, & Hosoda, 2014;Theurer, Huber, Delgado-Elorduy, & Wanderley, 1999). Feeding FOP and SOP silages mixture instead of FTY silage with a reduced concentrate input could therefore have an adverse effect on nitrogen utilization.

Our hypothesis in this study was that feeding FOP and SOP silages mixture instead of FTY silage would influence the DMI, nutrient digestibility, lactation performance, and N utilization of dairy cows. We expected that increasing the cow's NDF digestibility due to an equal replacement of FTY silage with FOP and SOP silages mixture would increase the DMI and then improve the milk production. We also expected that replacing FTY silage with FOP and SOP silages mixture while reducing the concentrate input could maintain the same level of milk production. Our objective in the present study, therefore, was to evaluate the effects of the replacement of FTY silage with FOP and SOP silages mixture while maintaining or reducing concentrate input on the DMI, milk production, nutrient digestibility, and N balance in dairy cows.

2 | MATERIALS AND METHODS

The method of feeding management used in this study was approved by the Animal Care and Use Guidelines of the NARO of Japan.

2.1 | Preparation of experimental grass silage

Fields with timothy and a mixture of orchardgrass–perennial ryegrass were established at adjacent locations on August 10, 2016 in Sapporo, Japan (43°0′N, 141°24′E). The timothy was sown at 20 kg/ha, and the orchardgrass and perennial ryegrass were sown at 15 and 15 kg/ha, respectively. The FTY silage was made from a timothy sward, and the FOP and SOP silages were made from a mixed orchardgrass–perennial ryegrass sward in 2018. The N-P-K fertilizers were applied at rates of 60-60-64 kg/ha for primary growth and 83-0-0 kg/ha for regrowth. The FTY was cut on June 18, 2018 (botanical composition [BC]: 85% timothy, 10% grass weed, 2% broad-leaved weed, 3% dead matter; DM yield: 7.1 t/ha). The FOP was cut on May 28, 2018 (BC: 65% orchardgrass, 29% perennial ryegrass, 2% white clover, 1% grass weed, 2% broad-leaved weed, 1% dead matter; DM yield: 4.8 t/ha) and SOP was cut on July 9, 2018 (BC: 54% orchardgrass, 31% perennial ryegrass, 2% white clover, 2% grass weed, 1% broad-leaved weed, 10% dead matter; DM yield: 2.1 t/ha). The grass was cut with a mower-conditioner, wilted for approximately 6 hr, and harvested with a precision-chop harvester. The chopped materials were transported and baled in roll form and wrapped with eight layers of polyethylene film using a chopping combi bale wrapper (TSW2020; IHI STAR Machinery), and then stored outdoors over 4 months.

2.2 | Cows, experiment design, diets, and management

Nine multiparous Holstein cows (3.4 ± 0.6 parity, 678 ± 21 kg of body weight, 131 ± 17 days in milk) were randomly assigned to a replicated 3 × 3 Latin square design with the three dietary treatments (TYL, OPL, or OPH). The experimental period was 21 day, with 14 day for treatment adaptation and 7 day for data collection. The three dietary treatments were (1) TYL (control treatment): the diet contained FTY silage as the sole forage source in which the forage-to-concentrate ratio was 50:50, (2) OPL: the diet replaced the FTY silage of the TYL diet with a mixture of FOP and SOP silage, and (3)

OPH: the diet contained that mixture of FOP and SOP silage as the forage source in which the forage-to-concentrate ratio was 60:40. The mixture ratio of FOP and SOP silage for the OPL and OPH diets was 70:30, according to the DM yield. The experimental diets each contained grass silage as a forage source and were supplemented with high-moisture shelled corn, soybean meal, commercial concentrate, calcium carbonate, and a vitamin mix. The ingredient composition of the commercial concentrate was 36.9% corn, 21.3% rapeseed meal, 19.3% corn gluten feed, 7.1% wheat bran, 5.1% palm kernel meal, 2.0% wheat, 2.0% corn distillers grains with solubles, 2.0% molasses, 1.0% barley, 1.0% soybean meal, 1.0% calcium carbonate, 0.5% heat-treated soybean, 0.5% alfalfa, and 0.1% NaCl. The vitamin mix contained 5,000,000 IU/kg of vitamin A, 600,000 IU/kg of vitamin D3, and 20,000 mg/kg of dL-α-tocopherol acetate. The TYL and OPH diets were formulated to contain approximately 33% forage NDF and 16% crude protein (CP). The chemical compositions of the feedstuffs included in the experimental diets are given in Table 1, and the ingredient and chemical compositions of the experimental total mixed rations are shown in Table 2. All experimental diets were formulated to meet or exceed requirements of the Japanese Feeding Standard for Dairy Cattle (NARO, 2017) for a 680 kg lactating cow producing 40 kg of milk/day. Throughout the experiment, the cows were kept in individual tie stalls and had free access to fresh water and a trace mineral salt block (containing, per 1 kg: Fe, 1,232 mg; Cu, 150 mg; Co, 25 mg; Zn, 500 mg; Mn, 500 mg; I, 50 mg; Se, 15 mg; Na, 382 g). The cows were offered the diets as a total mixed ration for free intake (10% refusals) twice daily (09:30 and 16:30 hr) and milked twice daily (09:00 and 19:00 hr).

2.3 | Data and sample collection

During days 15–20 of each 21-day period, the amounts of diets and orts were weighed, and representative samples were collected daily. The ort samples were pooled, and one sample was retained per cow per period. The diet and ort samples were dried at 60°C in an oven for 48 hr. The dried samples were ground through a 1-mm screen using a centrifugal mill (SM2000; Retsch & Co., Haan, Germany) and stored until analysis. The milk yield was measured daily and averaged over the collection period, and duplicate milk samples were taken at every milking. One set of duplicate samples was analyzed for fat, CP, and lactose concentrations by infrared spectroscopy (Milko-Scan

TABLE 1 Chemical composition of experimental feedstuffs

	Grass silage[a]			concentrate[b]		
Item	FTY	FOP	SOP	HMC	SBM	CC
Chemical composition						
DM (%)	35.2	26.0	25.6	69.0	87.1	86.7
OM (% of DM)	92.9	91.1	89.3	98.6	93.2	94.5
CP (% of DM)	8.6	11.8	15.6	8.2	51.6	21.2
Ether extract (% of DM)	4.0	5.2	5.0	2.7	1.0	3.4
NDF (% of DM)	66.4	55.5	52.9	8.8	11.0	26.7
ADF (% of DM)	43.0	32.9	33.4	2.3	8.5	12.7
iNDF (% of DM)	16.4	6.6	10.2	1.2	0.7	4.9
Starch (% of DM)	1.1	1.4	1.3	70.2	0.7	29.3
Fermentation profile						
pH	3.83	3.72	3.91	4.83	–	–
Lactic acid (% of DM)	5.58	9.77	9.86	0.46	–	–
Acetic acid (% of DM)	0.87	1.81	2.39	0.17	–	–
Propionic acid (% of DM)	ND	ND	0.02	ND	–	–
Butyric acid (% of DM)	0.02	ND	ND	ND	–	–
Volatile basic *N* (/ total *N*)	7.3	5.3	9.2	1.5	–	–

Abbreviation: DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; iNDF, indigestible NDF; ND, not detected.

[a]FTY, 1st-cut timothy harvested at the heading stage; FOP, 1st-cut orchardgrass–perennial ryegrass mixture grass harvested at the late vegetative stage; SOP, 2nd-cut orchardgrass–perennial ryegrass mixture grass after 42 days regrowth.

[b]HMC, high-moisture shelled corn; SBM, soybean meal; CC, commercial concentrate.

TABLE 2 Ingredient and chemical composition of experimental diets

Item[a]	Diet[b] TYL	OPL	OPH
Ingredient (% of DM)			
FTY silage	50.0	0.0	0.0
FOP silage	0.0	35.0	42.0
SOP silage	0.0	15.0	18.0
High moisture shelled corn	21.5	21.5	21.5
Soybean meal	13.0	13.0	10.0
Commercial concentrate	15.0	15.0	8.0
Calcium carbonate	0.3	0.3	0.3
Vitamin mix	0.2	0.2	0.2
Forage to concentrate ratio	50:50	50:50	60:40
Chemical composition			
DM (%)	47.5	39.2	32.8
OM (% of DM)	94.2	93.1	92.7
CP (% of DM)	16.2	18.2	16.4
Ether extract (% of DM)	3.2	3.8	4.0
NDF (% of DM)	40.5	34.7	38.0
Forage NDF (% of DM)	33.2	27.4	32.8
iNDF (% of DM)	9.3	4.9	5.3
ADF (% of DM)	25.0	20.0	22.2
Starch (% of DM)	20.1	20.3	18.3

Abbreviation: OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; iNDF, indigestible NDF.

[a]DM, dry matter; FTY, 1st-cut timothy harvested at the heading stage; FOP, 1st-cut orchardgrass–perennial ryegrass mixture grass harvested at the late vegetative stage; SOP, 2nd-cut orchardgrass–perennial ryegrass mixture grass after 42 days regrowth.

[b]TYL, diet contained FTY silage as solo forage source where forage-to-concentrate ratio was 50:50; OPL, diet contained FOP and SOP silages mixture where forage-to-concentrate ratio was 50:50; OPH, diet contained FOP and SOP silages mixture where forage-to-concentrate ratio was 60:40.

FT120; N. Foss Electric, Hillerød, Denmark; AOAC International, 2000). The second set of samples was refrigerated until the end of each collection period, composited by cow within the period according to daily milk yield, and frozen at −30°C until N analysis.

On days 15–17 of each 21-day period, fecal grab samples were collected twice daily (10:00 and 16:00 hr) and stored in a refrigerator at 5°C for determining the apparent digestibility and N balance. Just after collection, fecal samples were composited per cow per period. A portion of fresh feces was dried at 60°C for at least 72 hr in a forced-air oven followed by grinding through a 1-mm screen and stored until analysis, and the other portion was frozen at −30°C until N analysis. Urine spot samples were collected by perineal massage at the same fecal grab sampling time. Urine samples were acidified immediately after collection by diluting one volume of urine with four volumes of 0.072N sulfuric acid, composited by volume per cow per period, and then stored at −30°C until the N and creatinine analyses. The cows were weighed on day 15 of each period. Blood samples were collected into heparinized tubes from the jugular vein at 0 and 4 hr after the morning feeding on day 21 of each period. The samples were immediately centrifuged at 1,200 × *g* for 15 min at 5°C, and the plasma was stored at −30°C until analysis.

2.4 | Chemical analysis

The DM was determined by drying the sample at 135°C for 2 hr. The ether extract (EE), Kjeldahl N, and crude ash values were determined by the AOAC method (AOAC International, 2000 methods 920.39, 990.03 and 942.05, respectively). The organic matter (OM) was calculated as the weight loss on ashing. The NDF and acid detergent fiber (ADF) (NDF was assayed with a heat-stable amylase, sodium sulfite and expressed exclusive of residual ash; ADF was expressed exclusive of residual ash) were analyzed according to the methods of Van Soest, Robertson, and Lewis (1991) and the AOAC method (AOAC International, 2000 method 973.18). The indigestible NDF (iNDF) was determined by in situ incubation for 288 hr in 25-μm pore size bags according to Cabezas-Garcia et al. (2017). The starch was analyzed using a commercial kit (Total starch assay kit; Megazyme International Ireland, Wicklow, Ireland; McCleary, Solah, & Gibson, 1994). Urine creatinine was analyzed using a commercial kit (LabAssay Creatinine; FUJIFILM Wako pure chemical, Osaka, Japan). Urea-N in plasma was measured with an automatic analyzer (BM-8060; JEOL Ltd., Tokyo, Japan).

The fermentation products of the silage were determined using cold-water extracts. Wet silage (40 g) was homogenized with 140 ml of sterilized distilled water and stored at 4°C overnight (Cai, Benno, Ogawa, & Kumai, 1999). The pH was measured with a glass electrode pH meter (D-51; Horiba), and volatile basic nitrogen (VBN) was determined by steam distillation of the filtrates. The organic acid contents were measured using high-performance liquid chromatography (Ohmomo, Tanaka, & Kitamoto, 1993).

2.5 | Calculation and statistical analyses

The whole-tract apparent digestibility of the DM and nutrients was determined using the iNDF as an internal digestibility marker. We calculated the DM and nutrient digestibility using the following equation: DM digestibility (%) = 100 × [1−(DiNDF/FiNDF)], nutrient digestibility (%) = 100 × [1−(DiNDF/FiNDF) × (FN/DN)], in which DiNDF is the iNDF content (% of DM) of the actually consumed diet, FiNDF is the iNDF content (% of DM) of the feces, FN is the nutrient content (% of DM) of the feces, and DN is the nutrient content (% of DM) of the actually consumed diet. Fecal N excretion was calculated based on the equation: fecal *N* (g/d) = iNDFI/FiNDF × FNC, in which iNDFI is the iNDF intake (g/d), FiNDF is the iNDF content (% of DM) of the feces, and FNC is the N content (% of DM) of the feces. The daily urine volume was estimated as body weight × 22.8/urinary creatinine content (mmol/L) (Asai et al., 2005). The urinary N excretion was calculated by multiplying urine N × urine volume. The milk N secretion was calculated by multiplying milk N content × milk volume. The retention N was calculated by subtracting the fecal, urine, and milk N from the N intake. The fat corrected milk (FCM) yield was calculated as (0.4 × kg of milk) + 15(kg of milk × milk fat/100). The energy corrected milk (ECM) yield was calculated as (0.327 × kg of milk) + 12.95 (kg of milk × milk fat/100) + 7.2 (kg of milk × milk CP/100).

We used the MIXED procedure of the SAS software to analyze the data for all variables. The data for intake, milk production, digestibility, and N balance were analyzed as a replicated 3 × 3 Latin square using the following model:

$$Y_{ijkl} = \mu + a_i + b_j + c_k\left(a_i\right) + d_l + \varepsilon_{ijkl}$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, a_i is the fixed effect of the square (i = 1–3), b_j is the fixed effect of the period (j = 1 to 3), $c_k(a_i)$ is the random effect of animal within a square (k = 1–9), d_l is the fixed effect of dietary treatment (l = 1–3), and ε_{ijkl} is the residual error. The values of the plasma concentrations were analyzed as repeated measures by adding the fixed effects of the sampling times and their interaction with the dietary treatment to the previous model. A first-order autoregressive was the best covariance structure based upon the smallest Akaike's information criterion values. Other covariance structures tested included compound symmetry, heterogeneous autoregressive 1, heterogeneous compound symmetry, and unstructured. Multiple comparisons among the dietary treatments were carried out using the Tukey method, and significance was declared at $p < .05$.

3 | RESULTS

3.1 | Diet characteristics

Our analyses revealed large differences in the DM, CP, and NDF contents among the forages (Table 1). The DM contents of the FOP and SOP silage were at the same level, and lower than that of FTY silage reflecting the rainy weather conditions prior to harvest. The CP content was lowest for FTY silage, intermediate for FOP silage, and highest for SOP silage. Conversely, the NDF content was highest for FTY silage, intermediate for FOP silage, and lowest for SOP silage. The fermentation quality was good for all silages as indicated by low pH values (average: 3.82) and the contents of butyrate and VBN. The lactic acid concentrations of FOP and SOP silage were the same level and were higher than that of FTY silage.

Due to the equal replacement of FTY silage with FOP and SOP silages mixture as a forage source, the starch contents of the TYL and OPL diets were at the same level, whereas the CP content was higher and the fiber content was lower for the OPL diet compared to the TYL diet (Table 2). The NDF and starch contents were slightly lower for the OPH diet compared to the TYL diet, but the CP and forage NDF contents were similar between the OPH and TYL diets.

3.2 | DMI and milk production

The dietary treatment affected the DMI, milk yield, milk composition, and feed efficiency (except for the milk lactose content) (Table 3). The DMI and milk yield were greater for the cows fed the OPL compared to the cows fed the TYL or OPH diet (p = .01 and $p < .01$), and no differences in the DMI or milk yield were detected between the TYL and OPH diets. The FCM and ECM yields were higher for the cows fed the OPL or OPH diet compared to the yields for the cows fed the TYL diet ($p < .01$) and were similar between the OPL and OPH diets. The fat yield was higher for the cows fed OPH compared to those fed TYL ($p < .01$), with no difference between OPL and TYL or OPH. The CP yield was highest for the cows fed OPL, intermediate for the cows fed OPH, and lowest for the cows fed TYL ($p < .01$). The effect of the dietary treatment on the lactose yield was similar to the effect observed for the milk yield. The milk

TABLE 3 Dry matter intake, milk production, and feed efficiency in cows fed experimental diets

	Diet[‡]				
Item[†]	TYL	OPL	OPH	*SEM*	*p*-value
DMI (kg/d)	26.4[b]	27.6[a]	26.1[b]	0.13	.01
Yield (kg/d)					
Milk	37.7[b]	42.2[a]	39.1[b]	0.41	<.01
FCM	40.7[b]	43.4[a]	43.5[a]	0.33	<.01
ECM	44.2[b]	47.9[a]	47.3[a]	0.38	<.01
Fat	1.71[b]	1.77[ab]	1.86[a]	0.001	<.01
CP	1.35c	1.56a	1.44[b]	0.001	<.01
Lactose	1.70[b]	1.91[a]	1.76[b]	0.001	<.01
Milk composition (%)					
Fat	4.59[a]	4.22[b]	4.76[a]	0.007	<.01
CP	3.63[b]	3.71[a]	3.71[a]	0.001	.04
Lactose	4.50	4.52	4.49	<0.001	.52
Feed efficiency (kg/kg)					
Milk/DMI	1.42[b]	1.53[a]	1.50[a]	0.001	.02
FCM/DMI	1.54b	1.57[b]	1.67[a]	<0.001	<.01
ECM/DMI	1.67[b]	1.74[b]	1.81[a]	<0.001	<.01

[a,b,c]Means within a row without a common superscript differ ($p < .05$).
[†]DMI, dry matter intake; FCM, 4% fat corrected milk; ECM, energy corrected milk; CP, crude protein.
[‡]TYL, cows were fed diet contained FTY silage as solo forage source where forage-to-concentrate ratio was 50:50; OPL, cows were fed diet contained FOP and SOP silages mixture where forage-to-concentrate ratio was 50:50; OPH, cows were fed diet contained FOP and SOP silages mixture where forage-to-concentrate ratio was 60:40.

fat content was lower for the cows fed OPL than for the cows fed TYL or OPH ($p < .01$), with no significant difference between TYL and OPH. Feeding the OPL and OPH diets instead of the TYL diet increased the milk CP content ($p = .04$), whereas no significant difference in the milk CP content was revealed between OPL and OPH. The milk yield per DMI was increased by replacing timothy silage with OP silage ($p = .02$). However, the feed efficiency, expressed as FCM/DMI and ECM/DMI, was higher for the cows fed OPH compared to the cows fed TYL and OPL ($p < .01$), and it was similar between TYL and OPL.

3.3 | Whole-tract digestibility

The forage source had a large impact on nutrient digestibility (Table 4). The whole-tract digestibility of DM, OM, and NDF was higher for the cows fed the OPL and OPH diets than for those fed the TYL diet ($p < .01$) and was similar between the OPL and OPH diets. The CP digestibility was higher for the cows fed the OPL diet compared to those fed TYL ($p < .01$), with no difference between OPH and TYL or OPL. The ADF digestibility was highest for the cows fed the OPH diet, intermediate for the cows fed the OPL diet, and lowest for the cows fed the TYL diet. The starch digestibility was not affected by the dietary treatment.

3.4 | Nitrogen utilization

The nitrogen intake was influenced by the dietary treatment ($p < .01$) and was higher for the cows fed OPL compared to the cows fed the TYL or OPH diet (Table 5), which is a reflection of the differences in the DMI and CP content of the three diets. Significant differences were detected among the dietary treatments in milk N secretion, urinary N excretion, and N retention (all $p < .01$) with no dietary effect on fecal N excretion. The milk N secretion was highest for the cows fed the OPL diet, intermediate for the cows fed the OPH diet, and lowest for the cows fed the TYL diet. The urinary N excretion was highest for the cows fed the OPL diet, intermediate for the cows fed the TYL diet, and lowest for the cows fed the OPH diet. As a proportion of N intake, the milk N secretion was higher for OPH compared to TYL and OPL ($p < .01$); conversely, the urinary N excretion was lower for OPH than for TYL and OPL ($p < .01$), with no significant difference between TYL and OPL. The N retention as a proportion of N intake was not different among the dietary treatments.

Significant differences among the dietary treatments in plasma urea-N concentration were detected ($p < .01$). The plasma urea-N concentration was lower for OPH than for TYL and OPL, with no difference between TYL and OPL.

TABLE 4 Whole-tract digestibility in cows fed experimental diets

Item	Diet[‡] TYL	OPL	OPH	*SEM*	*p*-value
DM	68.2^{b}	78.7^{a}	78.2^{a}	0.54	<.01
OM	69.8^{b}	80.4^{a}	79.8^{a}	0.50	<.01
CP	65.9^{b}	71.6^{a}	68.2^{ab}	1.52	<.01
NDF	54.3^{b}	70.3^{a}	71.9^{a}	0.74	<.01
ADF	53.7^{c}	65.5^{b}	68.5^{a}	0.83	<.01
Starch	98.1	98.9	98.5	0.04	.30

Abbreviation: DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

[a,b,c]Means within a row without a common superscript differ ($p < .05$).

[‡]TYL, cows were fed diet contained FTY silage as solo forage source where forage-to-concentrate ratio was 50:50; OPL, cows were fed diet contained FOP and SOP silages mixture where forage-to-concentrate ratio was 50:50; OPH, cows were fed diet contained FOP and SOP silages mixture where forage-to-concentrate ratio was 60:40.

4 | DISCUSSION

4.1 | Effect of an equal replacement of FTY with FOP and SOP silages mixture (TYL versus OPL)

We observed that the whole-tract nutrient digestibility except for starch digestibility increased in response to the equal replacement of FTY silage with FOP and SOP silages mixture in total mixed ration. The nutrient digestibility of both FOP and SOP silages is higher than that of FTY silage (Yajima et al., 2020). Thus, the increase in the nutrient digestibility by the equal replacement of FTY silage with FOP and SOP silages mixture would reflect these forage dietary characteristics. It has been shown that the DMI increased when cows were fed more digestible NDF and/or lower NDF diets (Huhtanen et al., 2007;NRC, 2001), and our present results are consistent with those earlier reports. In the present study, an equal replacement of FTY silage with FOP and SOP silages mixture increased the cows' NDF digestibility and decreased the dietary NDF content. Feeds low in digestibility are thought to place constraints on the DMI because of their slow clearance from the rumen (NRC, 2001). Thus, the increased NDF digestibility and lower dietary NDF content due to an equivalent substitution of FOP and SOP silages mixture for FTY silage could lower the rumen fill and could result in an increase in the DMI.

We also observed that the milk yield was increased by equal replacement of FTY silage with FOP and SOP silages mixture. Feeding the OPL diet instead of the TYL diet increased the DM and nutrient digestibility. The DMI was also greater for the cows fed the OPL diet than for those fed the TYL due to the increased fiber digestibility and lower dietary fiber content. Thus, increasing the DM and nutrient digestibility and the DMI by equal replacement of FTY silage with FOP and SOP silages mixture would increase the milk yield. In some studies (Beauchemin, 1991;Yan et al., 2011), lower forage NDF in diet was closely linked to milk fat depression. The milk fat depression due to the feeding of a lower-forage NDF diet could be caused by low ruminal pH, an increase in the accumulation of trans-18:1

TABLE 5 Nitrogen balance and plasma urea-N concentration in cows fed experimental diet

Item	Diet[†] TYL	OPL	OPH	SEM	p-value
Nitrogen balance					
Nintake (g/day)	698.2[b]	800.8[a]	684.1[b]	41.08	<.01
Milk *N*					
g/day	215.3[c]	244.2[a]	229.1[b]	15.08	<.01
% of *N* intake	30.9[b]	30.5[b]	33.5[a]	0.34	<.01
Fecal excretion					
g/day	238.3	227.6	217.8	54.62	.18
% of *N* intake	34.1[a]	28.4[b]	31.8[ab]	1.52	<.01
Urinary excretion					
g/day	227.6[b]	257.0[a]	188.6[c]	34.75	<.01
% of *N* intake	32.6[a]	32.1[a]	27.6[b]	1.43	<.01
N retention					
g/day	17.1[b]	72.1[a]	48.5ab	27.40	0.04
%of *N* intake	2.4	8.9	7.0	4.57	0.06
Plasma urea-*N* (mg/dL)	17.2[a]	17.2[a]	14.1[b]	0.22	<.01

[a,b,c]Means within a row without a common superscript differ ($p < .05$).

[†]TYL, cows were fed diet contained FTY silage as solo forage source where forage-to-concentrate ratio was 50:50; OPL, cows were fed diet contained FOP and SOP silages mixture where forage-to-concentrate ratio was 50:50; OPH, cows were fed diet contained FOP and SOP silages mixture where forage-to-concentrate ratio was 60:40.

fatty acids (Yang et al., 2018) and a reduction of de novo fatty acid synthesis within the mammary gland (Bauman & Griinari, 2003). We observed that the milk fat content decreased in response to the equal replacement of FTY silage with FOP and SOP silages mixture. Thus, an equal replacement of FTY silage with FOP and SOP silages mixture as a forage source decreased the forage NDF in the diet and could change the rumen fermentation pattern, and if could then decrease the milk fat content. However, feeding the OPL diet instead of the TYL diet had no effect on milk fat yield. The same milk fat production response of the cows thus suggested that cows fed the OPL diet did not show a dramatic or acute milk fat depression.

The milk protein yield and protein content are related to the net energy and CP intakes (Broderick, 2003;Jenkins & McGuire, 2006). Several research groups reported that feeding higher digestible silage increased the milk protein yield and/or protein content (Alstrup, Søegaard, & Weisbjerg, 2016;Brunette, Baurhoo, & Mustafa, 2016;Kuoppala, Rinne, Nousiainen, & Huhtanen, 2008), and our present findings are consistent with those reports. Both FOP and SOP silages have higher TDN and CP contents compared to FTY silage (Yajima et al., 2020). Thus, the increased milk protein content and yield due to an equal replacement of FTY silage with FOP and SOP silages mixture could be explained by higher TDN and CP intakes, and may be reflected by an improved energy status and increased intestinal supply of amino acids to the cow (Alstrup et al., 2016;Rinne, Jaakkola, Kaustell, Heikkilä, & Huhtanen, 1999). However, our analyses revealed that feeding OPL instead of TYL did not influence the secreted N in milk, the urinary N excretion and N retention as a proportion of N intake, or the plasma urea-N concentration. Thus, an equal replacement of FTY silage with FOP and SOP silages mixture as a forage source did not improve the N utilization, and it might have little impact on the efficiency of rumen microbial protein synthesis.

4.2 | Effect of replacing FTY silage with FOP and SOP silages mixture while reducing concentrate input (TYL versus OPH)

The DM, OM, NDF, and ADF digestibilities were higher for the cows fed OPH than for those fed TYL, which reflects the differences in the forage dietary characteristics between the timothy and OP silage. The NDF content was lower for the OPH diet compared to the TYL diet. Thus, the cows' higher NDF digestibility and lower NDF content for OPH compared to TYL could be expected to increase the DMI because the dietary NDF content and NDF digestibility influence the DMI (Huhtanen et al., 2007;NRC, 2001). However, our present findings showed no difference in the DMI between cows fed the TYL diet and those fed the OPH diet. Some researchers suggested that increasing the forage-to-concentrate ratio in the diet reduces the DMI (Cabezas-Garcia et al., 2017;Steinshamn, Purup, Thuen, & Hansen-Møller, 2008). In the present study, the forage-to-concentrate ratio was 50:50 for the TYL diet and 60:40 for the

OPH diet. Thus, the higher forage-to-concentrate ratio for OPH compared to TYL could cancel the increase in the DMI that is due to increased NDF digestibility.

Some researchers suggested that improving forage quality can allow the concentrate supplementation to be reduced without compromising the milk production (Cabezas-Garcia et al., 2017;Huhtanen et al., 2013), which is consistent with our present findings. Here, the FCM and ECM yields were increased by replacing FTY silage with FOP and SOP silages mixture while reducing the concentrate input. Although the DMI was similar between the uses of TYL and OPH, the digestibility of DM, OM, and fiber was increased by feeding the OPH diet instead of the TYL diet. Thus, the similar DMI and increasing digestibility due to replacing the TYL diet with the OPH diet would increase the FCM and ECM yields, and then increase the feed efficiency. In addition, the milk fat content was similar between TYL and OPH, and the milk fat yield was increased by replacing the TYL diet with the OPH diet, although an equal replacement of FTY silage with FOP and SOP silages mixture as a forage source decreased the milk fat content. The dietary forage NDF content and the digestible fiber content have a large impact on milk fat production (NARO, 2017;NRC, 2001;Yang et al., 2018). The similar forage NDF and higher digestible NDF contents for OPH compared with TYL could maintain the milk fat content and improve the milk fat production.

It has been suggested that feeding lower starch and/or higher rumen-digestible CP diets increases the excess N not used for microbial protein synthesis, and then increases the urinary N loss and/or reduces the milk protein content (Miyaji & Nonaka, 2018;NARO, 2017;Theurer et al., 1999). In the present study, however, the urinary N loss was not increased and the milk protein was not decreased by feeding the OPH diet instead of the TYL diet, although replacing the TYL diet with the OPH diet reduced the dietary starch content and might increase the rumen-digestible CP content because grass silage has a higher rumen-digestible CP content (Tamminga, Ketelaar, & Vuuren, 1991;Yan & Agnew, 2004). Contrary to our expectations, feeding FOP and SOP silages mixture instead of FTY silage (along with a reduced concentrate input) increased the milk protein production and reduced the urinary N loss and plasma urea-N content. The reasons for this improvement in N utilization due to feeding the OPH diet instead of the TYL diet are not clear; however, the synchronization of the degradation rate of protein with that of carbohydrate is important for utilizing N effectively with ruminal microbes (Hoover & Stokes, 1991). Thus, the replacement of the TYL diet with the OPH diet could improve the ruminal N utilization by increasing the ruminal microbial protein synthesis based on the synchronization of the degradation of the starch of high-moisture corn and the fiber of OP silage with the degradation of the CP of OP silage, and this improvement could decrease the urinary N losses and increase the milk CP production in cows. The lower plasma urea-N concentrations in cows fed the OPH diet may support this hypothesis.

5 | CONCLUSIONS

Our present data demonstrated that an equal replacement of FTY silage with FOP and SOP silages mixture in the diet increased the DMI and nutrient digestibility, and thus increased the milk production by dairy cows. However, this equal replacement decreased the dietary forage NDF content and then decreased the milk fat content. An equal replacement of FTY silage with FOP and SOP silages mixture as the forage source could therefore be an effective strategy to improve the feed utilization and milk yield, but its decreased milk fat content should be considered. We also observed that feeding FOP and SOP silages mixture instead of FTY silage with an increased forage-to-concentrate ratio increased the nutrient digestibility, milk fat, and FCM and ECM yields and then improved the feed efficiency. This replacement of FTY silage with FOP and SOP silages mixture with a reduced concentrate input also decreased the urinary N loss and thus increased the milk protein composition and yield. Feeding FOP and SOP silage instead of FTY silage with an increased forage-to-concentrate ratio could thus be a good approach to improve milk production, feed efficiency, and N utilization with a reduced concentrate input.

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REFERENCES

Alstrup, L., Søegaard, K., & Weisbjerg, M. R. (2016). Effects of maturity and harvest season of grass-clover silage and of forage-to-concentrate ratio on milk production of dairy cows. *Journal of Dairy Science*, *99*, 328–340. https://doi.org/10.3168/jds.2015-9802

Asai, H., Hayashi, N., Takai, N., Yoshimura, Y., Nakamura, Y., Yokota, H., & Kita, K. (2005). Estimation of daily urinary potassium excretion using urinary creatinine as an index substance in prepartum dairy cows. *Animal Science Journal*, *76*, 51–54. https://doi.org/10.1111/j.1740-0929.2005.00237.x

Association of Official Analytical Chemists (AOAC) International (2000). *Official methods of analysis of the association of official analytical chemists*, 17th ed. Gaithersburg, MD, USA: Association of Official Analytical Chemists.

Bauman, D. E., & Griinari, J. M. (2003). Nutritional regulation of milk fat synthesis. *Annual Review of Nutrition*, *23*, 203–227. https://doi.org/10.1146/annurev.nutr.23.011702.073408

Beauchemin, K. A. (1991). Effects of dietary neutral detergent fiber concentration and alfalfa hay quality on chewing, rumen function and milk production of dairy cows. *Journal of Dairy Science*, *74*, 3140–3151. https://doi.org/10.3168/jds.S0022-0302(91)78499-3

Broderick, G. A. (2003). Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *Journal of Dairy Science*, *86*, 1370–1381. https://doi.org/10.3168/jds.S0022-0302(03)73721-7

Brunette, T., Baurhoo, B., & Mustafa, A. F. (2016). Effects of replacing grass silage with forage pearl millet silage on milk yield, nutrient digestion, and ruminal fermentation of lactating dairy cows. *Journal of Dairy Science*, *99*, 269–279. https://doi.org/10.3168/jds.2015-9619

Cabezas-Garcia, E. H., Krizsan, S. J., Shingfield, K. J., & Huhtanen, P. (2017). Effects of replacement of late-harvested grass silage and barley with early-harvested silage on milk production and methane emissions. *Journal of Dairy Science*, *100*, 5228–5240. https://doi.org/10.3168/jds.2016-12444

Cai, Y., Benno, Y., Ogawa, M., & Kumai, S. (1999). Effect of applying lactic acid bacteria isolated from forage crops on fermentation characteristics and aerobic deterioration of silage. *Journal of Dairy Science*, *82*, 520–526. https://doi.org/10.3168/jds.S0022-0302(99)75263-X

Deguchi, K. (2016). Invasion of rhizomatous grasses on timothy grassland in Hokkaido. *Japanese Journal of Grassland Science*, *62*, 153–157.

Hoover, W. H., & Stokes, S. R. (1991). Balancing carbohydrates and proteins for optimum rumen microbial yield. *Journal of Dairy Science*, *74*, 3630–3644. https://doi.org/10.3168/jds.S0022-0302(91)78553-6

Huhtanen, P., Jaakkola, S., & Nousiainen, J. (2013). An overview of silage research in Finland: From ensiling innovation to advances in dairy cow feeding. *Agricultural and Food Science*, *22*, 35–56. https://doi.org/10.23986/afsci.6632

Huhtanen, P., Rinne, M., & Nousiainen, J. (2007). Evaluation of the factors affecting silage intake of dairy cows: A revision of the relative silage dry-matter intake index. *Animal*, *1*, 758–770. https://doi.org/10.1017/S175173110773673X

Ishiguri, T. (1975). Nutritive value in grasses harvested at different cutting schedules. *Japanese Journal of Grassland Science*, *21*, 154–158.

Jenkins, T. C., & McGuire, M. A. (2006). Major advances in nutrition: Impact on milk composition. *Journal of Dairy Science*, *89*, 1302–1310. https://doi.org/10.3168/jds.S0022-0302(06)72198-1

Kitamura, T. (2016). Effect of rhizomatous grasses on fermentation quality and nutritive value of grass silage. *Japanese Journal of Grassland Science*, *62*, 158–162.

Kuoppala, K., Rinne, M., Nousiainen, J., & Huhtanen, P. (2008). The effect of cutting time of grass silage in primary growth and regrowth and the interactions between silage quality and concentrate level on milk production of dairy cows. *Livestock Science*, *116*, 171–182. https://doi.org/10.1016/j.livsci.2007.10.002

Masuko, T., Ono, A., Furukawa, N., & Otani, T. (1994). Changes in crude protein, ADF and water soluble carbohydrate (WSC) contents of temperate grass cut at three stages of growth in Hokkaido. *Japanese Journal of Grassland Science*, *40*, 227–229.

McCleary, B. V., Solah, V., & Gibson, T. S. (1994). Quantitative measurement of total starch in cereal flours and products. *Journal of Cereal Science*, *20*, 51–58. https://doi.org/10.1006/jcrs.1994.1044

Miyaji, M., Matsuyama, H., & Hosoda, K. (2014). Effect of substituting brown rice for corn on lactation and digestion in dairy cows fed diets with a high proportion of grain. *Journal of Dairy Science*, *97*, 952–960. https://doi.org/10.3168/jds.2013-7046

Miyaji, M., & Nonaka, K. (2018). Effects of altering total mixed ration conservation method when feeding dry-rolled versus steam-flaked hulled rice on lactation and digestion in dairy cows. *Journal of Dairy Science*, *101*, 5092–5101. https://doi.org/10.3168/jds.2017-13802

Miyaji, M., Yajima, A., Sudo, K., & Aoki, Y. (2020). The botanical composition and nutrient yield in a mixed orchardgrass (*Dactylis glomerata* L.) - perennial ryegrass (*Lolium perenne* L.) sward harvested 4 times a year and in a timothy (*Phleum pratense* L.) sward harvested twice a year. *Japanese Journal of Grassland Science*, *65*, 253–259.

Mowat, D. N., Fulkerson, R. S., Tossell, W. E., & Winch, J. E. (1965). The in vitro digestibility and protein content of leaf and stem portions of forages. *Canadian Journal of Plant Science*, *45*, 321–331.

National Agriculture and Food Research Organization (NARO) (2017). *Japanese feeding standard for dairy cattle*. Tokyo, Japan: Japan Livestock Industry Association.

National Research Council (NRC), 2001. National Research Council. *Nutrient requirements of dairy cattle, seventh revised edition*. Washington, DC: National Academy Press.

Ohmomo, S., Tanaka, O., & Kitamoto, H. (1993). Analysis of organic acids in silage by high-performance liquid chromatography. *Bulletin National Grassland Research Institute*, *48*, 51–56.

Rinne, M., Jaakkola, S., Kaustell, K., Heikkilä, T., & Huhtanen, P. (1999). Silages harvested at different stages of grass growth v. concentrate foods as energy and protein sources in milk production. *Animal Science*, *69*, 251–263. https://doi.org/10.1017/S1357729800051286

Steinshamn, H., Purup, S., Thuen, E., & Hansen-Møller, J. (2008). Effects of clover-grass silages and concentrate supplementation on the content of phytoestrogens in dairy cow milk. *Journal of Dairy Science*, *91*, 2715–2725. https://doi.org/10.3168/jds.2007-0857

Tamminga, S., Ketelaar, R., & van Vuuren, A. M. (1991). Degradation of nitrogenous compounds in conserved forages in the rumen of dairy cows. *Grass and Forage Science*, *46*, 427–435. https://doi.org/10.1111/j.1365-2494.1991.tb02403.x

Theurer, C. B., Huber, J. T., Delgado-Elorduy, A., & Wanderley, R. (1999). Summary of steam-flaking corn or sorghum grain for lactating dairy cows. *Journal of Dairy Science*, *82*, 1950–1959. https://doi.org/10.3168/jds.S0022-0302(99)75431-7

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Yajima, A., Sudo, K., Tada, S., Miyaji, M., & Aoki, Y.2020. Forage characteristics of grass silage made from orchardgrass and perennial ryegrass mixed meadow, harvested early and at short intervals in Hokkaido. Nihon Chikusan Gakkaiho 91, in press.

Yan, R., Chen, S., Zhang, X., Han, J., Zhang, Y., & Undersander, D. (2011). Effects of replacing part of corn silage and alfalfa hay with Leymus chinensis hay on milk production and composition. *Journal of Dairy Science*, *94*, 3605–3608. https://doi.org/10.3168/jds.2010-3536

Yan, T., & Agnew, R. E. (2004). Prediction of nutritive values in grass silages: II. Degradability of nitrogen and dry matter using digestibility, chemical composition, and fermentation data. *Journal of Animal Science*, *82*, 1380–1391. https://doi.org/10.2527/2004.8251380x

Yang, Y., Ferreira, G., Teets, C. L., Corl, B. A., Thomason, W. E., & Griffey, C. A. (2018). Effects of feeding hulled and hull-less barley with low- and high-forage diets on lactation performance, nutrient digestibility, and milk fatty acid composition of lactating dairy cows. *Journal of Dairy Science*, *101*, 3036–3043. https://doi.org/10.3168/jds.2017-14082

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