J. Dairy Sci. 101:1–13
https://doi.org/10.3168/jds.2018-14449

Effect of grain- or by-product-based concentrate fed with early- or late-harvested first-cut grass silage on dairy cow performance

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ABSTRACT

This study compared the effects of a grain-based conventional concentrate (GC) and a concentrate based on agro-industrial by-products (BC), fed with grass silage harvested at early (ES) or late (LS) maturity stage, on dairy performance, CH_4 and CO_2 emissions, and metabolic status of dairy cows. Twenty lactating Nordic Red cows averaging 81 d in milk and 31.9 kg of milk/d pre-trial were assigned to a replicated 4 × 4 Latin square design. Dietary treatments were in a 2 × 2 factorial arrangement. The silages were harvested 2 wk apart from the same primary growth grass ley. The GC was made from oats, barley and wheat, and soybean meal, whereas the BC contained sugar beet pulp, wheat bran, canola meal, distillers dried grains, palm kernel expeller, and molasses. The diets were fed ad libitum as total mixed rations and were formulated from 661 g/kg of silage, 326 g/kg of concentrate, and 13 g/kg of minerals on a dry matter basis. The BC supplied the cows with less energy. Despite this, milk yield and composition were unaffected by concentrate type, except that milk protein was 0.7 g/kg lower in cows fed BC than in those fed GC. These results were accompanied by a 44 g/kg decrease in total-tract digestibility of crude protein and a 54 g/kg increase in neutral detergent fiber digestibility for cows fed BC. Cows fed ES on average consumed 2 kg/d more dry matter and yielded 3.5 kg/d more milk, 149 g/d more protein, and 141 g/d more fat than cows fed LS. There were few interaction effects between concentrate and silage sources on daily intake and dairy performance. However, edible feed conversion ratio (human-edible output in animal/potentially human-edible feed) showed greater improvements with ES than LS when replacing GC with BC. Feeding diets with late-cut silage generally reduced digestibility and energy utilization efficiency, but improved N utilization efficiency. Feeding LS also led to greater CH_4 yield and CH_4/CO_2 ratio, and higher plasma concentration of nonesterified fatty acids. Plasma parameters reflecting energy metabolism and inflammation were all within the normal ranges, indicating that the cows were in good health during the experiment. In conclusion, a conventional concentrate can be replaced by agro-industrial by-products without compromising production in early lactation dairy cows. However, silage maturity has a stronger effect on the production traits of dairy cows than type of concentrate.
Key words: by-products, dairy cow, energy utilization, grass silage, milk production

INTRODUCTION

In many farming systems worldwide, a large proportion of feed resources fed to dairy cows could instead be used directly as human foods, or utilized with higher efficiency in poultry and pig production. Demand to increase food production and to secure national food supply is growing (FAO, 2011; Eisler et al., 2014). Feeding agro-industrial by-products has recently been suggested as an efficient option to improve sustainability in terms of human-edible output, calculated as animal products minus potentially human-edible input of feedstuffs, in dairy production systems (Ertl et al., 2015b, 2016).

A total recorded use of agro-industrial by-products of 535,989 t in commercial feeds for farm animals was recorded in Sweden in 2014 (Swedish Board of Agriculture, 2014). As much as 80% of the by-products was used for ruminants and most of that was produced nationally. Some previous studies have demonstrated that soybean meal (**SBM**) can be successfully replaced with canola meal (**CM**) in grass silage-based diets to dairy cows without compromising production (Shingfield et al., 2003; Huhtanen et al., 2011; Martineau et al., 2013). However, in Sweden imported canola by-products cover 20% of the total amount of agro-industrial by-products used in ruminant production systems, while at the

Received January 15, 2018.
Accepted April 6, 2018.
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same time there is a surplus of dried distillers grain (**DDG**), which is exported to Europe (Swedish Board of Agriculture, 2014). Efficient use of non-human edible feed resources produced nationally could improve the resource efficiency of dairy food production. Additionally, restrictions on use of animal protein and genetically modified crops in the European Union (**EU**) motivate use of available agro-industrial by-products as alternative dietary ingredients for dairy cows.

Previous research has mainly focused on replacing single feed ingredients with agro-industrial by-products in diets to dairy cows. Apart from replacing pulses, an objective has often been to investigate and develop lower starch feeding strategies, to improve farm profitability and animal health in dairy production (e.g., Voelker and Allen, 2003; Dann et al., 2014; Ertl et al., 2016). Wheat bran and sugar beet pulp (**SBP**) are the most widely used nonforage fiber sources (**NFFS**) derived from agro-industries in Swedish ruminant production systems (Swedish Board of Agriculture, 2014). While milk production responses vary, some studies report improved or equivalent dairy performance when replacing grain-based concentrate with NFFS (Bradford and Mullins, 2012). Ertl et al. (2016) concluded that inclusion of additional fiber sources such as SBP and wheat bran does not impair milk production compared with supplementing with an organic grain-based concentrate. However, care should be taken in extrapolating these results to dairy cows earlier in lactation.

Compared with CM, wheat DDG contains less CP (387 vs. 315 g/kg of DM; Franco et al., 2017) and is a poorer source of lysine (49 vs. 25 g/kg of CP; Maxin et al., 2013). Martineau et al. (2013) established in a meta-analysis that replacement of protein supplements other than SBM, including DDG, with CM induces positive responses in milk and milk protein yield across a variety of forages.

The objective of this study was to compare a concentrate made solely from agro-industrial by-products, supplemented with equal amounts of CM and DDG as protein sources, with a conventional grain-based concentrate supplemented with SBM as the protein source, fed with 2 grass silages harvested at different maturity stages to lactating dairy cows. We hypothesized that a concentrate made completely from agro-industrial by-products, containing equal amounts of CM and DDG as protein sources and combined with early harvested grass silage, would not compromise dairy production or negatively affect environmental emissions. Parameters studied in the experiment included dairy cow performance, diet digestibility, energy and N utilization, CH_4 and CO_2 emissions, and plasma blood parameters indicative of cow metabolic status.

MATERIALS AND METHODS

The experiment was carried out during autumn 2015 at Röbäcksdalen research station, Swedish University of Agricultural Sciences in Umeå, Sweden (63°45′N; 20°17′E). The study was carried out with the permission of the Swedish Ethics Committee on Animal Research (Umeå, Sweden) and in accordance with Swedish laws and regulations regarding EU Directive 2010/63/EU on animal research.

Cows, Experimental Design, and Diets

Twenty lactating Nordic Red cows (12 multiparous and 8 primiparous) were used in a replicated 4 × 4 Latin square design. At the beginning of the experiment, the multiparous cows were on average (mean ± SE) at 86 ± 9.1 DIM, 633 ± 20.8 kg of BW, and producing 34.4 ± 0.92 kg of milk/d. The corresponding figures for the primiparous cows were 72 ± 9.6 DIM, 538 ± 16.3 kg of BW, and 28.0 ± 1.15 kg of milk/d. The cows were housed in an insulated loose housing barn and milked twice per day, at 0600 and 1500 h. The cows were fed a TMR ad libitum and given free access to water. The diets were mixed using a TMR mixer (Nolan A/S, Viborg, Denmark) and delivered in the feed troughs 4 times per day by an automatic feeding wagon. The cows were blocked according to milk yield and parity, and randomly assigned to treatments within block. Each experimental period lasted 21 d, and data recordings and samplings were conducted during the last 7 d.

The dietary treatments were in a 2 × 2 factorial arrangement, consisting of a grain-based concentrate (**GC**) or a concentrate made from agro-industrial by-products (**BC**), fed with either early- (**ES**) or late-harvested grass silage (**LS**; Table 1). The grass silages had different predicted digestible OM concentration (**DOM**; 744 and 662 g/kg of DM) and were harvested 2 wk apart (June 17 and July 1, 2015) from primary growth of a third-year timothy (*Phleum pratense*) ley. An acid-based additive (Promyr XR 630, Perstorp, Sweden) was used at a rate of 3.5 L/t to preserve the silages, which were ensiled and stored in bunker silos after wilting overnight. The concentrates were produced by Lantmännen Lantbruk AB (Malmö, Sweden) and composed to be isonitrogenous. The GC was formulated from (g/kg of feed) oats (273), barley (273), wheat (273), and SBM (141); the BC consisted of (g/kg feed) SBP (579), wheat bran (42), palm kernel cake (30), DDG (160), and heat-treated CM (141). The diet combining GC and ES was formulated to support milk production up to 35 kg of ECM (LUKE, 2017).

Recordings and Sampling

Individual intake was recorded daily with Roughage Intake Control feeders (Insentec, B.V., Marknesse, the Netherlands) and daily milk yield with gravimetric milk recorders (SAC, S.A. Christensen and Co. Ltd., Kolding, Denmark) during the whole experiment. The BW of the cows was measured after morning milking for 3 d before the experiment and on the last 3 d of each period. Body condition score was measured by 2 skilled individuals on a scale of 0 to 5 with precision increments of 0.25 (Edmonson et al., 1989) before the experiment and on the last day of each period.

Mass fluxes of CH_4, CO_2, and O_2 were recorded daily by the GreenFeed emission monitoring (**GEM**) system (GreenFeed, C-Lock, Rapid City, SD) as described by Huhtanen et al. (2015b). Gas calibrations (N_2, and a mixture of CH_4, O_2, and CO_2) were performed once a week and CO_2 recovery tests were conducted every second week during the whole experiment. Average recovery (mean ± SE) was 100 ± 0.8%. The air filters of the GEM units were cleaned twice a week to maintain the airflow above 26 L/s. A commercial concentrate (Nötfor Idissla, Lantmännen Lantbruk AB, Malmö, Sweden) was given to the cows to ensure regular visits by the cows to the GEM system. The program was set to allow each cow to visit at minimum 5-h intervals and to give 8 drops of 50 g of concentrate during each visit. The interval between drops was set to be 40 s. In each period, gas emission data on all individual animals in the last 7 d were used for statistical analysis.

Milk samples were collected during the last 4 consecutive milking occasions of each period. The DM concentration was determined twice a week for the silages and once a week for the concentrates. All feed samples were oven-dried at 60°C for 48 h. The diets were adjusted twice weekly to account for changes in DM content. The dried feed samples were ground to pass through a 1-mm sieve in a cutter mill (SM 2000, Retsch Ltd., Haan, Germany) providing samples for ash, CP, crude

Table 1. Feed and diet composition (g/kg of DM unless otherwise stated; n = 4)

	Diet[1]				Dietary ingredient[2]				
Item	EG	EB	LG	LB	Early cut silage	Late cut silage	Grain concentrate	By-product concentrate	GreenFeed concentrate[3]
Dietary ingredient									
Early-cut silage	661	661	0	0					
Late-cut silage	0	0	661	661					
Grain concentrate	326	0	326	0					
By-product concentrate	0	326	0	326					
Mineral[4]	13	13	13	13					
Nutrient composition[5]									
DM, g/kg	390	389	457	457	270	338	851	846	846
OM	940	932	942	935	946	951	965	941	924
CP	174	173	135	134	173	108	178	178	203
Crude fat	NA[6]	NA	NA	NA	NA	NA	42	54	42
NDF	339	397	438	496	443	607	162	351	212
Indigestible NDF	38	42	75	79	39	99	35	50	48
pdNDF[7]	301	355	363	417	404	508	127	301	164
Starch	151	30	152	34	NA	NA	444	51	273
Gross energy, MJ/kg of DM	18.7	18.6	18.4	18.3	18.9	18.4	19.0	18.9	18.3
Feed value									
ME,[8] MJ/kg of DM	12.1	11.8	11.2	11.0	11.9	10.6	12.9	12.0	11.3
MP	95.8	96.9	87.2	88.4	90.9	75.9	101	105	144
PBV[9]	33.7	33.4	6.4	5.9	38.1	−6.0	30.0	31.8	12.3

[1]EG = early harvested silage and grain concentrate; EB = early harvested silage and by-product concentrate; LG = late harvested silage and grain concentrate; LB = late harvested silage and by-product concentrate.

[2]Early harvest of silage on June 17, 2015; late harvest of silage on July 1, 2015. Fermentation quality of early and late harvested silage: pH = 3.8 and 3.9; lactic acid = 74.9 and 56.6 g/kg of DM; acetic acid = 19.6 and 18.0 g/kg of DM; propionic acid = 1.2 and 0.3 g/kg of DM; butyric acid = 0.3 and 0.2 g/kg of DM, and NH_3-N = 52 and 54 g/kg of total N.

[3]Concentrate mixture used in GreenFeed (Nötfor Idissla, Lantmännen Lantbruk AB, Malmö, Sweden).

[4]The commercial product Mixa Optimal (Lantmännen Lantbruk AB).

[5]Nutrient composition of TMR + concentrate mix from GreenFeed.

[6]NA = not analyzed.

[7]pdNDF = potentially digestible NDF.

[8]Based on coefficients from feed tables (LUKE, 2017).

[9]PBV = protein balance in the rumen (LUKE, 2017).

fat, NDF, and starch analysis, or through a 2-mm sieve, providing samples for indigestible NDF (**iNDF**) analysis. Additionally, silages were sampled for 3 d in the last week of each period to obtain a composite sample for fermentation quality analysis. The silage samples were stored frozen at −20°C before and after grinding. Frozen silage samples were ground in a cutter mill (SM 2000) to pass through a 20-mm sieve.

Feces (approximately 250 g) and urine (70 mL) spot samples from 12 multiparous cows in 3 blocks were collected at 0530 and 1530 h on 3 d of the last week of each period. Both feces and urine samples were pooled by cow/period during collection. The feces samples were oven-dried at 60°C for 48 h and ground in a cutter mill (SM 2000) to pass through a 1-mm sieve. Feces samples used for analysis of iNDF were ground by pestle and mortar to pass through a 2.5-mm sieve. Urine samples were frozen at −20°C after collection. Blood samples from the tail vein of the same 12 cows were collected on the last day of each period after morning milking into evacuated tubes containing Li-heparin as an anticoagulant (PST Plasma Separation Tubes, VWR International AB, Stockholm, Sweden). The blood samples were centrifuged at 2,100 × *g* for 15 min at room temperature and the plasma was stored at −80°C until analysis.

Chemical Analysis

The concentrations of DM, ash, CP, and NDF in feeds and feces, and silage fermentation quality parameters were determined as described by Gidlund et al. (2015). The urinary N concentration was determined by official method AOAC-984.13 (AOAC, 1990) using a Foss Kjeltec 2400 Analyzer Unit (Foss Tecator AB, Höganäs, Sweden) and Cu as digestion catalyst. The crude fat concentration of concentrates was analyzed according to official method AOAC 920.39 (AOAC, 1990) using a Soxtec solvent extraction system (Foss Analytical Ltd., Hillerød, Denmark). The iNDF concentration was determined by a 12-d in situ incubation in 3 dairy cows fed a forage-based diet according to the procedure of Krizsan et al. (2015). The starch concentration of concentrates was analyzed by a spectrophotometric method according to Salo and Salmi (1968), using a Schimadzu double-beam UV-VIS spectrophotometer UV-1800 (Schimadzu Co., Kyoto, Japan). Gross energy (**GE**) analysis of feeds, feces, and urine samples was conducted according to Gordon et al. (1995), using Parr 6400 Oxygen Bomb Calorimeters (Parr Instrument Co., Moline, IL) with benzoic acid as standard.

The milk samples were analyzed for concentration of fat, protein, and lactose using a near-infrared reflectance analyzer (CombiFoss 6000, Foss Analytical Ltd.). The plasma samples were analyzed at 37°C for metabolites related to energy metabolism [i.e., cholesterol, glucose, nonesterified fatty acids (**NEFA**), and BHB] and inflammation parameters (i.e., albumin, globulin, haptoglobin, and paraoxonase) by a clinical auto-analyzer (ILAB 600, Instrumentation Laboratory, Lexington, MA) as described by Bionaz et al. (2007).

Calculations

Potentially digestible NDF (**pdNDF**) was calculated as NDF − iNDF. Metabolizable energy concentration of silage was calculated assuming 16 MJ of ME per kg of DOM according to MAFF (1975). The DOM of silage was calculated from silage OM concentration and OM digestibility (**OMD**), which was estimated based on concentrations of iNDF and NDF according to Huhtanen et al. (2013). Concentration of ME and MP, and ruminal protein balance value (**PBV**) in concentrates were calculated from analyzed composition and tabulated digestibility and degradability coefficients in Finnish feed tables (LUKE, 2017). The ECM yield and milk energy concentration were calculated according to Sjaunja et al. (1990). The human-edible proportion of feeds was estimated according to Wilkinson (2011) and Ertl et al. (2015b). Edible feed conversion ratio (**eFCR**) for energy and for protein were calculated according to Ertl et al. (2015b). Total-tract digestibility was calculated using iNDF as an internal marker. Daily fecal DM output was calculated as daily iNDF intake divided by iNDF concentration in feces.

Nitrogen utilization efficiency was calculated as milk N/N intake. Daily fecal N excretion was calculated from fecal N concentration and daily fecal DM output. Daily urinary excretion was estimated from urinary N concentration and estimated urinary N excretion (**UN**, g/d), which was calculated using the equation of Huhtanen et al. (2015a) including subtraction of scurf N:

$$\text{UN} = \text{N intake} - \text{fecal N} - \text{milk N} - \text{scurf N} - \text{retained N},$$

where scurf N was calculated according to NRC (2001), N retention was estimated from the calculated ME balance by assuming that BW gain corresponds to a ME balance of 34 MJ/kg, BW loss to −28 MJ/kg (LUKE, 2017), and BW change represents 25.2 g of N/kg (LUKE, 2017).

The respiratory quotient was calculated as the ratio between CO_2 produced and O_2 consumed on a molar basis (Brouwer, 1965). Energy losses in feces and urine were calculated based on their GE concentrations and daily excretions. Digestible energy (**DE**) was calculated by subtracting fecal energy from GE intake, and ME by subtracting energy loss through CH_4 and urine from DE intake. Heat production (**HP**) was calculated according to Brouwer (1965), using data from the GEM devices and urinary N excretion. Energy balance was calculated by subtracting milk energy and HP from ME intake. The efficiency of ME used for lactation ($\boldsymbol{k_l}$) was calculated according to (AFRC, 1990):

$$k_l = (E_l + aE_g)/(MEI - ME_m),$$

where E_l is milk energy output (MJ/d), E_g is tissue energy balance (MJ/d), MEI is ME intake (MJ/d), ME_m is the ME requirement for maintenance (MJ/d), and coefficient a = 0.84 if E_g <0 or a = 0.95 if E_g >0.

Statistical Analysis

Experimental data were subjected to ANOVA using the General Linear Model of SAS (release 9.3, SAS Institute Inc., Cary, NC). by applying a model correcting for the effect of block, period, cow within block, and dietary treatment:

$$Y_{ijkl} = \mu + B_i + P_j + C_k(B_i) + S_l + T_m + (S \times T)_{lm} + \varepsilon_{ijklm},$$

where Y_{ijkl} is the dependent variable and μ is the mean for all observations, B_i is the effect of block i, P_j is the effect of period j, $C_k(B_i)$ is the effect of cow k within block i, S_l is the effect of silage l, T_m is the effect of concentrate type m, $(S \times T)_{lm}$ is the interaction between silage and concentrate type, and $\varepsilon_{ijklm} \sim N(\mathbf{0}, \sigma^2_e)$ is the random residual error, with an expected mean of 0 and σ^2_e as the constant variance.

RESULTS

Experimental Feeds and Diets

Both silages were well-preserved, with low pH (mean 3.85), moderate lactic acid concentration (mean 66 g/kg of DM), and low levels of NH_3-N (mean 53 g/kg N) and butyric acid (mean < 0.3 g/kg of DM; Table 1). However, LS had higher concentrations of DM, NDF, and iNDF, but lower CP, GE, ME, and MP concentrations, than ES. Moreover, BC had higher crude fat, NDF, and iNDF concentrations, but lower concentrations of starch and ME, than GC. Dietary nutrient composition (Table 1) was based on observed daily intake of both TMR and GreenFeed concentrate. A difference was observed in dietary CP concentration between the diets, reflecting differences in CP between the silages, and differences were also present in dietary NDF and pdNDF concentrations, but resulting from differences in both silage maturity and composition of concentrate. More dietary starch was present in diets containing GC as the concentrate.

Intake, Milk Production, and Digestibility

Feeding LS compared with ES in the diets decreased (P < 0.01) total and silage DMI, and intake of CP, ME, and MP, but increased (P < 0.01) intake of NDF (Table 2). Replacing GC with BC decreased ($P \leq 0.01$) intake of CP, starch (from 3.4 to 0.7 kg/d; results not presented), and ME, but increased (P < 0.01) intake of NDF. Intake of concentrate from the GEM units was slightly higher (P < 0.01; results not presented) when cows were fed LS diets compared with ES diets (1.4 vs. 1.2 kg of DM/d).

Yields of milk and ECM, milk protein concentration, and yields of milk fat, protein, and lactose were higher (P < 0.01) when cows were fed ES diets compared with LS diets. Replacing GC with BC had no effect ($P \geq$ 0.08) on milk production, except that milk protein concentration decreased (P = 0.02). Feeding ES diets improved (P < 0.01) feed efficiency and BCS of experimental cows compared with feeding LS diets. The eFCR for protein and energy increased more with ES than LS when GC was replaced by BC ($P \leq 0.05$).

Feeding ES compared with LS in the diet increased (P < 0.01) the digestibility of OM, CP, and NDF (Table 3). Replacing GC with BC in the diet decreased (P < 0.01) the digestibility of CP, but increased (P < 0.01) the digestibility of NDF and pdNDF.

Gas Emissions

Feeding ES diets increased (P < 0.01) total emissions of CH_4 and CO_2, but decreased (P < 0.01) CH_4 intensity (g of CH_4/kg of ECM) and CH_4/CO_2 ratio compared with feeding LS diets (Table 4). Higher (P = 0.05) CO_2 emissions per kilogram of DMI, but a lower (P < 0.01) CH_4/CO_2 ratio, were observed when cows were fed BC rather than GC.

Energy Utilization

Feeding ES diets increased (P < 0.01) intake of GE and ME and increased energy excretion in urine, as CH_4, in milk, and in HP, but decreased (P < 0.01) en-

Table 2. Intake, milk production, and production efficiency in cows fed the experimental diets (n = 20)

Item	Diet[1]				SEM	*P*-value[2]		
	EG	EB	LG	LB		S	C	S × C
Intake, kg/d								
DMI	23.7	22.6	21.3	20.9	0.38	<0.01	0.06	0.34
Silage DMI	14.8	14.1	13.1	12.9	0.25	<0.01	0.07	0.33
GreenFeed concentrate DMI[3]	1.2	1.2	1.3	1.4	0.02	<0.01	0.79	0.12
CP	4.1	3.9	2.9	2.8	0.06	<0.01	0.01	0.20
NDF	8.0	9.0	9.3	10.4	0.17	<0.01	<0.01	0.72
ME,[4] MJ/d	285	266	239	229	4.4	<0.01	<0.01	0.25
MP	2.27	2.18	1.85	1.85	0.033	<0.01	0.17	0.25
Milk yield, kg/d	28.9	28.5	25.5	24.9	0.40	<0.01	0.34	0.99
ECM[5] yield, kg/d	32.4	32.0	29.3	27.5	0.53	<0.01	0.08	0.33
Composition, g/kg								
Fat	48.2	48.6	50.2	48.0	0.92	0.46	0.40	0.19
Protein	36.9	36.1	36.0	35.4	0.29	<0.01	0.02	0.91
Lactose	47.3	47.1	47.1	47.0	0.20	0.47	0.50	0.89
Yield, g/d								
Fat	1,357	1,354	1,262	1,167	38.2	<0.01	0.28	0.31
Protein	1,042	1,014	891	867	15.6	<0.01	0.11	0.84
Lactose	1,348	1,321	1,149	1,142	25.8	<0.01	0.62	0.55
eFCR[6] for energy, MJ/MJ of edible input	0.92	4.56	0.92	3.69	0.099	<0.01	<0.01	<0.01
eFCR for protein, g/g of edible input	0.94	4.15	1.00	3.61	0.161	0.14	<0.01	0.05
ECM/DMI, kg/kg	1.37	1.42	1.37	1.34	0.033	<0.01	0.85	0.17
BW, kg	608	608	603	605	2.2	0.10	0.58	0.53
BCS[7]	2.9	2.9	2.8	2.9	0.02	<0.01	0.41	0.13

[1]EG = early harvested silage and grain concentrate; EB = early harvested silage and by-product concentrate; LG = late harvested silage and grain concentrate; LB = late harvested silage and by-product concentrate.

[2]Probability of significant effect of silage maturity stage (S), concentrate type (C), and interaction between S × C.

[3]Concentrate mixture used in GreenFeed (Nötfor Idissla, Lantmännen Lantbruk AB, Malmö, Sweden).

[4]Based on coefficients from feed tables (LUKE, 2017).

[5]Calculated according to Sjaunja et al. (1990).

[6]eFCR = edible feed conversion ratio, calculated as human-edible output in animal/potentially human-edible feed input from both TMR and GreenFeed concentrate mix according to Wilkinson (2011) and Ertl et al. (2015b).

[7]Scored according to Edmonson et al. (1989).

ergy excretion in feces compared with feeding LS diets (Table 5). Feeding BC decreased ($P = 0.01$) urinary energy output when combined with ES rather than LS. Feeding ES resulted in a more positive energy balance in cows than feeding LS and the difference between silage sources was smaller when cows were fed BC compared with GC ($P = 0.02$).

The ratios DE/GE, ME/GE, and energy balance/ME intake and respiratory quotient were higher ($P < 0.01$) when cows consumed ES diets, but the ratios CH_4 energy/DE, HP/ME intake, and milk energy/ME intake were lower ($P \leq 0.02$), compared with cows fed LS diets. Feeding ES combined with GC gave the greatest ($P \leq 0.03$) k_l in experimental cows.

Table 3. Digestibility of dietary chemical components in experimental cows (n = 12)

Digestibility, g/kg	Diet[1]				SEM	*P*-value[2]	
	EG	EB	LG	LB		S	C
OM	808	793	722	718	6.1	<0.01	0.11
CP	775	729	672	631	10.4	<0.01	<0.01
NDF	689	740	623	680	11.8	<0.01	<0.01
pdNDF[3]	775	828	751	808	13.4	0.11	<0.01

[1]EG = early harvested silage and grain concentrate; EB = early harvested silage and by-product concentrate; LG = late harvested silage and grain concentrate; LB = late harvested silage and by-product concentrate.

[2]Probability of significant effect of silage maturity stage (S), concentrate type (C), and interaction between S × C; the interaction was not significant for any item ($P \geq 0.20$).

[3]pdNDF = potentially digestible NDF.

Table 4. Methane and carbon dioxide emissions for cows fed the experimental diets (n = 20)

Item	Diet[1]				SEM	P-value[2]	
	EG	EB	LG	LB		S	C
CH_4							
g/d	418	413	396	379	6.1	<0.01	0.06
g/kg of ECM	13.1	13.0	14.0	13.9	0.30	<0.01	0.65
g/kg of DMI	17.8	18.5	18.7	18.3	0.49	0.29	0.68
CO_2							
g/d	12,679	12,690	11,206	11,160	110.5	<0.01	0.87
g/kg of ECM	396	399	398	410	7.0	0.34	0.27
g/kg of DMI	540	567	532	540	9.2	0.06	0.05
CH_4/CO_2, g/kg	32.9	32.5	35.3	33.9	0.34	<0.01	<0.01

[1]EG = early harvested silage and grain concentrate; EB = early harvested silage and by-product concentrate; LG = late harvested silage and grain concentrate; LB = late harvested silage and by-product concentrate.

[2]Probability of significant effect of silage maturity stage (S), concentrate type (C), and interaction between S × C; the interaction was not significant for any item ($P \geq 0.12$).

Nitrogen Excretion

Feeding LS diets decreased ($P < 0.01$) urine weight, urinary N excretion, and urinary N/N intake ratio, but increased ($P < 0.01$) fecal DM excretion and fecal N/N intake ratio compared with feeding ES diets (Table 6). Compared with GC diets, cows fed BC diets had higher ($P \leq 0.01$) fecal N excretion and ratio of fecal N/N intake. Feeding ES diets decreased ($P < 0.01$) N utilization efficiency in cows compared with feeding LS diets.

Blood Plasma Parameters

Feeding ES diets decreased ($P \leq 0.05$) plasma concentrations of cholesterol and NEFA in cows compared with feeding LS diets (Table 7). Cows consuming BC

Table 5. Energy intake, output, and utilization in cows fed the experimental diets (n = 12)

Item	Diet[1]				SEM	P-value[2]		
	EG	EB	LG	LB		S	C	S × C
Energy intake and output (MJ/d)								
Gross energy (GE) intake	426	409	374	381	10.2	<0.01	0.59	0.22
ME intake	270	255	215	217	5.5	<0.01	0.20	0.13
Fecal energy	97.7	102	120	124	5.4	<0.01	0.43	0.99
Urine energy	35.5	30.9	16.8	18.3	1.15	<0.01	0.18	0.01
CH_4 energy	22.9	23.2	21.7	21.2	0.49	<0.01	0.86	0.46
Milk energy	106	106	98.0	92.6	2.33	<0.01	0.20	0.33
Heat production[3]	138	139	128	126	1.5	<0.01	0.70	0.20
Energy balance	26.5	8.6	−11.1	−2.1	5.65	<0.01	0.42	0.02
Energy use								
Digestible energy (DE)/GE	0.771	0.753	0.680	0.675	0.0074	<0.01	0.12	0.44
ME/GE	0.633	0.621	0.577	0.568	0.0055	<0.01	0.06	0.70
CH_4 energy/GE	0.054	0.057	0.058	0.057	0.0016	0.20	0.62	0.14
CH_4 energy/DE	0.070	0.076	0.086	0.084	0.0022	<0.01	0.37	0.10
HP/ME intake[4]	0.514	0.551	0.609	0.595	0.0159	<0.01	0.46	0.11
Energy balance/ME intake	0.090	0.026	−0.069	−0.035	0.0306	<0.01	0.61	0.11
Milk energy/ME intake	0.396	0.423	0.460	0.440	0.0171	0.02	0.82	0.17
k_l[5]	0.674	0.645	0.633	0.653	0.0112	0.17	0.67	0.03
RQ[6]	0.99	0.99	0.96	0.96	0.004	<0.01	0.75	0.69

[1]EG = early harvested silage and grain concentrate; EB = early harvested silage and by-product concentrate; LG = late harvested silage and grain concentrate; LB = late harvested silage and by-product concentrate.

[2]Probability of significant effect of silage maturity stage (S), concentrate type (C), and interaction between S × C.

[3]Calculated according to Brouwer (1965).

[4]HP = heat production.

[5]k_l = efficiency of ME use for lactation, calculated according to AFRC (1990).

[6]Respiratory quotient, calculated as $CO_{2\ \mathrm{eliminated}}/O_{2\ \mathrm{consumed}}$ on a molar basis.

Table 6. Nitrogen metabolism in cows fed the experimental diets (n = 12)

	Diet[1]					P-value[2]	
Item	EG	EB	LG	LB	SEM	S	C
Urine excretion							
Urine weight, kg/d	39.2	43.3	21.8	24.3	2.23	<0.01	0.14
N, g/d	282	273	144	149	7.5	<0.01	0.78
N, g/kg of N intake	444	433	330	325	13.2	<0.01	0.53
Fecal excretion							
DM, kg/d	5.0	5.2	6.2	6.5	0.28	<0.01	0.37
N, g/d	153	178	150	171	8.7	0.57	0.01
N, g/kg of N intake	241	279	341	363	11.3	<0.01	<0.01
N utilization efficiency,[3] g/kg	249	255	308	306	7.4	<0.01	0.80

[1]EG = early harvested silage and grain concentrate; EB = early harvested silage and by-product concentrate; LG = late harvested silage and grain concentrate; LB = late harvested silage and by-product concentrate.

[2]Probability of significant effect of silage maturity stage (S), concentrate type (C), and interaction between S × C; the interaction was not significant for any item ($P \geq 0.34$).

[3]Calulated as milk N/N intake.

diets had higher ($P \leq 0.05$) concentrations of cholesterol and glucose than those consuming GC diets. Regarding inflammation parameters, feeding ES diets decreased ($P = 0.02$) plasma paraoxonase concentration, but increased ($P \leq 0.03$) the concentrations of haptoglobin and myeloperoxidase compared with LS.

DISCUSSION

The primary objective of this study was to compare dairy cow production performance on replacing conventional grain-based concentrate with a concentrate made completely from agro-industrial by-products. A wider perspective of using agro-industrial by-products was adopted, by taking into account effects on energy metabolism, CH_4 and N emissions, and cow health status parameters. The aim was to explain mechanisms of effects of whole diets, rather than of specific feed ingredients. This is useful information in development of a sustainable feeding strategy that contributes to perceived greater value of dairy food production by society.

The formulation of experimental diets successfully reflected intended differences in dietary feed ingredients. The differences between ES and LS in dietary concentrations of ME and CP were as expected. The decline in predicted in vivo DOM concentration (5.9 g/d) with advancing maturity agrees with the values of 5.0 and 6.2 g/d reported previously for primary growth grass silages (Kuoppala et al., 2008; Randby et al., 2012; Cabezas-Garcia et al., 2017). Differences in DM concentration and fermentation quality between

Table 7. Concentrations of plasma metabolites related to energy metabolism and inflammation for cows fed the experimental diets (n = 12)

	Diet[1]					P-value[2]	
Item	EG	EB	LG	LB	SEM	S	C
Energy metabolism parameter							
Cholesterol, mmol/L	7.15	7.95	7.68	8.45	0.211	0.02	<0.01
Glucose, mmol/L	3.95	4.07	3.86	3.98	0.083	0.16	0.05
NEFA,[3] mmol/L	0.140	0.119	0.213	0.152	0.0254	0.05	0.12
BHB, mmol/L	1.01	0.98	0.97	0.86	0.085	0.37	0.40
Inflammation parameter							
Albumin, g/L	37.3	37.9	38.0	38.3	0.38	0.17	0.22
Globulin, g/L	45.2	43.6	42.6	43.9	0.85	0.17	0.82
Haptoglobin, g/L	0.194	0.169	0.119	0.144	0.0219	0.03	0.98
Paraoxonase, U/mL	103	111	113	119	3.6	0.02	0.06
Myeloperoxidase, U/L	435	421	380	364	19.1	<0.01	0.43

[1]EG = early harvested silage and grain concentrate; EB = early harvested silage and by-product concentrate; LG = late harvested silage and grain concentrate; LB = late harvested silage and by-product concentrate.

[2]Probability of significant effect of silage maturity stage (S), concentrate type (C), and interaction between S × C; the interaction was not significant for any item ($P \geq 0.11$).

[3]NEFA = nonesterified fatty acids.

the silages were small and were assumed not to be of any relevance with regard to feed intake (Krizsan and Randby, 2007). The dietary effect of the concentrates was mainly a change from high starch-low NDF to low starch-high NDF when replacing GC with BC in the diets.

Intake, Milk Production, and Digestibility

It is well established in the literature that early harvested, highly digestible grass silage is beneficial for dairy cow production performance in terms of daily DMI and milk production (Huhtanen et al., 2006; Kuoppala et al., 2008; Randby et al., 2012). Silage maturity at harvest affected most production traits in this study, with early harvesting generally improving production. The greater response in eFCR when BC was combined with ES rather than LS was mainly due to the superior milk production response of ES, but concentrate intake from the GEM unit was also slightly lower in cows fed ES compared with LS diets.

In agreement with Karlsson et al. (2016), total and silage DMI and yield of milk and milk solids were not affected by dietary concentrate source in this study. An exception was slightly lower protein content in milk from BC compared with GC cows. Lower milk protein content in cows fed fibrous supplements is sometimes ascribed to higher fat content of the supplements (Thomas, 1984). Only a small difference in crude fat concentration was observed between the diets in this study. Similarly, Ertl et al. (2015b, 2016) did not observe any changes in yield of milk or milk constituents when comparing a by-product-supplemented diet with a typical Austrian organic dairy farm diet. However, the effects of inclusion of by-products in dairy cow diets on production reported in the literature are inconsistent. Replacement of barley with NFFS is reported to result in greater milk yield, an effect explained by improved silage DMI (e.g., Huhtanen, 1993) or with no change in intake (Huhtanen, 1987). It has been speculated that greater milk yield with fibrous supplements, despite lower ME intake, may be related to positive associative effects from a combination of different concentrate carbohydrate sources compared with barley alone. Carbohydrates fermented at different rates compared with barley alone can improve microbial protein synthesis in grass silage-based diets (Huhtanen, 1987). Moreover, fish meal response in terms of milk protein yield tends to be greater with barley than with NFFS supplementation. The CP content of NFFS is sometimes higher than that in barley, which may explain a general increase in milk yield (Huhtanen, 1993). Alamouti et al. (2009) reported lower DMI and unaffected milk yield with partial replacement of grain with NFFS combined with alfalfa hay and corn silage, but their cows were in mid-lactation (i.e., had relatively lower demand for energy for milk production). In general, the variability in feed value could be assumed to be greater in individual by-products than in concentrate mixtures made from several ingredients. Furthermore, the dietary effect on milk production, and other performance traits, may depend on the relationships between the absorbed nutrients and subsequently the possibility of the animal to use dietary energy for milk production.

Huhtanen (1993) and Huhtanen et al. (1995) suggest that variable production responses in dairy cows fed NFFS supplements can be explained by the rumen fermentation profile. In cattle fed grass silage-based diets, unmolassed SBP and barley fiber supplements resulted in greater duodenal NAN flow and a higher proportion of propionate than barley, whereas barley-supplemented diets promoted a higher proportion of butyrate (Huhtanen, 1992). Ertl et al. (2015a) observed a lower acetate to propionate ratio in vitro for a diet supplemented with by-products compared with a control concentrate mixture and attributed this to more easily fermentable fiber such as pectin and hemicellulose in the by-products, which is assumed to stimulate propionate formation. Another explanation suggested for the higher observed propionate production in by-product-supplemented diets was higher abundance of *Prevotella* (Ertl et al., 2015a). Furthermore, those authors speculated that by-products that stimulate propionate formation and gluconeogenesis in dairy cows may be beneficial, particularly during early lactation, through improved energy efficiency.

Greater NDF and pdNDF digestibility for the diets supplemented with BC agrees with results reported by Huhtanen (1987) and Huhtanen et al. (1995) for grass silage-based diets supplemented with NFFS and CM for dairy cows. Sugar beet pulp is characterized by a higher concentration of pdNDF compared with barley (309 vs. 197 g of pdNDF/kg of DM; Franco et al., 2017), which explains the higher NDF digestibility of BC-supplemented diets in this study. Few protein supplements derived from the agro-industry have as high a CP concentration as SBM, and they generally have lower rumen protein degradability (Maxin et al., 2013). The reduction in CP digestibility could also be explained by heat damage of the protein in DDG during the drying process, which could explain the reduced milk protein content in cows fed BC diets.

Methane Emissions and Energy and N Utilization

The higher total CH_4 emissions from cows consuming ES diets can be attributed to the higher DMI and OMD (Ramin and Huhtanen, 2013). The reduced CH_4

intensity with ES diets is in line with findings by Warner et al. (2016). The lower CH_4/CO_2 ratio and greater intake of energy indicated that more of the ME was used for productive purposes, despite greater urinary and CH_4 energy losses by the cows consuming ES diets. This was supported by the greater energy digestibility (DE/GE) and metabolizability (ME/GE) in cows consuming ES rather than LS diets. Furthermore, energy partitioning toward milk production (k_l) was greater for cows consuming ES rather than LS when supplemented with GC in this study. Proportionally lower losses of CH_4 in relation to GE intake were caused by increases in ME intake. Yan et al. (2010) reported CH_4 proportional losses of 8.5 to 4.5% of GE intake for cows with intake ranging from maintenance to 5-fold the maintenance feeding level. Kuoppala et al. (2008) and Yan et al. (2010) suggest that increasing ME intake gives better dairy performance because increased feeding level reduces the proportion of energy used for maintenance. Increased ME intake would normally also result in greater utilization efficiency of feed energy to milk production, as evidenced by a low ratio of HP to ME intake in this study. The range of CH_4 energy of GE intake was 5.4 to 5.8% in this study, which is in line with results reported for grass silage-based diets for dairy cows (Yan et al., 2010). The CH_4 energy output as a proportion of DE in the present study was negatively related to dietary ME concentration ($R^2 = 0.36$), ME/GE ($R^2 = 0.38$) and k_l ($R^2 = 0.48$), and positively related to inefficiency of ME used for production ($R^2 = 0.77$; HP/ME intake). These results indicate greater energy expenditure for maintenance rather than production for cows fed LS compared with ES diets. The higher NDF intake of LS diets could be associated with greater work in rumination and digestion, which increases the energy cost of digestion by enhanced secretion of salt in digestive fluids, such as saliva and enzymes, accompanied by greater desquamation through physical action (Lobley, 1986).

The elevated N utilization efficiency in cows fed LS is in line with previous findings (Rinne et al., 1999; Randby et al., 2012). It is mainly attributable to lower dietary concentrations of CP and PBV in LS compared with ES diets. According to a meta-analysis by Huhtanen et al. (2008), dietary concentrations of CP and PBV have much stronger negative effects on N utilization efficiency than CP intake. Although decreases in dietary CP concentration can increase the risk of restricted microbial activity in the rumen, the PBV values for all diets in this study were still above zero, the recommended minimum allowance (LUKE, 2017). The major increase in manure N excretion can be attributed to urinary N excretion, as observed by Gordon et al. (1995) and in other studies with high CP diets (Hynes et al., 2016a, 2016b). According to Huhtanen et al. (2008), who reviewed a wide range of dietary treatments (n = 277), on average 84.4% of the incremental N from diets is excreted in urine when using N intake and DMI as independent variables in a bivariate model. In the present study, 89.2% higher urinary energy output was also observed when cows were fed ES compared with LS diets. In addition to increased urinary N excretion, elevated urinary energy output may also be explained by the greater amount of phenolic acids and their metabolites excreted in urine by ruminants fed early cut, highly digestible grass silage (Martin, 1969). However, it is possible that urinary energy excretion was overestimated due to underestimation of fecal N in this study. Total-tract digestibility data in this study was slightly high in cows fed ES diets. This uncertainty can contribute to errors in determination of total urine volume. For instance, if OMD were overestimated by 40 g/kg in comparison with the value reported by Kuoppala et al. (2010), who determined digestibility by the total feces collection method in cows fed similar diets (comparable silage iNDF), then urinary energy excretion would be on average 7.7% lower. However the difference in urinary energy excretion between early and late cut silage was greater in this study than reported for lactating cows by Gordon et al. (1995), who observed 77.4% greater energy output in urine with highly digestible silage, and in growing cattle by Beever et al. (1988), who recorded 18.2% greater energy output in urine with highly digestible silage.

Blood Plasma Metabolism and Inflammatory Parameters

Cows fed the BC-supplemented diets showed better energy status, as indicated by the higher glucose and numerically lower NEFA concentrations (Piccioli-Cappelli et al., 2014). These cows also showed better liver functionality, as seen from higher cholesterol, which suggests more pronounced synthesis of lipoproteins, and numerically higher paraoxonase, as previously observed by Bionaz et al. (2007) and Bossaert et al. (2012). These results could in part be explained by better inflammo-metabolic condition, which can improve synthesis of some common proteins (e.g., lipoproteins, paraoxonase) in the liver (Loor et al., 2013). It is possible that the less beneficial status in cows fed GC could derive from higher intake of fermentable carbohydrates (Minuti et al., 2014).

In the present study, energy retention significantly decreased and even led to a negative energy balance when the forage source switched from ES to LS, as also evidenced by lower BCS value (results not presented). Reduced energy balance was also reflected in lower plasma glucose concentration and higher NEFA concentration, which is in line with findings by Gross et al. (2011). Based on these results and on the elevated plasma cholesterol concentration, the cows fed LS had a lower inflammatory state, despite the negative energy balance (Gross et al., 2015). Indeed, cows consuming LS diets showed lower haptoglobin and myeloperoxidase activity, which suggests a lower challenge of the innate immune system (Bertoni and Trevisi, 2013; Trevisi et al., 2016). From these data, it is possible to conclude that the diet including LS did not completely cover the energy requirement of the cows and induced mild lipid mobilization, but did not negatively affect the inflammatory response compared with the diet containing ES. Considering that the response of the innate immune system can occur in many compartments (Trevisi et al., 2011, 2016), and that the silages mainly affected the digestive tract, it is probable that the immune system in the rumen or gut epithelium is challenged. Minuti et al. (2013, 2014) observed inflammo-metabolic changes in sheep, with altered permeability of the intestines and the rumen, compatible with those observed in this study.

CONCLUSIONS

Concentrate made from agro-industrial by-products was used to replace conventional grain-based concentrate in dairy cow diets without impairing feed intake, milk production, diet digestibility, or CH_4 emissions. Based on blood parameter values, there were indications of better energy status in cows fed the concentrate made of by-products compared with the grain-based concentrate. This could explain the maintained production level irrespective of dietary concentrate source. Feeding the by-products mixture reduced human-edible inputs and, combined with a highly digestible grass silage, increased eFCR for both energy and protein in dairy cows. However, silage digestibility had a stronger effect on production performance by dairy cows than source of concentrate.

ACKNOWLEDGMENTS

Degong Pang received a PhD studentship sponsored by the China Scholarship Council, Beijing, China. This work was funded by The Regional Foundation for Agricultural Research in Northern Sweden (RJN), Umeå, Sweden; The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS), Stockholm, Sweden; Lantmännen Lantbruk AB, Malmö, Sweden; and Valio Ltd., Helsinki, Finland. The authors thank the staff at the Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umeå, Sweden, and the Agri-Food and Biosciences Institute, Hillsborough, Co. Down, United Kingdom, for assistance in animal management and chemical analysis.

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