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Partial replacement of soybean meal with soybean silage in lactating dairy cows diet: part 1, milk production, digestibility, and N balance

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ABSTRACT

The high reliance of the European livestock sector on imported soybean meal (SBM), especially from South America, poses environmental problems, like greenhouse gas emissions for transportation and land-use change with the loss of carbon stock and biodiversity. Aim of the present study was to evaluate the partial substitution of SBM with whole-plant soybean silage in the diet of dairy cows. Thirty-six lactating Holstein cows were arranged according to a change-over design, with 2 weeks of adaptation and 5 days of sampling per period. A control diet (CON) was based on maize silage and SBM, representing 10.7% of total dry matter (DM). In a soybean silage diet (SBS) 35% (on DM basis) of SBM was replaced by soybean silage. The dietary treatment did not affect DM intake, milk production, and dairy efficiency while cows fed SBS resulted in lower milk crude protein (3.43 vs. 3.55%, $p < .001$) and higher milk urea (30.5 vs. 28.7 mg/dL, $p = .002$), in comparison with CON. Nutrients digestibility was lower for SBS than CON; particularly fibre digestibility was 31.5 vs. 38.8% ($p < .001$). The efficiency of nitrogen utilisation was higher for CON than SBS (32.7 vs. 31.3%, $p = .003$). Soybean silage did not penalise feed intake and milk production. However, to fully exploit this forage, digestibility, and nitrogen utilisation efficiency should be improved.

HIGHLIGHTS

- Soybean silage can substitute one-third of soybean meal in dairy cow diet
- Soybean silage inclusion in the diet did not affect milk yield and DMI
- Soybean silage inclusion in the diet reduced N use efficiency

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Introduction

Soybean meal (SBM) is the main protein feed source used in the EU (European Commission 2020). The reasons for its popularity are the high crude protein (CP) concentration (up to 53.8% of the DM, as reported by NRC (National Research Council) 2001 for decorticated soybean meal), the optimal amino acid profile, and, in particular, the high content of Lysine (6.29% of total CP; NRC (National Research Council) 2001). In the period 2019-2020 in the EU, 29.2 million tonnes of SBM were used as feed, and 97% of this amount was not produced in the EU. The data updated to April 2019 showed that the USA (36%) and Brazil (34%) are the main exporters of SBM to Europe (European Commission 2020). In Italy, SBM is among the most economically convenient protein sources on the market (granariamilano.org). It is by far the most used meal from oilseed, accounting for 76% of total oilseed meals in 2019; regarding the supply, 50% SBM used in Italy in 2019 was imported, and 33.8% was produced locally from imported seeds (ASSALZOO 2020).

In Brazil, significant areas of the Amazon forest and the *cerrado* have been cleared to increase the arable land needed for this crop. This was linked with the problem of land-use change and the related loss of biodiversity and carbon stock (Bickel and Dros 2003), causing a high environmental cost linked to the production of soybean. Transportation represents another source of greenhouse gases related to Brazilian SBM use, not only overseas but also within Brazil, due to the predominance of road transportation (Prudêncio da Silva et al. 2010).

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For these reasons, there is a need to reduce the inclusion of imported SBM in dairy cattle diets by finding alternative protein sources (Wilkinson and Young 2020). With this regard, self-produced whole-plant soybean silage could represent an alternative source to SBM. Compared to SBM (NRC (National Research Council) 2001), CP and RUP concentrations of soybean silage are lower (CP is around 20% of DM), but this silage is also a source of energy, in the form of NDF (45%) and, mostly, EE (up to 8% at maturity stage R7-8) (Tabacco and Comino 2019). Furthermore, from an agronomic and environmental standpoint, on farm cropping of grain legumes, like soybean, provides several benefits (Stagnari et al. 2017; Watson et al. 2017). Compared to lucerne or other perennial legumes, soybean has the advantage that the soil is not occupied over multiple years (Seiter et al. 2004), and for this reason, this crop can be inserted in a flexible rotation with maize silage. Despite the low concentration of water-soluble carbohydrates, and the high content of protein, oil, and ash, which could increase the buffering capacity, soybean can be well preserved as silage (Mustafa and Seguin 2003).

To the best of our knowledge, only a few studies determined the nutritive value of whole-plant soybean silage. Beside the above mentioned work of Mustafa and Seguin (2003), the *in vitro* study of Spanghero et al. (2015) found higher CP, EE, and NDF digestibility (NDFD) with advancing plant maturity (i.e. from R4 to R6).

As far as we know, three *in vivo* studies conducted outside Europe and one in an Italian commercial farm tested whole-plant soybean silage in the ration of dairy cows. In Vargas-Bello-Pérez et al. (2008), soybean silage was used in substitution of lucerne silage, while in Ghizzi et al. (2020) and Silva et al. (2021), in substitution of maize silage. In all of these works, DMI was reduced by the inclusion of soybean silage, probably due to lower NDFD leading to lower milk yield in Vargas-Bello-Pérez et al. (2008) and Ghizzi et al. (2020). In Silva et al. (2021), digestibility was not affected by the treatment and thus milk yield was not reduced. In contrast, in the study conducted in Italy (Tabacco and Comino 2019), soybean silage partially replaced SBM and cotton seeds, leading to increased DMI but had no effect on fat-corrected milk production. Regarding N balance, Ghizzi et al. (2020) found lower milk protein and Silva et al. (2021) lower N intake for the soybean silage diets.

Low NDFD and N use efficiency appear to be possible weak points of feeding soybean silage to dairy cows. On the other hand, reduction of SBM in the ration of high-yielding dairy cows seems to be feasible and not penalising for production, as found by Gislon et al. (2020). We hypothesised that a reduction of about 35% of SBM could lead to the same lactation performances of a control diet based on SBM as the main protein source.

Given these considerations, the aim of this study was to evaluate the effects of partial replacement of SBM with self-produced whole-plant soybean silage on DMI, milk yield, digestibility, and nitrogen balance.

Materials and methods

Silage preparation

This experiment was conducted in the experimental farm, Angelo Menozzi, of the University of Milan (Italy), located in Landriano (Pavia, Italy). Soybean (hybrid Buenos, class 1+; Limagrain Italy, Parma, Italy) was sown on 3 June 2018, in a medium consistency soil. The crop was harvested and chopped to a theoretical length of cut of 1.7 cm, on 27 September 2018, when the dry matter content of the crop was 26.2%, at the R6 stage (as described by Fehr et al. 1971). The silage was stored in silo tube bags for 50 days.

Two days prior to harvest, 5 plots of 1 m^2 were hand-harvested and from each plot a subsample of whole-plants was kept while the other plants were divided into stalks, leaves, pods, and seeds.

Lactation trial

Thirty-six Holstein cows (initial DIM = 159 ± 45.0 d, initial BW = 632 ± 75.0 kg, parity = 1.80 ± 0.90) were housed in a free-stall barn with cubicles and with free access to drinking water. Cows were fed once per day at 1000, and the TMR was pushed towards the cows several times per day. Each cow was milked twice per day, at 0900 and 2000 hours.

According to DIM and milk production at the beginning of the experiment, cows were divided into two groups, arranged in a change-over design. Two weeks for adaptation to the experimental diets were followed by five days of sampling collection for each of the two periods of the experiment; after the first period, cows had nine days of transition to the new diet.

The two experimental diets were formulated using the CNCPS model (version 6.5; Cornell University, Ithaca, NY) to meet the nutrient requirements of the lactating cows at the beginning of the experiment. The control diet (CON) included 10.7% SBM on DM (Table 1). The soybean silage diet (SBS) was

Table 1. Composition of a diet with partial substitution of soybean meal with soybean silage (SBS) and the control diet with soybean meal (CON) (% of DM).

Item	SBS	CON
High moisture maize	15.6	0.00
Soybean silage	12.4	0.00
Barley silage	10.2	10.3
Lucerne hay	9.43	9.52
Maize silage	9.22	25.5
Maize meal	8.72	16.4
Flaked maize grain	7.27	7.31
Soybean meal	6.91	10.7
Sunflower meal	4.94	4.97
Molasses cane	4.81	4.83
Barley grain	2.79	2.80
Wheat straw	1.95	1.95
Maize gluten feed	1.51	1.52
Wheat shorts	1.20	1.21
Wheat middlings	1.05	1.06
Sodium bicarbonate	0.76	0.76
Soybean oil	0.62	0.62
Calcium carbonate	0.31	0.31
White salt	0.11	0.11
Minvit[a]	0.09	0.09
Magnesium oxide	0.05	0.05

[a]Each kg contained: 31 g Fe, 70.5 g Zn, 30.4 g Mn, 100 mg Se, 2 g I, 60 mg Mo, 6.9 g Cu, 500 mg beta carotene, 4,000,000 U Vitamin A, 800,000 U Vitamin D3, 20,500 U Vitamin E, 2450 U Vitamin B1, 343 U Vitamin B6, 20 U Vitamin B12, and 52,000 U Vitamin PP.

characterised by the inclusion of 12.4% soybean silage on total ration DM and 6.91% SBM. Maize silage, high moisture maize, and maize meal were included in the two diets in different amounts in order to provide the same concentrations of NDF and starch (respectively 31.5 and 28.6% on average) (Table 2).

Samples collection

Samples of TMRs and the ingredients were collected three times in each experimental period. Spot samples of urine and faeces were collected twice, on day 1 and 5 of each experimental period, seven h after feeding. One aliquot of urine (10 ml) was added with 4.078 N sulphuric acid in the ratio of 20:1 (*vol/vol*) for the analysis of N concentration, while a second aliquot (10 ml) with 0.072 N sulphuric acid in a ratio of 1:4 (*vol/vol*), for the analysis of creatinine and purine derivatives (PD) concentrations. At the beginning and at the end of each experimental period, BW was recorded using a digital scale (with 4 weight sensors SB1, from PTM, Brescia, Italy). Individual daily milk production was electronically recorded during the two experimental weeks. A milk sample for every cow was collected on days 1, 3 and 5 of each experimental week in both morning and evening milking, with 2-bromo-2-nitro-1,3-propanediol as a preservative and stored at 4 °C before analysis. Milk samples were analysed for protein, fat, lactose, urea, casein, SNF, acetone and BHB content using a Fourier transform infrared (FTIR) analyser (MilkoScan FT6000; Foss Analytical A/S), while somatic cell count was carried out with differential count (Fossomatic™ 7; Foss A/S, Hillerod, Denmark). Fat and protein corrected milk (FPCM) was calculated according to NRC (National Research Council) (2001).

Table 2. Chemical analysis of a diet with partial substitution of soybean meal with soybean silage (SBS), the control diet with soybean meal (CON), soybean silage and maize silage used in these diets.

	Soybean silage	Maize silage	SBS	CON
DM, % AF	24.5	33.1	48.9	49.6
Ash, % of DM	9.00	4.33	6.38	5.90
OM, % of DM	91.0	95.7	93.6	94.1
CP, % of DM	23.0	8.03	15.2	14.7
Sol CP, % of CP	61.1	63.1	29.8	21.6
EE, % of DM	7.28	3.17	3.33	2.72
NDF, % of DM	43.2	44.7	30.4	30.6
ADF, % of DM	33.3	25.5	18.9	18.8
ADL, % of DM	7.09	3.63	3.89	3.81
NDIP, % of DM	1.84	1.35	1.34	1.32
ADIP, % of DM	1.76	1.14	0.96	1.03
NFC, % of DM	17.5	39.8	44.8	46.0
Lactic acid, %	5.27	8.16		
Acetic acid, %	0.88	1.92		
Butyric acid, %	0.28	0.33		
pH	5.30	3.81		
N–NH_3, % of total N	11.2	7.10	13.9	15.1

DM: Dry matter; AF: As fed; CP: crude protein; EE: Ether extract; ADF:acid detergent fibre; ADL: Acid detergent lignin; NDF: neutral detergent fibre; NDIP: neutral detergent insoluble protein; ADIP: acid detergent insoluble protein; NFC: Nonfibrous carbohydrate.

Individual DMI estimation

Individual DMI was estimated according to equation 11 of Dórea et al. (2017). This equation considers the ratio of allantoin : creatinine in the urine, BW, and milk yield.

Urine allantoin was measured through the method of Chen and Gomes (1992), using Biochrom Libra S11 Visible Spectrophotometer (Biochrom Ltd., Cambridge, United Kingdom); urines creatinine was measured using ILab Aries (Werfen, Milan, Italy) (Jaffé 1886).

Total urine output was estimated according to Valadares et al. (1999), using creatinine concentration in spot urine sample and considering a daily creatinine excretion rate of 29 g/kg of BW. A correction factor of 0.667 was used to take into account the diurnal variation of creatinine in the spot samples, according to the work of Lee et al. (2019).

Chemical analyses

All samples were stored at −20 °C. Before analysis, samples of feeds and faeces were thawed and oven-dried at 55 °C until constant weight and ground through a 1-mm screen (Pulverisette 19, Fritsch, Idar-Oberstein, Germany).

Feeds and faeces were analysed for the concentrations of DM, ash, ether extract, starch (AOAC International 1995, numbers 945.15, 942.05, 920.29, and 996.11, respectively), NDF corrected for insoluble ash and with the addition of α-amylase (aNDFom; Mertens et al. 2002), ADF and ADL (Van Soest et al. 1991), using the Ankom 200 fibre apparatus (Ankom Technology Corp., Fairport, NY). All samples, including urines, were analysed for CP (N × 6.25) (AOAC International 1995, numbers 990.03) using Rapid MAX N Exceed Elementar (Elementar Analysensysteme GmbH, Germany). For the two diets and the two main forages, protein fractions were estimated according to CNCPS method (Sniffen et al. 1992), following the analysis procedure of Licitra et al. (1996). A silage sample was divided into 2 subsamples. The first subsample was extracted for pH determination using a Stomacher blender (Seward Ltd., Worthing, United Kingdom) for 4 min in distilled water at a 9:1 water-to-sample material (fresh weight) ratio. The second subsample was extracted using a Stomacher blender for 4 min in 0.05 M sulphuric acid (H_2SO_4) at a 5:1 acid-to-sample material (fresh weight) ratio. A 40-mL aliquot of silage acid extract was filtered with a 0.20-μm syringe filter and used to quantify the fermentation products. Lactic, acetic, propionic, and butyric acids were determined by means of HPLC in the acid extract (Canale et al. 1984). Kjeldahl method was used for the determination of $N\text{-}NH_3$ of the silages.

In vitro *analyses*

Four *in vitro* incubation were conducted:

1. A 48 h incubation using glass syringes to determine the net energy for lactation (NEl) of whole-plant and separated plant components
2. A 48 h incubation using DaisyII incubator jars to determine NDFD of whole-plant and separated plant components
3. A 120 h incubation using a fully automated system (Gas Endeavour) to determine the kinetic of fibre fermentation of soybean and maize silages
4. A 288 h incubation using DaisyII incubator jars to determine uNDF of faeces and TMRs

NEl was estimated according to the gas production (GP) method of Menke and Steingass (1988), working with three replicates per sample, correcting for standards and blank (i.e. syringe without sample). Rumen fluid was collected from three fistulated dry Italian Friesian cows fed a diet composed of meadow hay, maize silage, ryegrass hay, SBM, maize meal, and mineral and vitamin integration (676, 96, 77, 70, 54 and 25 g/kg DM, respectively). The donor animals were handled as outlined by the Directive 2010/63/EU on animal welfare for experimental animals, according to the University of Milan Welfare Organism (OPBA) and with authorisation number 904/2016-PR from the Italian Ministry of Health. The cows were fed the TMR twice daily (0700 and 1900 hours) to achieve a DMI of 8 kg/d. Rumen liquor was collected two h after the morning feeding. The incubation was run in 100-mL glass syringes (Haberle Labortechnik, Germany), according to Menke and Steingass (1988). Equation 12b (Menke and Steingass 1988) was used to estimate NE_l for seeds, while equation 12c was used for the other components and the whole-plant.

In the second incubation, NDFD was evaluated at 48 h *in vitro* incubation using the DaisyII incubator jars (Ankom Technology, Macedon, NY, USA). For each sample, 0.500 g was weighted, with three replicates per sample, in F57 bags. The bags were pre-treated, washing them with NDS and α-amylase before being incubated, according to Battelli et al. (2020). Each jars of DaisyII incubator contained standards and blanks (i.e. bags without sample). Rumen fluid and the fistulated cows were treated as explained above. The buffer was composed by two solutions as reported by Ankom protocol. The inoculum was mixed with the buffer in a ratio of 450 g/L, for a total of 1.6 L, while rumen fluid was added at a dose of 400 mL/jar, using a 1:4 ratio with the buffer into each pre-warmed (39 °C) jar. After 48 h of incubation, jars were emptied and the F57 bags were rinsed thoroughly with cold tap water and analysed for aNDFom content using the Ankom 200 fibre analyser.

The kinetic of fibre fermentation of soybean silage and maize silage samples was assessed using a fully automated system (Gas Endeavour, Bioprocess Control AB, Lund, Sweden) for the real-time monitoring of GP in rumen fermentation batch processes. The substrates analysed consisted of 2 (±0.01) g of pure NDF residue, previously obtained treating the samples with neutral detergent solution using the Ankom 200 fibre analyser and filter bags (Sefar Petex® 12 × 6 cm; 15 μm pore size) with 3.75 g of sample. Three replicates per sample for each period were used. The incubation was run into 500 mL reactors and blanks in triplicate were also included. Particularly, the final incubation medium contained the buffer solution, prepared according to Menke and Steingass (1988), and rumen liquor in a 2:1

Table 3. Chemical analysis and nutritive value of whole-plant soybean and plant components.

	Pods	Leaves	Seeds	Stalks	Whole-plant	s.e.	*p*-Value
% of Whole-plant DM	15.2^{b}	15.7^{b}	34.1^{a}	35.0^{a}		1.80	<.001
DM, % AF	24.7^{c}	30.5^{b}	41.9^{a}	23.4^{c}	29.5^{b}	0.60	<.001
Ash, % DM	8.67^{b}	11.2^{a}	5.38^{d}	8.81^{b}	7.87^{c}	0.18	<.001
CP, % DM	12.0^{c}	20.8^{b}	37.6^{a}	9.09^{d}	20.7^{b}	0.54	<.001
EE, % DM	0.96^{d}	2.35^{c}	12.2^{a}	0.64^{d}	5.76^{b}	0.41	<.001
aNDFom, % DM	51.2^{b}	30.3^{d}	19.1^{e}	64.1^{a}	38.4^{c}	0.90	<.001
ADFom, % DM	39.0^{b}	16.2^{d}	12.8^{e}	52.2^{a}	29.3^{c}	0.78	<.001
ADL, % DM	6.63^{b}	4.28^{c}	0.25^{d}	10.5^{a}	4.72^{c}	0.34	<.001
NDFD, %NDF	38.0^{c}	55.0^{b}	92.5^{a}	21.3^{d}	38.6^{c}	2.05	<.001
NE_l^{f}, MJ/kg DM	4.70^{b}	4.95^{b}	7.22^{a}	3.21^{c}	5.13^{b}	0.22	<.001
Gas^{g} 6 h, ml	22.2^{a}	22.4^{a}	18.1^{b}	14.5^{c}	19.4^{ab}	1.16	<.001
Gas^{g} 24 h, ml	42.3^{a}	38.2^{ab}	40.7^{a}	26.3^{c}	34.7^{b}	2.04	<.001
Gas^{g} 48 h, ml	47.7^{a}	43.7^{ab}	44.9^{ab}	31.0^{c}	39.4^{b}	2.57	<.001

[a,b,c,d,e]Means in the same row with different superscripts are statistically different at $p<.05$

[f]NE_l: -1.04 + 0.1195 × Gas production + 0.0051 × CP + 0.0152 × EE, for seeds; NE_l: 0.81 + 0.0816 gas production + 0.0046 × CP + 0.0135 × EE, for pods, leaves, stalks, and whole-plant.

[g]Gas production from 200 mg DM.

DM: Dry matter; AF: As fed; CP: crude protein; EE: Ether extract; aNDFom: Amylase-treated, ash free NDF (neutral detergent fibre); ADFom: Ash free ADF (acid detergent fibre); ADL: Acid detergent lignin; NDFD: NDF (neutral detergent fibre) digestibility; NEl: Net energy of lactation; Gas: Gas production from 200 mg DM.

ratio, treating the rumen liquor as explained above. Each batch contained 300 mL of the medium, and was kept in continuous stirring at 39 °C. The incubation lasted 120 h, with continuous and automated measurement and registration of the gas produced, normalised at 0 °C and 101.3 kPa. Potential GP (pGP) (mL/g NDF) at time t was estimated following the model derived from that reported by McDonald (1981), as explained in the equation below:

$$pGP = b \times \left(1 - e^{-k_{GP} \times (t - l)}\right)$$

with b: potential GP (mL/g NDF); k_{GP}: GP rate (%/h); t: incubation time (h); l: lag phase (h).

The model for pGP kinetic was fitted to net gas volume data using the algorithm of Levenberg Marquardt employed in the NLIN procedure of SAS 9.4.

To assess digestibility of DM, OM, CP, and NDF (DMD, OMD, CPD, and NDFD, respectively), the undigested NDF (uNDF) of TMRs and faeces, estimated at 288 h in vitro incubation, was used as internal marker according to the following equations:

$$DMD = 100 - \left(100 \times \frac{\%uNDF\ in\ TMR}{\%uNDF\ in\ faeces}\right)$$

$$\text{NutrientD (OMD, CPD, and NDFD)} = 100 - \left(100 \times \frac{\%uNDF\ in\ TMR}{\%uNDF\ in\ faeces} \times \frac{\%Nutrient\ in\ faeces}{\%Nutrient\ in\ TMR}\right)$$

Faecal samples were pooled per period for each cow, and SBS and CON TMRs and the silages where pooled per period. In addition, sample of soybean silage and maize silage were incubated to determine their uNDF and potentially degradable NDF (pdNDF) at 288 h, according to the following equation:

$$pdNDF = 100 - \left(100 \times \frac{\%\ uNDF}{\%\ NDF}\right)$$

Incubations were conducted as explained above, using the DaisyII incubator jars.

Statistical analysis

Using the proc univariate procedure (normal option) of SAS 9.4, the Shapiro-Wilk normality test was used to determine whether or not the residuals were normally distributed. All of them resulted normally distributed ($p>.05$). The data collected were statistically analysed by the proc mixed procedure of SAS 9.4, with the following model:

$$Y_{ijklm} = \mu + SEQ_i + P_j + T_k + LACT_l + COW_m(SEQ_i) + \varepsilon_{ijklm}$$

where Y_{ijklm} is the dependent variable; μ is the overall mean; SEQ_i is the treatment sequence effect (i = 1, 2); P_j is the period effect (j = 1, 2); T_k is the treatment effect (k = 1, 2); $LACT_l$ is the number of lactation effect (l = 1, 7); COW_m is the random animal effect (m = 1, 36), and ε_{ijklm} is the residual error.

The data regarding the chemical analysis and the nutritive value of whole-plant and separated plant components were statistically analysed by the proc glm procedure of SAS 9.4, with the following model:

$$Y_{ij} = \mu + C_i + F_j + \varepsilon_{ij}$$

where Y_{ij} is the dependent variable; μ is the overall mean; C_i is the plant components effect (i = 1, 5); F_j is the field plot effect (j = 1, 5), and ε_{ij} is the residual error.

Table 4. Ruminal fermentation of NDF of soybean silage and maize silage.

	Soybean silage		Maize silage	
	Mean	S. D.	Mean	S. D.
Gas endeavour				
b^a, mL/g NDF	98.5	15.1	162	5.47
k_{GP}^b, %/h	4.74	0.36	3.01	0.18
l^c, h	1.41	0.72	3.50	0.98
DaisyII				
pdNDF 288 h, % NDF	54.1	0.49	83.5	0.81

[a]b: potential gas production.
[b]k_{GP}: gas production rate.
[c]l: lag phase.
pdNDF 288 h: Potentially degradable NDF (neutral detergent fibre) at 288 hours; S. D.: Standard Deviation.

Least squares means estimates are reported. For all statistical analyses, significance was declared at $p \leq .05$ and trends at $0.05 < p \leq 0.10$.

Results

Nutritive value of whole-plant soybean and plant components

The chemical composition of the soybean and maize silages used in the experiment is shown in Table 2. Compared to maize silage, the forage with the highest inclusion in CON diet, soybean silage had higher pH and lower concentration of lactic and acetic acid while butyric acid was low for both silages. Protein fractions A and B2 were higher for soybean silage than for maize silage.

Relative contribution to total DM, chemical analysis and nutritive value of whole-plant soybean and plant components at harvesting are reported in Table 3. More than 1/3 of soybean whole-plant DM was represented by the stalk and another 1/3 by the seeds. These two components were characterised by the lowest and the highest nutritive value. Stalks had the highest content (% DM) of NDF with the lowest NDFD (% of NDF) while seeds the lowest NDF concentration and the highest digestibility of NDF ($p < .001$). Seeds had the highest concentration (% DM) of CP followed by leaves; EE concentration (% DM) was highest ($p < .001$) for seeds as well, while it was much lower for the other components (in particular, below 1% for pods and stalks). Table 4 reports the data concerning ruminal fermentation of NDF of soybean and maize silages. Since the replicates were not independent, no statistical analysis was applied. The NDF of soybean silage was fermented faster than that of maize silage (k_{GP} of soybean silage was higher than maize silage) and the lag phase was shorter. However, the NDF of soybean silage was less fermentable because the potential GP (mL/g of NDF) was lower and this result was found also after 288 h of incubation (pdNDF).

Table 5. Intake, milk yield and composition of cows fed a diet with partial substitution of soybean meal with soybean silage (SBS) and the control diet with soybean meal (CON).

	SBS	CON	S.E.	*p*-Value
DMI, kg/d	23.8	23.6	0.511	.659
DMI (NRC)[a], kg/d	25.3	24.9	0.509	.263
Milk, kg/d	33.2	32.7	1.68	.377
FPCM[b], kg/d	34.7	34.2	1.48	.474
Dairy efficiency	1.40	1.39	0.053	.783
Fat, %	4.46	4.44	0.146	.806
Fat yield, kg/d	1.50	1.41	0.069	.024
Protein, %	3.43	3.55	0.060	<.001
Protein yield, kg/d	1.13	1.15	0.050	.378
Lactose, %	5.00	4.98	0.037	.261
Linear score	1.76	1.72	0.445	.825
Urea, mg/dL	30.5	28.7	0.743	.002
Casein, %	2.70	2.78	0.050	<.001
Casein, % of total N	78.7	78.4	0.221	.004
Acetone, mM	0.016	0.006	0.004	.008
BHB, mM	0.034	0.019	0.005	<.001
Live weight, kg	645	642	17.4	.444

[a]FPCM: Milk × (0.122 × fat + 0.072 × protein + 0.052 × lactose) (adapted from NRC (National Research Council) 2001).
[b]DMI (NRC): (0.372 × (fat corrected milk) + 0.0968 × (body weight) ^0.75) × (1 - EXP(-0.192 × ((week of lactation) + 3.67))) (adapted from NRC (National Research Council) 2001).
SE: Standard Error.

Dry matter intake and milk production

The partial substitution of SBM with soybean silage did not affect milk production or FPCM (Table 5). Milk production was, on average, 33.0 and 34.5 kg/d, respectively, for milk and FPCM, and both of them were not significantly different between the dietary treatments ($p = .377$ and $p = .474$, respectively). Also dairy efficiency and DMI, either estimated through the model of Dórea et al. (2017) or with the NRC (National Research Council) (2001) equation, were not affected by the treatment. Considering DMI estimated with the model of Dórea et al. (2017), the average values of dairy efficiency and DMI of the two treatments were 1.40 and 23.7 kg ($p = .783$ and $p = .659$, respectively). The fat yield was higher for cows fed SBS than CON ($p = .024$), but the treatment did not affect milk fat concentration ($p = .806$). The SBS diet resulted in lower milk protein concentration ($p < .001$) and higher milk urea than CON ($p = .002$). As for protein, milk casein concentration was higher for CON ($p < .001$) but, when expressed as a percentage of total N, was higher for SBS ($p = .004$).

Digestibility

The values of total tract digestibility of cows fed the two dietary treatments are reported in Table 6. The

Table 6. Total tract digestibility of a diet with partial substitution of soybean meal with soybean silage (SBS) and the control diet with soybean meal (CON).

	SBS	CON	S.E.	*p*-Value
DMD, % of DMI	65.2	68.6	0.491	<.001
OMD, % of OM intake	66.4	69.8	0.460	<.001
NDFD, % of NDF intake	31.5	38.8	0.776	<.001
CPD, % of CP intake	60.0	62.5	1.05	.065

DMD: DM (dry matter) digestibility;DMI: Dry matter intake; OMD: OM (organic matter) digestibility; NDFD: NDF (neutral detergent fibre) digestibility; CP D: CP (crude protein) digestibility.

Table 7. Nitrogen (N) balance of cows fed a diet with partial substitution of soybean meal with soybean silage (SBS), and the control diet with soybean meal (CONadd.

		SBS	CON	S.E.	*p*-Value
N intake	g/d	572	555	12.2	.098
N faeces	g/d	229	207	9.12	.013
	% of N intake	40.0	37.5	1.53	.065
N urines	g/d	183	158	8.24	.001
	% of N intake	32.3	28.9	1.42	.005
N milk	g/d	178	181	7.89	.378
	% of N intake	31.3	32.7	1.04	.003
N retained	g/d	−17.5	9.32	12.1	.006
	% of N intake	−3.53	0.92	2.19	.012

S.E.: Standard Error.

SBS resulted in lower digestibility ($p < .001$) than CON for DM, OM, and NDF. The cows fed CON diet had a tendential ($p = .065$) higher CP digestibility than SBS diet.

N balance

There was a tendency ($p = .098$) for higher N intake for cows fed SBS diet than CON (Table 7). The faecal N excretion was higher for SBS, but the difference was significant only when expressed in g/d ($p = .013$). The diet affected urinary N excretion when expressed both in g/d and as % of N intake ($p < .001$ and $p = .005$, respectively), with higher values for SBS than CON. Milk N excretion (g/d) was not different between treatments; however, N efficiency (N milk/N intake × 100) was higher for CON than SBS ($p = .003$). The soybean silage diet resulted in a higher N mobilisation than CON ($p = .006$ and $p = .011$, respectively, for g/d and percentage balance). On the opposite, cows fed CON stored N (Table 7).

Creatinine and PD

Urine volume, urine content of N (%), and creatinine, uric acid, and allantoin (mmol/L) were not affected by the treatment. The same result was found when the excretion of creatinine, uric acid, and allantoin was expressed in mmol/d (Table 8).

Table 8. Urine volume, protein and creatinine, uric acid and allantoin excretion from cows fed a diet with partial substitution of soybean meal with soybean silage (SBS) and the control diet with soybean meal (CON).

		SBS	CON	S.E.	*p*-Value
Urine	L/d	19.3	19.1	1.70	.912
Nitrogen	%	0.985	0.923	0.053	.179
Creatinine	mmol/L	6.04	6.57	0.445	.111
	mmol/d	110	110	2.98	.444
Uric acid	mmol/L	1.27	1.41	0.121	.180
	mmol/d	23.8	23.7	2.05	.965
Allantoin	mmol/L	17.4	19.5	1.11	.068
	mmol/d	325	334	19.0	.589

S.E.: Standard Error.

Discussion

Nutritive value of whole-plant soybean and plant components

The soybean silage used in the present study proved to be a good source of CP and EE. These two chemical parameters are higher than those of the soybean silage used in the experiments of Silva et al. (2021), Ghizzi et al. (2020), and Vargas-Bello-Pérez et al. (2008), where the focus was to evaluate its potential in substitution of another forage and not as a replacement of protein source. In the soybean silage used in the present study, NDIP and ADIP concentrations were lower in comparison with the CNCPS feed bank, (1.84% and 1.76% vs. 4.33% and 2.14% of DM in the CNCPS), while NH_3 was higher (11.2 vs. 8.6% of total N). In Silva et al. (2021), NDIP (2.63%) was lower than the CNCPS one as well. As expected, most of the protein in the soybean plant derived from the seeds; however, the leaves, with 20.8% of CP on DM, also contributed significantly to the total plant CP. Overall, the chemical composition of soybean components in the present study and that of R6 stage soybean silage in Spanghero et al. (2015) are very close to each other, except DM concentration, because in the present experiment soybean was ensiled without a preliminary wilting phase. However, NDFD resulted in being closer to the R5 stage soybean of Spanghero et al. (2015), especially for stalks (19.3% R5 and 8.2% R6 vs. 21.3% in the present study) and whole-plant (38.8% R5 and 46.5% R6 vs. 38.6% in the present study). Whole-plant NDFD was in line with what was found by Mustafa and Seguin (2003) for soybean harvested between R5 and R6 stage (35.5%), measured in situ up to 96 h incubation. Regarding gas production, the present data for whole-plant resulted higher than the study mentioned above for both 6 h and 24 h incubations.

Dry matter intake and milk yield

The lactation trial results demonstrated that soybean silage used as a self-produced protein source at the inclusion level of 12.4% of diet DM did not hamper milk production. Differently, the results reported by Ghizzi et al. (2020) (where soybean silage was included as 0, 8, 16, and 24% of diet total DM) and Vargas-Bello-Pérez et al. (2008) (inclusion was 36% of diet total DM), showed a reduction of milk yield with a reduction of DMI, due to a greater NDF concentration in the diet (Vargas-Bello-Pérez et al. 2008) or to greater intakes of longer feed particles and EE for soybean silage diets (Ghizzi et al. 2020). Intake was reduced in the study of Silva et al. (2021) (with soybean silage representing the 8% of diet DM), because of higher proportion of long particles (>19 mm) in the soybean silage diet; however, milk production was not affected. In the present study, the NDF values were 30.4% for SBS and 30.6% for CON while in Vargas-Bello-Pérez et al. (2008), NDF concentrations were 36.7% for the soybean silage diet and 34.1% in the lucerne control diet. The EE content of the diets of the present study was similar (3.33% of SBS and 2.72% of CON), while in Ghizzi et al. (2020), the EE of the experimental diets ranged between 3.33% and 4.45%. However, the higher EE concentration of soybean silage of the present study (7.28% on DM) may be responsible for the higher yield of milk fat of SBS treatment.

In the present study, it was not possible to determine the individual DMI gravimetrically. However, according to the conclusions of the meta-analysis of Dorea et al. (2017), PD can be used as an alternative method to estimate feed intake in dairy cattle in research trials, so individual DMI was estimated through it. This method takes into account factors that directly influence feed intake (i.e. fat corrected milk, body weight and the week of lactation). Moreover it considers the fact that the DM ingested in turn influences the microbial development and consequently the microbial protein yield with the associated urine PD (Dórea et al. 2017). The equation n. 11 (Dórea et al. 2017) was selected because of the lowest root mean squared error (0.49) and one of the highest R^2 (0.91) among the models evaluated for dairy cattle. However, being an estimation, the values obtained have to be considered with caution. The prediction of microbial protein yield might be improved also considering diet composition, rather than just DMI (Oldick et al. 1999). Secondly, the urinary recovery of duodenal purines, like allantoin, might affect the performance of the model used (González-Ronquillo et al. 2004).

Nevertheless, this method gave reasonable results if compared with DMI obtained through the NRC equation: for the two diets, the estimation through PD gave, on average, an intake 5.6% lower than that predicted by the NRC equation. In both estimations, DMI was not affected by the diet. By contrast, in the study of Tabacco and Comino (2019), the diet with the inclusion of soybean silage (8.7% on total DM) resulted in higher DM intake in comparison with control (23.2 vs. 22.3 kg/d). Creatinine, uric acid, and allantoin were not statistically different according to the dietary treatment. The daily production amounts of these metabolites were within the ranges found by Dórea et al. (2017), namely 96–208 mmol/d for creatinine, 5–118 mmol/d for uric acid, and 169–713 mmol/d for allantoin.

Milk quality

The main effect on milk quality is related to milk N compounds. Lower milk protein percentage and higher milk urea of the SBS diet can be associated with an unbalanced ratio of protein/energy provided with the diet (Oltner and Wiktorsson 1983). In particular, the rumen degradability of protein is high for legume silages, so, in order to incorporate more efficiently this dietary N into microbial protein, it is advisable to increase the concentration of readily fermentable carbohydrates, as found by Broderick (2003). High degradable N is also due to protein degradation during silage storing (Dewhurst et al. 2003). These results are in agreement with the findings of Ghizzi et al. (2020), where milk protein concentration decreased linearly with increasing inclusion levels of soybean silage, probably because of lower CPD, (from 75.7% with 0% soybean silage inclusion to 67.6% with 24% inclusion). However, the authors found that milk urea nitrogen (MUN) was numerically but not significantly higher. In Silva et al. (2021), no statistical differences were found for milk protein, milk protein yield, and MUN between the soybean silage and the maize silage diet. In contrast, Tabacco and Comino (2019) found higher milk protein for soybean silage (3.60 vs. 3.45%) and no difference in milk urea. However, the two diets of the above cited work had lower protein concentration (13.9% for control and 13.3% for the soybean silage diet). Moreover, the two experimental diets had high but very similar inclusions of maize silage and high moisture maize (on average for the two diets: 29.6 and 12.3% of total diet DM respectively). Higher N excretion through urine and higher, although not alarming, concentrations of acetone and BHB in the milk of the cows fed SBS also confirmed the insufficient energy provided by SBS. Vargas-Bello-Pérez et al. (2008) found higher MUN content with soybean silage too. However, higher MUN has to be taken into account when SBM is reduced into the diet in favour of other legume protein source (Volpelli et al. 2009a, 2009b). Nevertheless, protein yield was not affected by the treatment in the present study.

Digestibility

The cows fed the SBS diet had a lower total tract digestibility than CON. This result is in agreement with Ghizzi et al. (2020), who found decreasing values of DMD, OMD, and NDFD with increasing inclusion of soybean silage. By contrast, in Silva et al. (2021), the diet with the inclusion of soybean silage was not less digestible than the one based solely on maize silage as forage source; even NDFD was only numerically different despite low NDFD for soybean silage (27.4%). Moreover, in Ghizzi et al. (2020), soybean was harvested at stage R5.5 (with silage DM of 23.6%) while in the present study at R6 (24.5% DM), which should guarantee higher NDF and CP digestibility (Spanghero et al. 2015). However, the value of NDFD (31.5%) in the present study is very close to the value found by Vargas-Bello-Pérez et al. (2008) (31.2% for NDF ruminal digestibility) in a diet where soybean silage was included for 36% of total DM. As hypothesised by Ghizzi et al. (2020), poor NDFD was probably the main driver in reducing the overall DMD of SBS compared to CON. Tabacco and Comino (2019) found lower in vitro NDFD with advancing phenological stage (from 53.6% of R4-5 to 51.5% of R7-8) but associated with higher NE_l (from 6.11 to 6.51 MJ/kg DM); by contrast, Spanghero et al. (2015) reported an increase of NDFD with advancing phenological stage (from 31.9% of R4 to 46.5% of R6). A possible explanation can be given considering the different plant components, with lower NDFD found for stalks. Another option could be using lower size varieties. In Tabacco et al. (2018), the authors found higher NDFD for silages of a variety of soybean with low size plant compared to one with medium-tall size plant (51.6 vs. 46.4%, on average). This could be due to the lower contribution of stalks on total DM in favour of pods, as suggested by higher CP content of the low size plant (22.8 vs. 19.5%), and by the more lignified fibre of medium-tall size plant (lignin concentration was 8.7 vs. 6.4% of the low size variety). Low NDFD was confirmed also considering NDF fermentation kinetic. The values k_{GP} in the present study was lower than the k_d found by Silva et al. (2021) (4.74 vs. 6.74%/h), but in line with Vargas-Bello-Pérez et al. (2008) and Mustafa and Seguin (2003) (4.8 and 5.1%/h, respectively). In Mustafa and Seguin (2003), the lag phase was (0.1 h) shorter than the one found here (1.41 h). Regarding OMD, Silva et al. (2021) found a value for the soybean silage diet (69%) not far from the one reported here. Another key factor in improving the quality of whole-plant soybean silage is CPD, especially if the goal is to increase the farm protein self-sufficiency. In the work of Spanghero et al. (2015), CPD was improved by advanced maturity stage at harvest time (i.e. at R6), due to the higher protein accumulation in the pods, as supposed by the authors. It can be assumed that delaying the harvest of soybean in the present study would have improved CPD as well, because the present values of NDFD are closer to R5 than to R6 soybean of the work of Spanghero et al. (2015). In the work of Rigueira et al. (2015), digestibility of DM, NDF, CP, and NFC of a diet containing soybean silage for beef cattle was improved by treating chopped soybean with microbial inoculant and molasses before ensiling. The authors explained this result by a better fermentation of the treated silage, which led to lower losses of cellular content, more digestible than the cell wall components (fibre). In the present study, whole-plant soybean silage pH after 50 d storage was 5.30, very close to Vargas-Bello-Pérez et al. (2008) (5.29), but higher compared to the pH of soybean silage in the study of Rigueira et al. (2015) or in the study of Touno et al. (2014) (4.78). Even if this pH value may suggest poor fermentation of soybean silage in the present study, no sign of spoilage was detected and the NDF content was in line with Touno et al. (2014) (43.2 vs. 45.3% on DM) and lower than Rigueira et al. (2015) (52.4% on average).

Nitrogen balance

Cows fed SBS had a lower dietary N use efficiency compared to CON. This could be due to unbalanced ratio protein/energy and a numerically lower CPD. Cows fed SBS had higher N faecal excretion (in g/d) and N urine excretion (as % of N intake). Higher excretion of N is detrimental for the environment as well; however, it has to be taken into account a reduction of N coming from outside the farm gate due to a lower use of SBM. According to the estimation made by Wilkinson and Young (2020), 700,000 tonnes of N coming from imported SBM have been excreted by livestock in EU in 2018/2019. The negative, even if close to zero, N retained value for SBS caused the mobilisation of body reserve, without affecting milk production. Maybe this was because the cows were far from the lactation peak (159 DIM, on average at the beginning of the study), so with lower metabolisable protein requirements. Long-term experiments could better elucidate if the negative N balance found with the present experimental conditions could negatively impact milk production.

Conclusions

Whole-plant soybean silage proved to be an adequate forage and protein source to be included in the ration of lactating cow at 12.4% of the DM, allowing a reduction of one-third of SBM (more than 1 kg/head per day), without affecting feed intake and milk production. Thus, environmental sustainability of milk production can be enhanced thanks to protein source grown on farm. Future research should be aimed at quantifying the environmental impact of SBS compared to CON. Possible limitations of soybean silage are that digestibility and protein use efficiency have to be improved in order to fully exploit its potential. More studies are advisable to better understand the effect of management and harvest practices aimed at increasing digestibility and to improve the protein/energy ratio in the diet, for example including higher amount of water soluble carbohydrates sources like sugarcane molasses.

Ethical approval

The experiment was conducted according to the Univeristy of Milan Welfare Organism (OP BA) and with authorization number 954/2016-P R from Italian Ministry of Health.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, A. R. G., upon reasonable request.

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