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Considering greenhouse gas emissions from feed production in diet formulation for dairy cows as a means of reducing the carbon footprint

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ABSTRACT

Dairy production often faces conflicting goals, such as reducing greenhouse gas emissions, increasing food production and achieving self-sufficiency without transgressing planetary boundaries. This study examined ways to decrease emissions intensity per kg of milk from high-producing cows by selecting feed ingredients with a low carbon footprint while also considering local alternatives. Diets comprising of grass-legume mixture silage and three concentrate mixtures (standard commercial, based on by-products, and domestic crops grown on-farm) were randomly allotted to three groups of high-producing Swedish Holstein cows (N = 48). Over 7 weeks, no differences were observed (mean ± SEM) in feed DM intake (commercial: 24.3, by-products: 24.7, domestic: 24.2 kg/day, ± 0.51 kg/day), energy-corrected milk (**ECM**) yield (commercial: 38.3, by-products: 38.5, domestic: 37.8, ± 0.98 kg/day) or enteric methane production (commercial: 387, by-products: 378, domestic: 402 g/day, ± 17.3 g/day) among the diets. However, an evaluation of the primary carbon footprint of feed production (excluding transportation emissions) showed that the by-products and domestic diets gave lower emissions than the commercial diet, 9.4, 10.2, and 11.9 Feed CO_2 equivalents (**$CO_{2\text{-eq}}$**) kg/day, respectively (SEM: ± 0.38 Feed $CO_{2\text{-eq}}$ kg/day). The emission intensity, expressed as feed emissions per kilogram of ECM yield, showed that the by-product-based and domestic diets generated lower carbon footprints, with emissions of 254 and 284 g Feed $CO_{2\text{-eq}}$/kg ECM, respectively, in comparison to 320 g Feed $CO_{2\text{-eq}}$/kg ECM observed for the commercial diet (SEM: ± 10.7 g Feed $CO_{2\text{-eq}}$/kg ECM). Considering greenhouse gas emissions from feed production in diet formulation resulted in a lower overall feed carbon footprint and lower emission intensity per ECM. These findings can assist in formulating dairy rations for high-yielding dairy cows that balance conflicting goals while maintaining productivity.

Implications

Feed production carbon footprint is an important parameter to consider when formulating dairy rations aiming to improve the environmental sustainability of dairy production. In this study, diets based on by-products reduced feed carbon footprint and emission intensity per kilogram energy–corrected milk both by 21%, while domestically produced feeds resulted in reductions of 14 and 11%, respectively, compared to a commercial mix. Our results contribute to developing sustainable dairy cow feeding strategies by designing rations that optimise productivity, lower carbon footprint, and promote local agricultural production. These findings help distinguish high-producing dairy systems based on their inputs and carbon footprint.

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Introduction

It is generally recognised that approximately 12% of the total anthropogenic greenhouse gas emissions are attributable to livestock production. Ruminant production systems cause the majority of the greenhouse gas emissions from livestock production and consist of enteric CH_4, CO_2 and N_2O (IPCC, 2019). Animal nutrition is a key action target for improved sustainability (FAO, 2023) since feed ration formulation can directly affect animal health, productivity and enteric fermentation. In the coming decades, increases in the global population will increase the demand for food, while the expected improvement in living standards will lead to increased demand for animal-source food (FAO, 2018; Enahoro et al., 2021; van Dijk et al., 2021). These environmental challenges and the risk of exceeding the Earth's biophysical limits (Steffen et al., 2015) create a need to sustainably produce food (Muscat et al., 2021). Different perspectives exist on achieving this (Billen

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et al., 2021). Focusing on the demand perspective has led some to suggest eliminating or reducing the consumption of animal-source foods and switching to a plant-based diet (Poore and Nemecek, 2018; Theurl et al., 2020). From a production perspective, some claim that intensification of production will lower emission intensity, defined as environmental impact per unit of animal-source food produced (Gerber et al., 2011), although this may exacerbate the problem of feed-food competition (Van Zanten et al., 2018). Many have suggested that food production should be prioritised on arable land, while feed production should be considered a secondary priority. Livestock production would then be based on low-opportunity-cost biomass or ecological leftovers (by-products) (Röös et al., 2016; Karlsson et al., 2018b; van Hal et al., 2019; van Selm et al., 2022).

A by-product-based animal-feeding system can address challenges like poor land suitability, feed-food competition, incomplete nutrient cycles and excessive reliance on external inputs (van Zanten et al., 2016; Frehner et al., 2022). It would thus help decrease overall greenhouse gas emissions and increase net food production (Wilkinson, 2011; Patel et al., 2017; Cheng et al., 2022). However, livestock reared in such a system would be subjected to various trade-offs, with adverse effects on productivity that could increase emission intensity. Despite its potential benefits, using by-products as feed has not been sufficiently studied in high-producing dairy cows. One study reported decreased productivity in high-producing dairy cows (Takiya et al., 2019), but most studies have been performed on cows with lower milk production levels (Pang et al., 2018; Karlsson et al., 2019; Guinguina et al., 2021). Furthermore, the global COVID-19 pandemic and the armed conflict in Ukraine affected the agricultural supply chain, creating uncertainty and commodity and labour shortages, resulting in food price volatility and affecting the availability of products (Workie et al., 2020; Lin et al., 2023). This highlights the importance of self-sufficiency and resilience to external shocks. One way to withstand such challenges is to grow most animal feed crops on-farm or have access to other domestically produced feedstuffs.

This study evaluated production responses in high-yielding dairy cows fed concentrates based on by-products or domestically produced feeds, compared with a commercial concentrate. The aim was to address knowledge gaps regarding milk production, enteric CH_4 and associated emissions from feed production. The hypotheses were that (i) feeding a concentrate based on by-products would result in lower milk production and higher CH_4 emissions compared with a commercial concentrate, (ii) using domestically (on-farm) produced ingredients would not impair productivity or result in higher CH_4 emissions compared with a commercial concentrate.

Material and methods

Animals and study design

The study was conducted at the company Lantmännen's experimental dairy farm "Nötcenter Viken" in Falköping, Sweden, from May to July 2022. A total of 48 Swedish Holstein cows were used, 15 primiparous and 33 multiparous (mean ± SD; 2.8 ± 1.0 lactations). At the start of the experiment, the cows averaged 185 ± 50 days in milk, with an energy-corrected milk (**ECM**) yield of 43.3 ± 5.32 kg/day and an average BW of 675 ± 54 kg. The cows were divided into two blocks based on parity level, and within each block, cows were randomly assigned to one of three dietary treatments. The treatments consisted of a partial mixed ration composed of grass-legume mixture silage and one of three types of pelleted concentrate: (i) Control (**CON**; a commercial mix (Komplett Maxa 175, Lantmännen Malmö, Sweden), (ii) by-product (**BYP**) and (iii) domestic (**DOM**). The experiment followed a randomised complete block design with the use of a covariate, with 2 weeks of adaptation to the diets and 7 weeks of data collection. Dry matter intake (**DMI**) (mean ± SD, CON: 22.5 ± 2.81, BYP: 22.6 ± 2.47, DOM 22.1 ± 2.72 kg/day), ECM production (CON: 43.5 ± 5.50, BYP: 43.2 ± 5.34, DOM 43.1 ± 5.49 kg/day) and BW (CON: 690 ± 49.8, BYP: 669 ± 57.4, DOM 671 ± 54.2 kg/day) were collected the week before the start of the experiment and were used as covariate data in the statistical analysis.

The cows were housed in a free-stall pen with sufficient cubicles covered with rubber mats and peat as bedding material. The cows had *ad libitum* access to their allocated partial mixed ration, salt licks, and water. A unique radio-frequency ear tag facilitated individual cows' identification, enabling automatic recognition in the feeding stations, BW scale (at the start and end of the experiment), milking unit, and the unit for enteric CH_4 emissions recording. The cows were milked voluntarily in a free cow traffic single-station voluntary milking system (310TM system; DeLaval International AB, Tumba, Sweden). Individual daily feed intake was recorded automatically using feed mangers on scales (BioControl, CRFI, Rakkestad, Norway). A single GreenFeed system unit (C-Lock Inc., Rapid City, SD, USA) was used for continuous measurements of emissions of enteric CH_4, respiratory CO_2 and O_2.

Dietary treatments

The dietary treatments (silage and concentrate pellets) were optimised using NorFor – the Nordic feed evaluation system (2011) to support a dairy cow producing 45 kg ECM per day. The silage-to-concentrate ratio was set at 45:55 on a DM basis for all rations. The rations were formulated to be as similar as possible, with prioritisation in descending order based on net energy, CP, starch, fat and NDF content (Table 1). The chemical composition of the ingredients used during ration formulation is presented in Supplementary Table S1.

All cows received the same silage, consisting of a grass-legume mixture, from the first cut of multiyear leys. The silage was a mixture of timothy (Phleum pratense L.), meadow fescue (Festuca pratensis L.) and perennial ryegrass (Lolium pratense L.) with less than 25% of red clover (Trifolium pratense L.) and white clover (Trifolium repens L.). The primary difference between treatments lay in the type of pelleted concentrate feed included in the dairy rations. The CON group was fed a commercially available pelleted concentrate mix (Komplett Maxa 175, Lantmännen Malmö, Sweden) chosen to represent a typical pelleted concentrate used by high-producing Swedish dairy herds (Lantmännen communication). For the BYP concentrate, ingredients were selected from by –products available in sufficient quantities in the Swedish market, either through domestic production or international trade. Priority was given to cereal by-products (e.g., wheat middlings), which were included at a minimum level of 40% of DM concentrate, and cereals were added to achieve a minimum of 170 g of starch per kg of DMI. For the DOM concentrate, ingredients were limited to those that could be supplied through domestic production, such as cereals, oilseed by-products, sugar by-products, and legume grains. During the formulation of the BYP and DOM concentrates, each ingredient's carbon footprint was taken into account, incorporating emissions in the form of fossil CO_2, N_2O and excluding land-use change. The carbon footprint was expressed as CO_2 equivalents (**CO_{2-eq}**) and was sourced in descending priority order from country-specific datasets, international datasets, and scientific publications (Garcia-Launay et al., 2014; GFLI, 2019; Lindberg et al., 2021; RKFS, 2021; Supplementary Table S2). All concentrates were pelleted by Lantmännen Lantbruk AB (Malmö, Sweden) (Table 2). The pelleting process (3.8 mm pellet) included milling,

Table 1
Chemical composition (mean ± SD; g/kg DM unless otherwise stated) of silage, control, by-product-based and domestic concentrates and of sugarbeet pulp pellets.

Item	Silage	CON	BYP	DOM	Betfor®
DM (g/kg)	275 ± 11.4	902 ± 3.7	903 ± 4.6	902 ± 2.9	925 ± 3.1
Ash	90.1 ± 2.70	67.4 ± 1.58	71.3 ± 2.16	75.6 ± 1.10	72.9 ± 0.98
CP	175 ± 5.8	178 ± 1.1	185 ± 1.4	181 ± 1.9	87.1 ± 1.35
aNDFom	488 ± 7.2	166 ± 9.3	241 ± 11.6	199 ± 7.2	348 ± 4.7
iNDF	54.5 ± 2.46	43.5 ± 2.69	66.6 ± 2.66	46.8 ± 0.50	27.2
Starch	NA	360 ± 3.8	300 ± 14.3	311 ± 5.8	15.8 ± 4.47
Ether extract	37.8 ± 1.67	54.8 ± 0.23	41.2 ± 0.57	44.4 ± 0.91	5.37 ± 0.018
IVOS (%)	88.7 ± 0.79	NA	NA	NA	NA
NEL (MJ/kg DM)	6.75[†]	7.36[†]	6.68[†]	7.05[†]	6.39[†]

Abbreviations: CON = Control mix; BYP = By-product based mix; DOM = Domestically produced mix; Betfor = Sugarbeet pulp pellets; aNDFom = amylase NDF organic matter; iNDF = indigestible NDF; IVOS = Ruminal fluid digestible organic matter; NA: Not analysed; NEL = Net energy for lactation.
[†] Based on the chemical composition according to NorFor (2011).

blending and heat treatment according to European and Swedish feed regulations (EC, 2005; SJVFS, 2018).

During the trial, the silage was mixed with the respective concentrate into three different partial mixed rations using a stationary mixer (Feed Mixer-Multimix, Cormall, Sønderborg, Denmark) and provided once daily *ad libitum* via an automatic feeding wagon (Free Stall Feeder M2000 XL, GEA, Düsseldorf, Germany). Silage DM content was determined twice per week throughout the experiment to adjust the composition of the partial mixed ration as needed. Additionally, cows received approximately 2 kg of concentrate (CON, BYP or DOM) per milking in the voluntary milking system (average 3.1 milking occasions per day), and sugar beet pellets (Betfor®, Nordic Sugar AB, Malmö, Sweden) were offered as attractant feed in the GreenFeed unit. These feedstuffs were included in the DMI calculation presented in Table 3.

Sample collection and analyses

Feed

During milk and faeces sampling weeks (1, 4 and 7), silage and concentrate samples were collected four times per week (Monday to Thursday). In other weeks, silage samples were collected five times per week (Monday to Friday), while concentrate samples were collected twice weekly (Monday and Thursday). All samples were stored at −20 °C until analysis. At the end of each week, frozen silage and concentrate samples were pooled per treatment and

Table 2
Composition (% of fresh matter) and estimated carbon footprint of silage, control, by-product-based, and domestic concentrate feeds used in the experiment with Swedish Holstein cows.

	Feed				
Ingredient	Silage	CON	BYP	DOM	CF ($CO_{2\text{-eq}}$ g/kg)[1]
Oat hulls	–	–	1.2	–	89[††]
Wheat bran	–	4.0	–	–	89[††]
Distillers' grain[2]	–	–	10.0	–	214[††]
Wheat middlings	–	–	41.1	–	289[††††]
Field beans	–	–	–	11.5	336[††††]
Barley	–	18.4	28.0	36.5	361[††††]
Molasses	–	2.5	3.0	3.0	370[††]
Grass-legume mixture silage	100	–	–	–	390[†]
Oats	–	–	3.0	8.5	390[††††]
Wheat	–	8.0	–	–	400[††]
Heat-treated rapeseed meal[3]	–	20.0	6.0	15.5	460[††††]
Dried sugar beet pulp (unmolassed)	–	6.6	2.0	15.0	460[††]
Rapeseed meal	–	5.3	–	–	506[††††]
Rapeseed cake	–	3.0	–	–	493[††††]
Maize	–	25.3	–	–	605[††††]
Crushed rapeseeds	–	–	2.0	5.6	917[††††]
Vegetable fats					
AkoFeed® Gigant75	–	2.8	–	–	1 000[††]
AkoFeed® Cattle	–	0.5	–	–	2 300[††]
Rumen-protected amino acids					
MetaSmartDry	–	0.2	–	–	3 000[†††]
LysiGEM BB	–	0.1	–	–	4 300[†††]
Minerals[4]	–	3.3	3.7	4.4	42[††] - 1 168[††††]
Pellet CF ($CO_{2\text{-eq}}$ g/kg)[5]		525	338	425	

Abbreviations: CON = Control mix; BYP = By-product based mix; DOM = Domestically produced mix; $CO_{2\text{-eq}}$ = Carbon dioxide equivalent; CF = Primary estimated carbon footprint.
[1] Primary carbon footprint expressed as $CO_{2\text{-eq}}$ g/kg fresh matter, except for Grass-legume mixture silage, which is expressed as $CO_{2\text{-eq}}$ g/kg DM.
[2] Fibre and yeast cells from ethanol manufacturing (Agrow Drank 90, Lantmännen Agroetanol, Norrköping, Sweden).
[3] Solvent-extracted and heat-moisture-treated rapeseed meal (ExPro®, AAK Sweden AB, Karlshamn, Sweden).
[4] Containing minerals, vitamins and trace elements. The values in the table describe the variation in $CO_{2\text{-eq}}$ among all included ingredients within this category; however, as these are small added quantities, they do not significantly impact the overall results.
[5] Primary carbon footprint expressed as g $CO_{2\text{-eq}}$ per kg product.
[†] Source: Lindberg et al. (2021).
[††] Source: Lantmännen's estimated value based on RKFS (2021) for calculating the carbon footprint of feeds.
[†††] Source: Synthetic amino acids impact based on Garcia-Launay et al. (2014).
[††††] Source: GFLI dataset (2019).

Table 3
Effect of the control, by-product-based and domestic dietary treatments assessed across the entire experimental period on feed intake, daily milk yield, milk yield-to-feed intake ratio and BW in Swedish Holstein cows.

Item	Diet			SEM[1]	*P*-value
	CON	BYP	DOM		
Number of cows	16	15	15		
DMI (kg/day)	24.3	24.7	24.2	0.51	0.707
Silage DMI (kg/day)	10.6	11.0	10.5	0.50	0.701
Concentrate DMI (kg/ day)[†]	13.4	13.2	12.9	0.43	0.650
Silage/DMI (%)	43.7	44.9	44.7	0.78	0.437
Milk yield (kg/ day)[†]	39.6[a]	36.0[b]	38.7[ab]	0.97	0.017
Milk yield/DMI[†]	1.62	1.48	1.59	0.054	0.103
BW (kg)	697	680	688	5.7	0.071

Abbreviations: CON = Silage plus control mix; BYP = Silage plus by-product based mix; DOM = Silage plus domestically produced mix; DMI = DM intake.
[†] Significant effect of days in milk.
[1] Greatest SEM value obtained.
[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$ after adjustment for multiple testing using Tukey's procedure.

week. All analyses were performed by the laboratory at the Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Uppsala, Sweden. The DM content of the silage was determined by a two-step procedure according to Åkerlind et al. (2011), first drying at 60 °C overnight and milling and then drying at 103 °C for 16 h overnight. The DM content of the concentrates was determined by drying at 103 °C for 16 h (Jennische and Larsson, 1990). Ash content for all feeds was determined by ignition at 550 °C for three hours (Jennische and Larsson, 1990). The other analyses were performed on samples dried at 60 °C for 16–20 h and allowed to stabilise for at least 4 h at room temperature. CP was analysed using an automated Kjeldahl procedure (Foss, Hillerød, Denmark; Nordic Committee on Food Analysis, 1976). The concentrates were analysed enzymatically for starch (including maltodextrin) according to Larsson and Bengtsson (1983). All feeds were analysed for amylase NDF organic matter (**aNDFom**) according to Chai and Udén (1998) and indigestible NDF (**iNDF**) according to Åkerlind et al. (2011). The pelleted feeds were pooled for ether extract analysis according to the batch delivered to the farm. The CON and sugar beet pulp pellets were composited in one sample each for the entire experiment, while for DOM and BYP, two samples were composited per feed by pooling weeks 1–4 and weeks 5–7. Silage and pelleted feed samples were analysed for ether extract according to European Commission regulations (EC, 2009). The silage samples were also analysed for *in vitro* organic matter digestibility (**OMD**). The net energy for lactation content in the concentrates and silage was calculated according to the NorFor system (Volden and Nielsen, 2011).

Milk

Milk yield was recorded automatically at each milking for all cows throughout the experiment, and the data were retrieved from the DelPro (DeLaval International AB, Tumba, Sweden) system. Milk samples were collected over two consecutive 24-hour periods one week before the adaptation period (used as a covariate) and then again during weeks 1, 4 and 7. Samples were collected automatically from the milking unit into 20-mL tubes containing bromo-2-nitropropane-1.3-diol on every milking occasion and stored at +4°C until analysis (performed within 7 days). Milk samples were analysed for concentrations of milk fat, milk protein, milk urea nitrogen (**MUN**), lactose and somatic cell count using IR Fourier-transform spectroscopy (CombiScope FTIR 300 HP, Delta Instruments B.V., Drachten, the Netherlands). Lactose was corrected for lactase monohydrate by dividing by 1.053. Due to the irregular milking intervals that occur in automatic milking, individual milk production per cow and day was calculated according to Nielsen et al. (2010). During week 4, due to a delayed changing of the sampling cassette, nine tubes were filled with milk samples from two animals, and these tubes were thus discarded. Energy-corrected milk yield was calculated based on fat, protein and lactose content according to Sjaunja et al. (1990):

$$ECM\,(kg) = Milk\ yield\,(kg) \times \left(\frac{38.3 \times fat\left(\frac{g}{kg}\right) + 24.2 \times protein\left(\frac{g}{kg}\right) + 16.54 \times lactose\left(\frac{g}{kg}\right) + 20.7}{3140} \right)$$

Faeces and digestibility

Faecal grab (~400 g) samples were collected from the rectum of each cow once daily on three consecutive days (Tuesday to Thursday) in weeks 1, 4 and 7 (Mehtiö et al., 2016). These samples were pooled per cow and week, stored at −20 °C until required, thawed, subsampled, subjected to freeze-drying, milled and analysed for DM, ash, CP, NDF and iNDF. The total amount of faeces was estimated from the total intake of iNDF and the content of iNDF in the faeces. For the iNDF analysis, composite faeces samples were freeze-dried, milled and analysed according to Åkerlind et al. (2011). Total-tract apparent digestibility was calculated from the estimated feed intake, faecal excretion and their chemical composition:

$$\text{Apparent total tract digestibility} = \frac{\text{Feed intake} - \text{Faecal output}}{\text{Feed intake}}$$

The calculation was based on data from the corresponding days of each sampling week.

Enteric gas emissions

Exhaled gases (O_2, CO_2, CH_4) were measured individually using a GreenFeed system unit (C-Lock Inc.; Zimmerman et al., 2011) throughout the whole experiment (weeks 1–7). The unit was equipped with a head position sensor, and data were excluded when head position criteria were unmet. All animals could visit the GreenFeed unit voluntarily, with a minimum interval of five hours between visits (maximum five visits/day). A sugar beet pulp-based pelleted bait was used to attract cows and maintain correct head positioning, dispensed at 30 g per cup drop, with up to 8 drops per visit and with 1 cup drop per 40 s. Gas emissions were calculated by subtracting background concentrations from those recorded during each visit and adjusting for airflow, temperature, and pressure using the ideal gas law. GreenFeed used a non−dispersive near-IR analyser to measure CH_4, O_2, and CO_2, calibrated every third day with standard gases provided by C-Lock Inc. to account for signal drift. Monthly recovery tests using known

CO_2 amounts confirmed an average recovery rate of 99.5%, and flow coefficients were adjusted accordingly (C-Lock Inc.). The calibration and recovery process were performed based on the manufacturer's recommendations (https://greenfeed.c-lockinc.com). Data were uploaded every 24 h through a web-based system (C-Lock Inc.), and the validated data were used for the statistical analysis.

Data management and statistical analysis

All data were analysed in R Studio (Posit Team, 2022; R Core Team, 2022) using basic R commands and the packages tidyverse (Wickham et al., 2019) and ggplot2 (Wickham, 2016). During the experimental period, two cows were excluded due to health issues unrelated to the experiment. One cow from the DOM treatment group suffered a mouth injury, while another from the BYP group developed pneumonia. Furthermore, one cow from the BYP group lost her ear identification tag during the experiment, resulting in abnormal feed intake values and milk and ECM values. The animal was identified as an outlier during the statistical analysis, and milk composition values and ECM values were removed from the dataset. Additionally, due to an error, one cow from the CON group had access to the wrong diet for 24 h during the experiment, and thus, her feed intake values were removed for that day. A successful visit to the GreenFeed was defined as a visit event with a duration of at least three minutes. A cut-off value of 20 successful visit events per animal during the entire experiment was used to ensure reliable data (Manafiazar et al., 2016). Animals with a lower number of successful visits were removed from the dataset. This resulted in 24 remaining animals (nine CON (three primiparous, six multiparous), six BYP (three primiparous, three multiparous) and nine DOM (three primiparous, six multiparous)). This resulted in a total of 1 494 successful visits for the entire experiment and an unbalanced design, with 679, 277 and 538 successful visits for the CON, BYP and DOM groups, respectively.

Data were averaged by cow and week, and a linear mixed-effects model with a continuous AR(1) correlation structure "corCAR1" was fitted for each response variable using the "nlme: Linear and Nonlinear Mixed Effects Models" package (Pinheiro et al., 2022). ANOVA was performed using the "car: Companion to Applied Regression" package (Fox and Weisberg, 2019) with the options type III option and Kenward-Roger approximation method. Treatment, week, and parity groups were used as fixed effects, while animal was used as a random effect to account for repeated measurements. A statistical model with the variables days in milk as a covariate to account for different stages of lactation, two-way interactions (treatment × week and treatment × parity group) and three-way interactions (treatment × week × days in milk) was tested, and explanatory variables were removed from the model if non-significant. Average milk yield, ECM yield, DMI and BW in the week before the adaptation period were included as covariates for milk, ECM yield, DMI, and BW, respectively. All residuals were tested for normality, and log transformation was performed if needed for the statistical analysis (stated in the following results tables where relevant). Statistical significance was set at $P < 0.05$, and pairwise comparisons adjusted using Tukey's method were performed using the means package (Lenth, 2023).

Results

There were no differences in total feed intake or intake of concentrates and silage between cows in the CON, BYP and DOM treatments. Milk yield differed between treatments throughout the entire experimental period. Cows on the BYP treatment produced 9% less milk compared to those on the CON treatment, while the DOM treatment group did not differ from either (Tables 3 and 4). On milk sampling days, no differences were observed between the treatments in milk yield, ECM yield, milk protein, lactose and somatic cell count. Milk fat content from cows on the BYP treatment was 8% higher compared to those on the CON treatment. However, MUN was 19% higher for BYP and 12% higher for DOM compared to CON. No treatment × week interaction was observed for any parameters reported in Tables 3 and 4.

Enteric CH_4 emissions, respiratory CO_2 and feed primary $CO_{2\text{-eq}}$ are presented in Table 5. There were no differences between the treatments in enteric CH_4 production or the CH_4 emissions intensity. Feed primary $CO_{2\text{-eq}}$ expressed as g/day differed between the treatments, as planned. Animals receiving the BYP and DOM diets had 21 and 14% lower Feed primary $CO_{2\text{-eq}}$ than those receiving CON. Feed primary $CO_{2\text{-eq}}$ expressed in g/kg milk was 15% lower in BYP cows compared with CON cows, while feed primary $CO_{2\text{-eq}}$ expressed as g/kg ECM was 21 and 11% lower in cows on the BYP and DOM diets, respectively, compared with those on the CON diet (Table 5). No treatment × week interaction was observed for any of the parameters reported in Table 5.

Intake and apparent digestibility results per treatment are presented in Table 6. Intake of aNDFom was 16% higher in BYP than in CON cows, whereas DOM cows did not differ from those in the other two treatments. Similarly, iNDF intake was 27% higher in BYP compared with CON cows, while no difference was observed between DOM and CON cows. Starch intake was 18% lower in BYP and 16% lower in DOM cows than in CON cows, while ether extract intake was 14% lower for BYP and DOM cows compared to CON cows. A treatment × week interaction ($P < 0.001$) was observed for iNDF and starch intake; however, posthoc comparisons suggest these changes were not consistently large or statistically distinct at each week (Supplementary Figs. S1 and S2). Animals consuming the BYP and DOM diet had consistently lower starch intake values and higher iNDF intake values throughout the experiment (weeks 1–7) than CON while no difference was observed between BYP and DOM.

DM and apparent OMD were lower by 3.5 percentage units in BYP cows compared with the other two diets, while no difference was observed between CON and DOM cows. The apparent digestibility of aNDFom differed between all treatments, with BYP cows having the lowest value (3.5 percentage units decrease compared to CON) and DOM cows the highest (2.9 percentage units increase compared to CON). A treatment × week interaction was observed for the apparent digestibility of DM, organic matter and aNDFom (Supplementary Table S3). Specifically, for the BYP group, DM digestibility was lower during weeks 1 and 7 compared to CON, but no difference was observed during week 4. Digestibility of organic matter was lower for animals that received BYP compared to CON throughout weeks 1, 4 and 7. Digestibility of aNDFom was lower for animals receiving BYP compared to CON throughout weeks 1 and 7, but no difference was observed during week 4. Animals in DOM had higher aNDFom digestibility during week 4 compared to CON, while no difference was observed between weeks 1 and 7.

Discussion

Intake and digestibility

No differences in DMI were found between the diets, for either concentrate or silage, aligning with findings from previous studies comparing by-product-based and cereal-based diets (Karlsson et al., 2018a; Guinguina et al., 2021). In a previous comparison of conventional and by-product-based diets, Takiya et al. (2019)

Table 4
Effect of the control, by-product-based and domestic dietary treatments assessed on sampling days during weeks 1, 4 and 7 on daily milk yield, energy-corrected milk yield, milk components and the ratio of milk yield and energy-corrected milk yield to DM intake in Swedish Holstein cows.

Item	Diet			SEM[1]	*P*-value
	CON	BYP	DOM		
Number of cows	16	14	15		
DMI (kg/day)	24.3	24.6	24.1	0.45	0.678
Milk yield (kg/day)	39.5	37.1	38.5	0.977	0.188
ECM (kg/day)	38.3	38.5	37.3	0.988	0.635
Fat (%)[†]	3.97[b]	4.29[a]	4.01[b]	0.078	0.004
Fat yield (kg/day)	1.53	1.57	1.50	0.039	0.323
Protein (%)[†]	3.42	3.45	3.36	0.036	0.143
Protein yield (kg/day)	1.31	1.28	1.25	0.037	0.484
Lactose (%)	4.56	4.62	4.63	0.032	0.208
Lactose yield (kg/day)[†]	1.78	1.70	1.75	0.052	0.500
Milk urea N (mg/100 mL)	12.0[b]	14.2[a]	13.4[a]	0.260	<0.001
Somatic cell count (1 000/ml)[††]	87.5	137.5	86.5	34.7	0.205
ECM/DMI	1.59	1.58	1.55	0.050	0.806

Abbreviations: CON = Silage plus control mix; BYP = Silage plus by-product based mix; DOM = Silage plus domestically produced mix; DMI = DM intake; ECM = Energy −corrected milk.
[†] Significant effect of days in milk.
[††] Back-transformed from log-transformed values (antilog scale) for interpretability.
[1] Greatest SEM value obtained.
[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$ after adjustment for multiple testing using Tukey's procedure.

Table 5
Effect of control, by-product-based and domestic concentrate diets on enteric gas emissions and feed primary carbon footprint in Swedish Holstein cows.

Item	Diet			SEM[1]	*P*-value
	CON	BYP	DOM		
Number of cows	9	6	9		
Successful visits per animal[2]	75 ± 35	46 ± 25	60 ± 20		
DMI (kg/day)[3]	24.4	24.5	24.2	0.49	0.885
Enteric CH_4 (g/day)	387	378	402	17.3	0.500
CH_4/Milk (g/kg)[4 ††]	10.39	9.98	11.43	0.797	0.351
CH_4/ECM (g/kg)	10.83	9.82	11.57	0.814	0.241
CH_4/DMI (g/kg)	16.4	15.8	17.3	0.60	0.119
Exhaled CO_2 (g/day)	12 941	13 042	13 070	396.0	0.954
CO_2/Milk (g/kg)[4 † ††]	351	358	368	28.1	0.858
CO_2/ECM (g/kg)	363	340	377	28.8	0.599
CO_2/DMI (g/kg)	552	548	564	19.0	0.762
CH_4/CO_2 (g/kg)[†]	29.8	28.6	30.9	0.75	0.048
Number of cows	16	15	15		
Feed primary $CO_{2\text{-eq}}$ (g/day)[††]	11 907[a]	9 423[b]	10 191[b]	378.0	<0.001
Feed primary $CO_{2\text{-eq}}$ /Milk (g/kg)[2 † ††]	311[a]	264[b]	279[ab]	10.8	0.004
Number of cows	16	14	15		
Feed primary $CO_{2\text{-eq}}$ /ECM (g/kg)	320[a]	254[b]	284[b]	10.7	<0.001

Abbreviations: CON = Silage plus control mix; BYP = Silage plus by-product based mix; DOM = Silage plus domestically produced mix; DMI = DM intake; ECM = Energy −corrected milk; $CO_{2\text{-eq}}$ = Carbon dioxide equivalent.
[†] Significant effect of days in milk.
[††] Back-transformed from log-transformed values (antilog scale) for interpretability.
[1] Greatest SEM value obtained.
[2] Total number of successful visits per cow during the entire experiment (weeks 1–7).
[3] DM intake used in methane and carbon dioxide yield calculations.
[4] Milk yield during the entire experimental period.
[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$ after adjustment for multiple testing using Tukey's procedure.

found no effect of diet on DMI in Holstein dairy cows postpeak lactation (150 days in milk). However, a decrease in DMI was observed in cows fed the by-product-based diet during late lactation (231 days in milk). In our study, the apparent total tract digestibility of DM, organic matter and aNDFom differed between the CON, BYP and DOM diets, where the BYP treatment group showed reduced digestibility of all the mentioned parameters. By-products, in general, vary in chemical composition, and by-products used in ruminant diets may, at large, be based on fibrous feeds or legume crops (Halmemies-Beauchet-Filleau et al., 2018). The BYP diet resulted in a higher intake of iNDF compared to CON and DOM, which could explain the lower digestibility of DM, OM, and aNDFom observed in BYP compared to CON and DOM. Similarly, Guinguina et al. (2021) observed decreases in DM, organic matter and NDF digestibility and no treatment effect on CP digestibility for diets based on sugar beet pulp, wheat middlings, barley fibre and wheat bran compared with cereal-based diets. Also, Karlsson et al. (2018a) observed decreased OMD for by-product-based diets composed mainly of sugar beet fibre, dried distillers' grains with solubles and rapeseed meal, compared to a cereal-based diet.

The similar DMI and OMD levels observed between CON and DOM indicate that domestically produced ingredients such as cereals and field beans can successfully replace maize kernels and heat-treated rapeseed meal without a negative response in performance. This finding is in agreement with previous studies that

Table 6
Effect of control, by-product-based and domestic concentrate diets on intake and apparent total-tract digestibility in Swedish Holstein cows.

Item	Diet			SEM[1]	*P*-value
	CON	BYP	DOM		
Number of cows	16	15	15		
Intake					
Organic matter (kg/day)	22.1	22.5	21.5	0.08	0.605
aNDFom (kg/day)[††]	7.41^{b}	8.58^{a}	7.69^{ab}	0.309	0.006
iNDF (kg/day)	1.16^{b}	1.47^{a}	1.16^{b}	0.045	<0.001
CP (kg/day)	4.19	4.38	4.12	0.153	0.447
RDP (kg/d)[†† †††]	2.80^{b}	3.29^{a}	2.97^{b}	0.094	<0.001
Starch (kg/day)[†]	4.62^{a}	3.81^{b}	3.87^{b}	0.131	<0.001
Ether extract (kg/day)	1.11^{a}	0.95^{b}	0.95^{b}	0.035	<0.001
Net energy lactation (MJ/day)[††††]	174	168	165	5.12	0.371
Net energy balance (%)[††††]	102.2	100.3	99.6	2.54	0.718
Apparent digestibility (%)					
DM	66.8^{a}	63.3^{b}	66.7^{a}	0.61	<0.001
Organic matter	68.2^{a}	64.7^{b}	68.2^{a}	0.58	<0.001
aNDFom	60.0^{b}	56.5^{c}	62.9^{a}	0.75	<0.001
CP	59.4	59.2	60.1	0.84	0.699

Abbreviations: CON = Silage plus control mix; BYP = Silage plus by-product based mix; DOM = Silage plus domestically produced mix; aNDFom = amylase NDF organic matter; iNDF = indigestible NDF; RDP = Rumen degradable protein.
[†] Significant effect of days in milk.
[††] Back-transformed from log-transformed values (antilog scale) for interpretability.
[†††] Calculated in IndividRAM software (Växa, 2008), according to NorFor (2011), based on feed intake and dairy ration composition during the entire experiment (weeks 1–7).
[††††] Calculated in IndividRAM software (Växa, 2008), according to NorFor (2011), based on feed intake, dairy ration composition and energy-corrected milk production on weeks 1, 4 and 7.
[1] Greatest SEM value obtained.
[a,b,c] Values within a row with different superscripts differ significantly at $P < 0.05$ after adjustment for multiple testing using Tukey's procedure.

investigated the effect of replacing rapeseed meal (Räisänen et al., 2023) or soybean meal (Cherif et al., 2018; Johnston et al., 2019) with field beans. The higher aNDFom digestibility in DOM could result from the higher inclusion of ingredients with high content of potentially degradable NDF, such as sugarbeet pulp and barley (NorFor, 2011). Milk production

Milk yield measured during the entire experiment was lower for the cows receiving the BYP diet compared with CON cows, while no differences were observed for the DOM group compared with the other two groups. The pattern was similar on sampling days, with the lowest numerical yield observed in the BYP group, but no statistical difference was observed between the treatments. Both parameters are presented to maintain transparency and inform about the milk yield on the specific days selected for sampling and analysis of milk composition. The difference in milk production during the entire experiment can be attributed to the lower OMD observed in BYP compared to CON and DOM, since DMI levels were similar between treatments. Incorporating by-products in dairy cow diets poses challenges due to variations in the chemical composition of available by-products, leading to inconsistent effects on DMI and milk yield (Pang et al., 2018; Takiya et al., 2019; Guinguina et al., 2021). This variation was evident in this study's larger SD values for BYP concentrate composition. The higher milk fat content in the BYP group compared to the CON group can be attributed to the higher aNDFom intake, which acts as a lipogenic nutrient (Van Knegsel et al., 2007). Other feed trials examining the production response of dairy cows in mid to late lactation have reported similar effects of by-product-based versus cereal-based concentrates on production performance. For instance, Ertl et al. (2016) replaced cereal grains and pulses with a mixture of wheat bran and sugar beet pulp without any adverse effects on ECM yield or milk composition. Karlsson et al. (2018a) observed no adverse effects on ECM yield but higher milk fat content when cereal grains and soybean meal were substituted by a combination of sugar beet pulp, dried distillers' grains with solubles, and rapeseed meal. Guinguina et al. (2021) replaced cereal-based concentrates with by-product-based diets for dairy cows in early lactation, observing no reductions in milk yield or alterations in milk composition.

Milk protein content and milk protein yields were similar among treatments. However, MUN levels were higher for animals in BYP and DOM compared to CON. Dietary CP content is the primary nutritional factor influencing MUN (Nousiainen et al., 2004) and did not differ among diets. The increased MUN levels could, thus, indicate differences in protein quality among treatments. Higher amounts of soluble CP and higher CP degradation rates in the rumen are expected to impact the ability of rumen microbes to fully utilise the produced ammonia (Hof et al., 1997; Nocek and Russell, 1988). The main protein source in the CON concentrate pellet was heat-treated rapeseed meal, which resulted in the lowest MUN values. On the contrary, BYP and DOM concentrate pellets consisted mainly of ingredients with high levels of rapidly available CP and high overall ruminal CP availability. Specifically, based on their CP content and inclusion levels, wheat middlings and barley constitute approximately 60% of BYP concentrate CP content. The difference in MUN levels between CON and BYP can thus be attributed to the higher intake of rumen-degradable protein by the BYP group. Field beans and barley constitute approximately 45% of the DOM concentrate CP, partially replacing the heat-treated rapeseed meal in the DOM pelleted concentrate. Compared with CON, the increased MUN levels in the DOM group agree with previous studies' findings, where field beans replaced rapeseed expeller (Räisänen et al., 2023). Furthermore, Puhakka et al. (2016) found that MUN levels tend to increase as rapeseed meal is replaced by field beans on dairy rations with high CP levels. Despite the differences between the treatments, MUN levels from CON and DOM cows were within the acceptable range (9.0–14.0 mg/100 ml) identified by Sawa et al. (2011), while BYP cows had slightly higher levels. Increased MUN levels could indicate decreased protein use efficiency and higher urinary nitrogen excretion.

Greenhouse gas emissions

Feeding the different concentrate mixtures did not result in differences in CH_4 production (g/day), yield (g/kg DMI), or intensity (g/kg milk or ECM) despite the lower milk yield in the BYP group. This agrees with previous findings of no difference in CH_4 production, yield or intensity between cereal-based and by-product-based diets with similar ingredients as the one used in the current experiment containing sugar beet pulp, wheat bran, rapeseed meal, dried distillers' grains with solubles, palm kernel expeller and molasses (Pang et al., 2018). The replacement of soybean meal with field beans in dairy rations has also resulted in no differences in CH_4 production, yield or intensity (Cherif et al., 2018; Johnston et al., 2019). In contrast to our results, Guinguina et al. (2021) reported decreased CH_4 production (g/d) and a lower amount of CH_4 yield (g/kg DMI) for grass-legume mixture silage-based diets containing unmolassed beet pulp, wheat middlings, barley fibre and wheat fibre compared with diets containing barley, oat and wheat grains.

Production of enteric CH_4 is mainly correlated with DMI (Mills et al., 2003; Yan et al., 2006; Ramin and Huhtanen, 2013; Beauchemin et al., 2022). The similar levels of CH_4 production observed for the CON, BYP and DOM diets were mainly due to the lack of differences in DMI between cows in these treatments. Other dietary parameters, such as OMD and NDF, fatty acid and CP intake (Nielsen et al., 2013; Niu et al., 2021; Donadia et al., 2023), as well as animal parameters such as BW and milk yield (Yan et al., 2006; Donadia et al., 2023), also influence CH_4 yield. No difference was observed in enteric CH_4 production or yield despite the difference in OMD and intakes of NDF and ether extract among treatments. The enteric CH_4 emissions were comparatively low in terms of production, yield and intensity relative to other studies (Pang et al.,2018; Karlsson et al., 2019; Guinguina et al., 2021). This outcome may be attributed to the higher observed DMI, the lower observed apparent total tract digestibility, the inferred faster passage rate and differences in the dietary fat content (Patra, 2013). Furthermore, the forage inclusion was lower in this experiment (45%) compared to the aforementioned studies (59–62%), which could also explain the lower enteric CH_4 production (Aguerre et al., 2011).

The higher aNDFom in the BYP group and the lower starch and ether extract intake in the BYP and DOM group did not affect CH_4 yield. However, higher CH_4 yield values in the BYP and DOM group may have been expected, at least because of the lower starch concentration, since rapidly fermentable starch increases propionate production. Propionate production serves as an alternative metabolic hydrogen sink to methanogenesis (Nielsen et al., 2013; Niu et al., 2021; Beauchemin et al., 2022). In the present study, the ether extract concentration was below 5% in all diets, and the difference between the diets was not large enough to result in a significant effect on CH_4 production. The lack of treatment effect on the CH_4/CO_2 ratio indicates no difference in the efficiency of microbial fermentation of the feed or metabolisable energy utilisation (Madsen et al., 2010).

Increased dietary inclusion of vegetable oils is often proposed as an enteric CH_4 mitigation strategy (Nielsen et al., 2013; Niu et al., 2021; Beauchemin et al., 2022; Donadia et al., 2023); however, their efficacy is influenced by several factors such as source, quantity, degree of saturation and carbon chain length of the fatty acids (Beauchemin et al., 2022). Vegetable oils rich in C16:0, such as those in the CON diet, are commonly included in dairy cow rations to enhance milk fat production. However, these vegetable oils are mainly derived from palm or palm kernel, leading to long transport distances and a high carbon footprint (GFLI, 2019; RKFS, 2021). This raises concerns about potential trade-offs, including natural habitats, peatland drainage, biodiversity loss and increased risk of forest fires (Meijaard et al., 2020). To address these challenges, the BYP and DOM diets used crushed rapeseed, which has a lower carbon footprint per kilogram and can be sourced domestically or from other European countries. In this study, ECM yield and CH_4 production were similar across treatments, while BYP and DOM had lower Feed $CO_{2\text{-eq}}$ values. This suggests that using vegetable fat in dairy rations involves uncertainties and trade-offs, and their production benefits must be weighed against their carbon footprint. Selecting fat sources with a lower carbon footprint and shorter supply chains could be one step towards more sustainable dairy production.

A significant practical challenge faced during this study was the reluctance of the animals to visit the GreenFeed unit. A plausible explanation is that the animals received up to 7 kg/day of concentrate feed from the automatic milking station, so the maximum intake of pellets from the GreenFeed unit (1 200 g/day) may have been insufficient attraction (mean pellet DMI per animal 363 g/d). We compensated for the reluctance of the animals to visit the unit by using a cut-off point of 20 successful visits per animal. The use of a low cut-off point might have resulted in increased residual variance for daily CH_4 for the animals with fewer visits (Arthur et al., 2017; Dressler et al., 2023), but allowed us to consider more data points in our analysis. Using a cut-off point of 30 successful visits would have resulted in excluding two animals from the BYP group and one animal from the DOM group. This would result in 59 ± 20 and 64 ± 16 successful visits (mean ± SD) for BYP and DOM, respectively, while enteric CH_4 production values would be 373 and 399 ± 21.1 g/day (estimated marginal mean ± SEM) for BYP and DOM, respectively.

The results of this study, in which dairy cow diets were optimised based on greenhouse gas emissions from the production of feed ingredients, highlight the importance of diet formulation for the environmental sustainability of dairy production. It is especially relevant when feeding strategies and the inclusion of certain ingredients are adjusted in order to mitigate enteric CH_4 emissions, as specific choices can result in trade-offs. Feed primary $CO_{2\text{-eq}}$ emissions and feed primary $CO_{2\text{-eq}}$ per kg ECM were lower for the BYP and DOM diets compared with CON, while feed primary $CO_{2\text{-eq}}$ per kg milk was lower only for the BYP diet compared with CON. We did not observe any differences in CH_4 production and CH_4 yield or intensity, and can thus conclude that the BYP and DOM diets outperformed CON in terms of carbon footprint when $CO_{2\text{-eq}}$ from feed production and enteric CH_4 are considered. Further research can incorporate the greenhouse gas contribution of ingredient transportation and manure management, providing a more nuanced comparison of the treatments. These results suggest that high-yielding milk production systems can be maintained even with high dependence on by-products and domestic feeds without compromising milk production or increasing greenhouse gas emissions from feed and enteric CH_4.

Limitations of this study

It is important to note that this study focused on the environmental sustainability of dairy production, focusing only on greenhouse gas emissions from feed production and direct emissions from animals. The calculations exclude emissions that occur during feed transport, processing, manure storage and handling. The significance of these emissions may vary based on factors such as transport methods, the use of renewable energy, technologies and geographical location (Henriksson et al., 2014). Specifically, the environmental impact of feed transport is influenced by the transportation methods and the length of the supply chain (Mogensen et al., 2014). For instance, emissions from short transportation distances (e.g. 100 km), such as those anticipated for the DOM diet, contribute less than 1% of the dairy ration carbon footprint. However, for long-distance transportation (e.g. 300 km), these emissions increase to approximately 3% of the dairy ration carbon footprint (Henriksson et al., 2014). Considering that the ingredients of BYP and DOM are sourced either domestically or from Northern Europe, the additional transportation emissions are expected to have a minor impact on the comparison among treatments. Emissions occurring during manure management and storage are mainly in the form of CH_4 and N_2O, and the magnitude of emissions is dependent on, e.g. storage system, temperature and cover (Kupper et al., 2020). A life cycle assessment may be used in a complementary study to make a comprehensive sustainability evaluation. The experimental diets are relevant for intensive dairy production in Scandinavia and northern Europe. However, diet composition varies across countries and regions due to factors such as ingredient quality and availability, climate conditions, soil type and infrastructure. The diets in this study were formulated for high milk yield, requiring high proportions of concentrate and using first-cut grass-legume mixture silage. The scenario may differ in practical dairy farming, e.g. late lactation animals may receive a mix of second- and third-cut silage.

The carbon footprint of feed ingredients, determined through economic allocation, is susceptible to price fluctuations and market conditions (Ardente and Cellura, 2012). Furthermore, changes in industrial processes that alter the feed value may have an impact on production and CH_4 emissions. Adoption of DOM or BYP diets on a large scale might result in challenges of resource availability and price fluctuations, which, in turn, affect the results of economic allocation. We assumed a marginal effect of our diets on the food system, but exploring these changes could be the focus of future modelling studies.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101544) can be found at the foot of the online page, in the Appendix section.

Ethics approval

The experiment complied with Swedish regulations and the guidelines set by EU Directive 2010/63/EU (European Union, 2010) on animal research. The procedures reported here were conducted with the approval of the Gothenburg Research Animal Ethics Committee (Dnr 5.8.18-14151/2022).

Data and model availability statement

None of the data were deposited in an official repository. The data and the model used are available upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors utilised OpenAI ChatGPT4 for grammar and spelling checks. The authors have reviewed and edited the content as needed and took full responsibility for the content of the publication.

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Declaration of interest

None.

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