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Performance and utilization of nutrients in dairy cows fed with sunflower meal

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Abstract

Non-decorticated sunflower meal (SFM) is a potential protein source for dairy cows with high-fibre content but high ruminal degradability. The effect of replacement of soybean meal (SBM) and wheat middlings (WM) with SFM on the intake, digestibility, microbial protein synthesis, nitrogen utilization and milk production of dairy cows was evaluated. Twelve Holstein cows were blocked by days in milk and distributed in three 4 × 4 Latin squares. Diets were formulated to be isonitrogenous and contained 550 g maize silage/kg dry matter (DM). Treatment diets were no SFM (CON) or 70, 140 and 210 g/kg DM of SFM replacing fixed mixture of SBM and WM (536 and 464 g/kg of the mixture, respectively). The inclusion of SFM in diet did not affect DM intake, but intake of rumen degradable protein increased linearly. Inclusion of SFM reduced or tended to reduce total-tract digestibility of non-fibre carbohydrate, total digestible nutrients and excretion of purine derivatives. Milk production, milk protein content and efficiency of nitrogen use for lactation were reduced with increasing levels of SFM in the diet. The use of non-decorticated SFM as a replacement for SBM–WM mixture in diet reduces performance and efficiency of nutrient use in lactating dairy cows. The outcome of the current study is attributed to reduced fibre digestibility in SFM hulls. Therefore, future studies should evaluate the use of decorticated SFM.

Introduction

Sunflower (*Helianthus annuus* L.) seeds are a prominent source of vegetable oil for human consumption and biofuel production (USDA, 2019). Sunflower seeds contain between 350 and 450 g oil/kg and 250 and 300 g hulls/kg (Finn *et al.*, 1985; Economides, 1998; NRC, 2001). Sunflower meal (SFM) is a by-product obtained after oil extraction and contains 280–500 g crude protein (CP)/kg dry matter (DM) depending on cultivar, method of oil extraction and degree of seed decortication (Hesley, 1994; Canibe *et al.*, 1999). Sunflower hulls have low CP and high level of lignified fibre (Arija *et al.*, 1998). Therefore, the variation in nutritional value of SFM is determined mostly by degree of seed decortication. However, non-decorticated SFM is commercialized, probably due to the extra cost of decortication.

SFM exhibits similar intestinal digestibility of undegradable protein to soybean meal (SBM), but with lower contents of lysine and threonine, and higher content of methionine (NRC, 2001; Branco *et al.*, 2006; Rodriguez *et al.*, 2008). However, it has been demonstrated that non-decorticated SFM contains a greater proportion of rumen degradable protein (RDP) than traditional protein sources such as SBM, cottonseed and canola meal (Erasmus *et al.*, 1994; Branco *et al.*, 2006; Rodriguez *et al.*, 2008), but lower ruminal DM degradation than SBM, possibly due to high content of hulls (Rodriguez *et al.*, 2008).

Previous studies using low-producing dairy cows (<20 kg of milk/day) showed no detrimental effect on milk production when 110 g/kg DM of low-oil non-decorticated SFM (Schingoethe *et al.*, 1977) or 150 g/kg DM of high-oil non-decorticated SFM (Silva *et al.*, 2005) was included in the diet as a substitute for SBM and maize. In another study, replacing SBM with SFM reduced milk yield of high-producing dairy cows (Yildiz *et al.*, 2015). The current authors hypothesized that replacing SBM and wheat middlings (WM) with non-decorticated SFM would increase RDP supply and decrease diet digestible energy, which would consequently reduce the performance of dairy cows. Therefore, the current study evaluated the effects of replacing SBM and WM with non-decorticated SFM on milk production, total-tract diet digestibility and nitrogen (N) metabolism of lactating dairy cows.

Materials and methods

Cows and diets

Twelve multiparous Holstein cows (31 ± 4.1 kg milk/day; 128 ± 38.2 days in milk; and 627 ± 48.1 kg body weight [BW]) were blocked by days in milk and assigned randomly within squares to treatment sequences in three replicated 4 × 4 Latin squares. Treatment sequences within Latin squares were balanced for carry-over effects with four 21-day periods, which included 14 day for diet adaptation and 7 days for data and sample collection.

Cows were housed in individual tie-stalls (215 × 125 cm) with rubber beds and had free access to water. The chemical compositions of the maize silage (MS), SFM, SBM, WM and ground maize grain used in the current trial are shown in Table 1. SFM (BUNGE Alimentos, S.A. Brazil) was obtained after solvent oil extraction of non-decorticated whole sunflower seeds. Cows were fed with four isonitrogenous experimental diets as a total mixed ration (TMR) containing four levels of SFM (0, 70, 140 and 210 g/DM), partially or fully replacing a SBM–WM mixture (536 g SBM/kg and 464 g WM/kg DM of the mixture) (Table 2). All diets were formulated to meet nutrient requirements of 650 kg cows producing 30 kg/day of milk and 38 g/kg milk fat (NRC, 2001). The TMR was prepared by blending MS and concentrate mixtures. The concentrate mixtures were prepared for each 21 day period. Diets were offered twice daily at 07.00 and 16.00 h. Amounts of feed offered to the cows were adjusted daily to allow refusals equal to proportions of 0.05–0.10 of intake. DM content from weekly composites of the silage and concentrate mixture was used to adjust the as-fed TMR composition to maintain constant dietary nutrient supply throughout the trial.

Animal measurements and sampling

Individual concentrate ingredients were sampled during each mixture preparation (21 days) and kept in a freezer (−15 °C) for subsequent grinding and chemical analysis. Daily DM intake (DMI) and diet component intakes were determined by differences between the weights of feed offered and feed refused. MS, concentrate mixture offered and diets refused were weighed twice daily for each cow. Approximately 100 g of the MS offered and refusal were sampled twice daily and stored (−15 °C). At the end of each collection period (7 days), the refusal samples from each animal were removed from the freezer, thawed at room temperature and blended manually to obtain a composite sample per animal for each period. The composite samples of MS (7 days) and refusal were pre-dried in a forced-air oven at 55 °C for 72 h.

Faecal samples were collected directly from the rectum once daily from day 15 to 19 of each period, at 08.00, 10.00, 12.00, 14.00 and 16.00 h, respectively. The daily faecal samples of each cow in each period were kept in a freezer (−15 °C) for later pre-drying in a forced-air oven at 55 °C for 72 h. After pre-drying and grinding, a single composite faecal sample was obtained per animal for each period.

Cows were milked twice daily (06.00 and 15.00 h) and milk yield recorded at each milking. Milk samples from the morning and afternoon milkings were collected on day 18 and 19 of each period. Composite samples were prepared daily according to milk production and three different aliquots were sampled. The first aliquot (60 ml) was stored at 6 °C with a preservative (bronopol-B2) for analysis of fat, lactose, solids and solid non-fat content. The second aliquot was analysed immediately for CP (N × 6.38). The third aliquot (10 ml) was deproteinized with 5 ml 250 g trichloroacetic acid/l and filtered on Whatman #1 filter paper; the filtrate was analysed for N content and the remainder stored at −15 °C for subsequent analysis of allantoin and urea. Fat-corrected milk (FCM; 3.5 g/100 g milk) was estimated according to the Gaines (1928) model: FCM (kg/day) = 0.432 × milk yield (kg) + 0.1623 × milk fat concentration (g/100 g). BWs were measured in the morning and afternoon (after milking) on day 7 and 21 of each period.

Blood samples were taken in ethylenediaminetetraacetic acid tubes from the coccygeal vessels of each cow 4 h after feeding on day 19 of each period, centrifuged immediately (2300 ***g***, 15 min, room temperature) and plasma was stored at −15 °C for urea analysis. Spot urine samples were obtained at approximately 0, 3 and 6 h post-feeding on day 17 of each period by manual stimulation of the vulva. After collection, 10 ml of urine was filtered and pipetted into a specimen container with 40 ml of 0.072 N sulphuric acid (H_2SO_4) and stored at −15 °C. Before urinary analysis, the urine samples for each time of collection from each cow were thawed, centrifuged at 2000 ***g*** for 15 min (room temperature) and combined into composite samples (10 ml for each time) for each cow in each period. These samples were analysed for N, urea, creatinine, allantoin and uric acid.

In situ *ruminal degradability*

In situ ruminal degradability of each dietary ingredient was obtained according to NRC (2001). Briefly, 5 g of pre-dried feed sample (2 mm) were added to 50-µm nylon bags (Tenyl Tecidos Tecnicos Ltda, Guarulhos, Brazil) measuring 16 × 8 cm^2 in triplicate and placed into the rumen of two lactating dairy cows (BW 550 kg and 20 kg milk/day). The cows were managed under similar conditions as described previously and fed with the experimental diet containing no SFM *ad libitum*. CP and DM degradation were determined over incubation times of 0, 2, 4, 8, 16, 24 and 48 h; samples were further incubated for 72 and 96 h for WM, MS and SFM (NRC, 2001). An additional incubation time (240 h) was used for determination of ruminal neutral detergent fibre (NDF) degradability in all feeds. Upon removal from the rumen, bags were washed carefully in tap water and dried at 55 °C in a forced-air oven for 72 h: residues were analysed later to determine the concentrations of DM, CP and NDF.

Ruminal CP degradation kinetics were estimated by fitting degradation data to the exponential model proposed by Ørskov and McDonald (1979):

$$Y = A + B \times (1 - \exp^{(-k_d \times t)})$$

where Y = degradability at time t, A = soluble fraction, B = potentially degradable fraction and k_d = rate of degradation of B(/h). Effective degradable (ED) fraction of the DM and RDP of each feed was calculated as:

$$\text{ED or RDP} = A + B\,[k_d/(k_d + k_p)]$$

where A is the soluble fraction and k_p is the ruminal rate of passage (/h) (Ørskov and McDonald, 1979). The rate of passage (k_p) was estimated using the equation of NRC (2001):

$$k_p \text{ of wet forage (\%/h)} = 3{\cdot}054 + 0{\cdot}614 \times \text{DMI (g/100 g BW)}$$

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Table 1. Chemical composition of MS and concentrate feeds

Item[a]	MS	GMG	SBM	WM	SFM
Dry matter (DM) (g/kg)	288	884	886	883	905
Organic matter (g/kg DM)	951	985	937	955	938
Crude protein (g/kg DM)	70.4	95.2	519	197	375
Non-protein N (g/kg N)	582	216	214	490	362
NDIN (g/kg N)	231	83.6	105	211	159
ADIN (g/kg N)	151	51.6	57.8	31.8	50.5
Ether extract (g/kg DM)	19.4	38.6	15.4	32.9	13.5
aNDFom (g/kg DM)	534	132	117	453	407
Non-fiber carbohydrate (g/kg DM)	328	720	285	272	142
Acid detergent fibre (g/kg DM)	358	36.1	84.9	150	273
$Lignin_{sa}$ (g/kg DM)	35.1	5.60	4.90	14.7	49.6
$Lignin_{sa}$ (g/kg aNDFom)	65.8	42.6	41.9	32.4	122
iNDF (g/kg DM)	248	24.4	11.8	150	235
iADF (g/kg DM)	140	6.70	4.2	75.5	177

MS, maize silage; GMG, ground maize grain; SBM, soybean meal; WM, wheat middlings; SFM, sunflower meal.
[a]NDIN, neutral detergent insoluble nitrogen; ADIN, acid detergent insoluble nitrogen; aNDFom, neutral detergent fibre corrected for ash and nitrogen; iNDF, indigestible neutral detergent fibre obtained after *in situ* ruminal incubation for 264 h; iADF, indigestible acid detergent fibre obtained after *in situ* ruminal incubation for 264 h.

$$k_p \text{ of concentrate (\%/h)} = 2.904 + 1.375 \times \text{DMI (g/100 g BW)} - 0.002 \times \text{concentrate in diet (g/kg DM)}.$$

RDP intake was calculated as:

$$\text{RDP intake (kg/day)} = \text{DMI (kg/day)} \times \text{RDP in diet (g/kg DM)}/1000.$$

RDP in diet was calculated from RDP content of each feed.

Ruminal NDF degradation kinetics was estimated by fitting degradation data to the Mertens and Loften (1980) exponential model:

$$Y = B \times \exp^{((-k_d \times (t-L))} + U$$

where Y = residue remaining at time t, B = potentially degradable fraction, k_d = rate of degradation of B(/h), L = discrete lag time (h) and U = undegradable fraction.

ED fraction of the NDF and RDP was calculated as:

$$\text{ED or RDP} = B \times [k_d/(k_d + k_p)]$$

Chemical analysis

The pre-dried MS, refusals and faecal samples, and original samples of SFM, SBM and WM were ground in a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) with 1-mm screen for chemical analysis and 2-mm screen for ruminal incubation *in situ*. Samples of feed, refusals and faeces were analysed for concentrations of DM (method no. 934.01), organic matter (OM, method no. 942.05), CP (method no. 954.01) and ether extract (EE, method no. 920.39) according to AOAC (2005). NDF was determined using heat stable amylase without sodium sulphite and corrected for residual ash (Mertens, 2002) and N (Licitra *et al.*, 1996) (aNDFom). Both NDF and acid detergent fibre (ADF) (sequential) were analysed with an Ankom® fibre analyser (Ankom Technology, Fairport, NY, USA). Concentrations of non-protein N (NPN), neutral detergent insoluble N (NDIN) and acid detergent insoluble N (ADIN) were measured according to Licitra *et al.* (1996). Lignin concentration was determined by solubilization of cellulose by hydrolysing the ADF residue with 72% H_2SO_4 (wt/wt) (Van Soest *et al.*, 1991). Non-fibre carbohydrate (NFC) was calculated by difference according to Hall (2000):

$$\text{NFC} = 100 - [(\text{CP} - \text{CP derived from urea}) + \text{urea} + \text{EE} + \%\text{ ash} + \text{aNDFom}]$$

Indigestible ADF was used as the internal marker to estimate apparent nutrient digestibility and faecal output (Cochran *et al.*, 1986). Indigestible ADF in feeds, refusals and faeces was obtained after ruminal incubation in a polyester bag (Ankom®, filter bag 57) for 264 h (Casali *et al.*, 2008).

Total digestible nutrient (TDN) was obtained from digestion trial according to Weiss (1998):

$$\text{TDN (g/100 g DM)} = \text{CP (g/100 g DM)} \times \text{dCP} + \text{aNDFom (g/100 g DM)} \times \text{daNDFom} + \text{NFC (g/100 g DM)} \times \text{dNFC} + \text{EE (g/100 g DM)} \times \text{dEE} \times 2{\cdot}25$$

where dCP is the total-tract digestible CP, daNDFom is the total-tract digestible aNDFom, dNFC is the total-tract digestible NFC and dEE is the total-tract digestible EE, all expressed as coefficients.

Milk fat and lactose were analysed by infrared spectrophotometry (IDF, 1996). Nitrogen in milk and deproteinized milk were

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Table 2. Ingredient and chemical composition of experimental diets

	SFM in diet g/kg DM[a]			
Item	0	70	140	210
Ingredient composition (g/kg DM)				
Maize silage	550	550	550	550
Ground maize grain	211	211	211	211
Soybean meal	112.5	75.0	37.5	
Wheat middlings	97.5	65.0	32.5	
Sunflower meal (SFM)		70.0	140	210
Urea : ammonium sulphate (9 : 1)	10.0	10.0	10.0	10.0
Limestone	9.7	9.7	9.7	9.7
Dicalcium phosphate	3.0	3.0	3.0	3.0
Salt	4.8	4.8	4.8	4.8
Mineral premix[b]	1.5	1.5	1.5	1.5
Chemical composition[c]				
Dry matter (DM) (g/kg)	414	414	414	415
Organic matter (g/kg DM)	959	960	961	962
Crude protein (g/kg DM)	162	162	162	163
Non protein nitrogen (g/kg N)	458	470	483	495
NDIN (g/kg N)	129	133	138	142
ADIN (g/kg N)	65.8	65.7	65.5	65.3
Rumen degradable protein (g/kg DM)	107	109	111	113
Ether extract (g/kg DM)	23.8	23.1	22.4	21.6
aNDFom (g/kg DM)	379	388	397	407
Non-fiber carbohydrate (g/kg DM)	410	402	394	386
Acid detergent fibre (g/kg DM)	229	240	251	262
$Lignin_{sa}$ (g/kg DM)	22.5	25.3	28.1	30.9
$Lignin_{sa}$ (g/kg aNDFom)	59.4	65.2	70.7	76.0
iNDF (g/kg DM)	158	169	180	191
iADF (g/kg DM)	86.1	95.9	106	116
Net energy of lactation (MJ/kg DM)[d]	6.04	5.94	5.93	5.70

[a]SFM replaced mixture of SBM and WM.
[b]Provided (per kg of DM): 383 g of Mg, 161 g of S, 30 mg of Zn, 9 mg of Mn, 7 mg of Cu, 0.4 mg of I, 0.2 mg of Se, 0.07 mg of Co.
[c]NDIN, neutral detergent insoluble nitrogen; ADIN, acid detergent insoluble nitrogen; aNDFom, neutral detergent fibre corrected for ash and nitrogen; iNDF, indigestible neutral detergent fibre obtained after *in situ* ruminal incubation for 264 h; iADF, indigestible acid detergent fibre obtained after *in situ* ruminal incubation for 264 h.
[d]Estimated according to NRC (2001): NEL (MJ/kg DM) = [0.245 × TDN (g/kg DM) − 0.12] × 4.184, were total digestible nutrients (TDN) were obtained from digestion trial (Table 4).

analysed by the micro-kjeldahl method (AOAC, 2005). Urea in milk, plasma and urine were measured using an enzymatic-colorimetric assay with urease (Urea CE Ref. 27, Labtest Diagnostica SA, Lagoa da Santa, Minas Gerais, Brazil). Urinary uric acid was quantified using the enzymatic-Trinder method (Ácido úrico Liquiform Ref. 73, Labtest Diagnostica SA, Lagoa da Santa, Minas Gerais, Brazil; Junge *et al.*, 2004). Allantoin concentrations in milk and urine samples were determined by colorimetry (Young and Conway, 1942). Creatinine in urine was measured by an enzymatic-colorimetric assay (Creatinina Ref. 35, Labtest Diagnostica SA, Lagoa da Santa, Minas Gerais, Brazil). Total urine volume was estimated using creatinine concentration as a marker and assuming daily creatinine excretion of 24 mg/kg of BW (Cobianchi *et al.*, 2012). Excretion of purine derivatives (PD) was calculated as the sum of allantoin and uric acid excreted in urine, and allantoin secreted in milk. Excretion of PD per TDN intake was used as an index of energy efficiency, while PD excretion per intake of CP and RDP was used as an index of nitrogen efficiency for microbial protein synthesis (MPS) in the rumen. Milk N efficiency was calculated as the ratio of N in milk (g/day) to N intake (g/day).

Statistical analysis

Data were analysed using PROC MIXED in SAS (SAS Institute, 1999–2000) for a replicated 4 × 4 Latin square design. The

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Table 3. *In situ* ruminal degradation kinetics of MS and concentrate feeds

Item[a]	MS	GMG	SBM	WM	SFM
DM					
A	0.272	0.115	0.303	0.292	0.217
B	0.528	0.885	0.697	0.481	0.522
k_d (/h)	0.029	0.028	0.057	0.118	0.089
ED	0.504	0.381	0.626	0.600	0.517
CP					
A	0.497	0.190	0.158	0.279	0.228
B	0.283	0.811	0.842	0.655	0.713
k_d (/h)	0.04	0.01	0.06	0.30	0.12
RDP	0.650	0.324	0.566	0.814	0.687
NDF					
B	0.732	0.887	0.978	0.673	0.452
U	0.268	0.113	0.022	0.327	0.548
k_d (/h)	0.021	0.024	0.051	0.041	0.050
Lag time (h)	1.76	2.29	0.01	0.10	0.02
ED	0.265	0.349	0.568	0.356	0.259

MS, maize silage; GMG, grain maize ground; SBM, soybean meal; WM, wheat middlings; SFM, sunflower meal.
[a]*A*, soluble fraction; *B*, insoluble potential degradable fraction; *U*, undegradable fraction; k_d, degradation rate of *B* fraction; ED, effective degradable fraction, with passage rate according to the NRC (2001); RDP, rumen degradable protein, with passage rate according to the NRC (2001); NDF, neutral detergent fibre; *A*, *B*, *U*, ED and RDP are expressed as coefficients.

following model was fitted to all variables:

$$Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + E_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the effect of square i, P_j is the effect of period j, $C_{k(i)}$ is the effect of cow k (within square i), T_l is the effect of treatment l, ST_{il} is the interaction between square i and treatment l, and E_{ijkl} is the residual error (0; σ^2). All terms were considered fixed except for $C_{k(i)}$ and E_{ijkl}, which were considered random. Significance was declared at $P \leqslant 0.05$, with trends at $P > 0.05$ and $\leqslant 0.10$. Dietary SFM levels were tested by partitioning degrees of freedom for diet into single degree of freedom variables corresponding to linear, quadratic and cubic effects. Cubic effects were not statistically significant for any of the variables and are not reported. All reported values were least squares means.

Results

The major change in the chemical composition of the diets with SFM inclusion was the increased concentration of aNDFom and lignin as a fraction of aNDFom (Tables 1 and 2). As a result, effective rumen degradation of DM in SFM was 15.8% lower than that of the SBM–WM mixture (0.517 *v.* 0.614, Table 3). As expected, SFM had greater RDP (0.687) than SBM (0.566), but lower RDP than WM (0.814) (Table 3). Potentially rumen-degradable NDF in SFM (0.452) was lower than those of SBM (0.978) and WM (0.673), probably due to its higher lignin concentration. Consequently, SFM had lower effective rumen degradable NDF (0.259) than SBM and WM (0.568 and 0.356 respectively; Table 3).

Sunflower meal inclusion did not affect BW ($P = 0.159$), DMI ($P = 0.118$) or CP intake ($P = 0.137$). Increasing levels of SFM in the diet resulted in linear increases of RDP ($P = 0.014$) and aNDFom ($P < 0.001$) intakes, and linear reductions in NFC ($P < 0.001$) and TDN ($P = 0.042$) intakes (Table 4). In addition, SFM inclusion did not affect total-tract digestibility of CP ($P = 0.112$) and EE ($P = 0.278$); however, linear reductions in total-tract digestibility of DM ($P = 0.036$) and TDN ($P = 0.031$) were observed, as well as a tendency to reduce digestibility of OM ($P = 0.075$) and NFC ($P = 0.076$) (Table 4).

Increasing levels of non-decorticated SBM in the diet linearly reduced milk production ($P < 0.001$), 3.5% FCM ($P < 0.001$), feed efficiency for milk production ($P = 0.024$) and milk component yields ($P < 0.05$) (Table 4). Concentrations of milk lactose ($P = 0.213$), NPN ($P = 0.872$) and fat ($P = 0.229$) were unaffected by dietary treatment (Table 4).

The concentrations of urea-N in milk ($P = 0.339$) and blood ($P = 0.324$) were not affected by SFM inclusion (Table 5). SFM inclusion did not affect milk allantoin secretion ($P = 0.481$) or urinary uric acid excretion ($P = 0.946$), but tended to reduce urinary allantoin excretion ($P = 0.072$) and PD excretion ($P = 0.079$) (Table 5). SFM inclusion did not affect PD excretion per TDN intake ($P = 0.256$), however, PD excretion as a proportion of CP intake ($P = 0.069$) tended to linearly reduce while PD excretion as a proportion of RDP intake ($P = 0.026$) linearly reduced with increasing levels of SFM in the diet. As a result, milk N production and milk N efficiency (milk N/N intake) were reduced ($P \leqslant 0.001$) (Table 5). Dietary treatment did not affect ($P > 0.10$) N intake, faecal N, urinary N or N balance of the dairy cows.

Discussion

The results of the current study confirmed the hypothesis that replacing the SBM and WM mixture (536 and 464 g/kg of the mixture, respectively) with non-decorticated SFM would increase intake of RDP and reduce intake of digestible energy, which would lead to reduced efficiency of N use and milk production of dairy cows. The lack of effect of SFM inclusion on DMI agrees with Schingoethe *et al.* (1977), who reported no change in DMI of dairy cows fed with a diet containing 110 g non-decorticated SFM/kg DM (370 g CP/kg DM and 212 g ADF/kg DM). Though SFM had greater concentrations of indigestible NDF and ADF, there is a potential for low ruminal fill due to high degradation rate of potentially degradable NDF, probably due to small particle size and high density of particles which reduce selective retention of feeds in the rumen (Lund *et al.*, 2007). The reduced TDN intake observed in the current study was probably due to reduced total tract digestibility of NFC, which could be attributed to high level of hull seeds in non-decorticated SFM (Arija *et al.*, 1998).

The reduction in milk production and milk protein synthesis of the dairy cows with increasing levels of SFM in the diet was a result of reduced digestible energy intake and PD excretion. Because there is evidence that urinary PD excretion, mainly allantoin, has high correlation with intestinal flow of microbial nucleic acid (Perez *et al.*, 1996; Valadares *et al.*, 1999; González-Ronquillo *et al.*, 2003), it was assumed that reduced PD excretion indicates reduced MPS in the rumen. According to NRC (2001), when RDP supply is not limited, the efficiency of energy use for MPS is fixed. This probably explains the lack of effect on the efficiency of energy utilization for MPS, measured by PD excretion per TDN intake. Reduced PD excretion per RDP intake indicates that SFM inclusion reduced RDP conversion to microbial protein probably due to reduced availability of carbon skeletons and energy for the rumen microbiota.

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Table 4. Effects of replacement of SBM and WM mixtures with SFM in the diet on intake, total tract digestibility, milk production and composition of lactating dairy cows

	SFM (g/kg DM diet)[b]					*P*-value[d]	
Item[a]	0	70	140	210	SEM[c]	L	Q
BW (kg)	642	647	635	643	9.2	0.878	0.159
Intake (kg/day)							
Dry matter	22.0	21.4	21.2	21.9	0.45	0.872	0.118
Crude protein	3.8	3.7	3.7	3.8	0.08	0.683	0.137
Rumen degradable protein	2.3	2.3	2.3	2.4	0.05	0.014	0.079
aNDFom	7.9	7.9	8.0	8.6	0.17	<0.001	0.176
Non-fibre carbohydrate	8.3	7.8	7.5	7.5	0.17	<0.001	0.157
Ether extract	0.5	0.5	0.5	0.5	0.01	<0.001	0.111
Total digestible nutrient	13.5	12.9	12.8	12.7	0.33	0.042	0.289
Dry matter (g/100 g BW)	3.4	3.3	3.3	3.4	0.09	0.891	0.239
aNDFom (g/100 g BW)	1.2	1.2	1.3	1.3	0.04	<0.001	0.251
Total-tract digestibility coefficient							
Dry matter	0.65	0.64	0.64	0.62	0.011	0.036	0.563
Organic matter	0.63	0.62	0.63	0.60	0.019	0.075	0.287
Crude protein	0.69	0.70	0.70	0.67	0.012	0.292	0.112
ANDFom	0.49	0.48	0.48	0.47	0.016	0.272	0.689
Non-fibre carbohydrate	0.72	0.70	0.72	0.69	0.014	0.076	0.942
Ether extract	0.86	0.87	0.88	0.85	0.013	0.351	0.278
Total digestible nutrient (g/100 g DM)	64	63	63	61	1.1	0.031	0.567
Production (kg/day)							
Milk	30	29	29	27	1.2	<0.001	0.696
3.5% FCM[e]	31	29	29	28	1.2	<0.001	0.722
Lactose	1.3	1.3	1.3	1.2	0.05	0.022	0.519
Protein	0.96	0.92	0.89	0.86	0.029	<0.001	0.882
Fat	1.10	1.02	1.03	0.98	0.044	<0.001	0.517
Feed efficiency[f] (kg/kg)	1.4	1.4	1.4	1.3	0.02	0.024	0.487
Milk composition (g/100 g)							
Lactose	4.4	4.5	4.5	4.5	0.05	0.213	0.526
Protein	3.2	3.2	3.1	3.1	0.08	0.043	0.563
NPN	0.29	0.28	0.28	0.28	0.011	0.872	0.833
Fat	3.7	3.6	3.6	3.6	0.97	0.229	0.358

[a]aNDFom, neutral detergent fibre corrected for ash and; BW, body weight.
[b]SFM replaced mixture of SBM and WM.
[c]s.e.d. = standard error of the least squares means.
[d]Probability of a significant effect linear (L) or quadratic (Q) of the SFM level.
[e]FCM = fat-corrected milk, estimated according Gaines (1928): FCM (kg/day) = 0.432 × milk production (kg) + 0.1623 × milk fat concentration (g/100 g).
[f]Milk production/DMI.

Digestible energy and MPS are the main factors contributing to duodenal flow of glucose and essential amino acids (EAA) such as lysine, methionine and histidine which are substrates that drive milk and milk protein synthesis in the mammary gland (Kronfeld, 1982; Schwab and Broderick, 2017). In addition, lower lysine content of SFM (NRC, 2001; Branco *et al.*, 2006; Rodriguez *et al.*, 2008) may have also caused reduced intestinal flow of lysine from the rumen. Therefore, the depressed milk production and milk protein synthesis with SFM inclusion is probably due to reduced glucose supply to the mammary gland and/or poor match between metabolizable protein supply and amino acid requirements for optimum milk production.

SFM inclusion did not affect indices associated with the pool of circulating urea, such as milk urea-N, blood urea-N and urinary urea-N and N balance. However, reduced milk N efficiency was observed with SFM inclusion, which is probably due to

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Table 5. Effects of replacement of SBM and WM mixtures with SFM on nitrogen (N) metabolism and efficiency of lactating dairy cows

	SFM (g/kg DM diet)[b]					*P*-value[d]	
Item[a]	0	70	140	210	SEM[c]	L	Q
Urea metabolism							
Milk urea-N (mg/dl)	17.9	17.7	16.9	17.9	0.80	0.809	0.338
Blood urea-N (mg/dl)	18.9	17.6	16.4	17.4	0.79	0.801	0.324
Urinary urea-N (g/day)	199	197	190	196	11.6	0.754	0.697
Urinary urea-N (g/g N)	0.85	0.83	0.80	0.77	0.036	0.089	0.789
Purines derivatives (PD)							
Milk allantoin (mmol/day)	2.9	2.8	2.8	2.7	0.20	0.481	0.918
Urinary allantoin (mmol/day)	412	392	402	349	22.1	0.072	0.441
Urinary uric acid (mmol/day)	43	47	41	44	3.2	0.847	0.946
Total PD (mmol/day)	458	442	446	396	30.9	0.079	0.438
Total PD (mmol/kg TDN intake)	34	34	35	31	2.0	0.359	0.252
Total PD (mmol/g CP intake)	0.12	0.12	0.12	0.10	0.007	0.069	0.214
Total PD (mmol/g RDP intake)	0.20	0.19	0.19	0.16	0.011	0.026	0.228
N balance (g/day)							
Intake	604	593	592	608	12.4	0.681	0.138
Milk N	150	144	139	135	4.6	<0.001	0.889
Faecal N	186	180	176	199	9.2	0.228	0.157
Urinary N	233	236	237	254	11.7	0.186	0.497
N balance	35	33	40	23	13.2	0.467	0.468
N efficiency (g/100 g N intake)							
Milk N	24.8	24.3	23.5	22.2	0.69	<0.001	0.227
Faecal N	30.8	30.4	29.7	32.7	1.23	0.297	0.117
Urinary N	38.6	39.8	40.0	41.8	1.82	0.189	0.897
N balance	5.8	5.6	6.8	3.3	2.20	0.438	0.436

[a]Total DP, Total PD, milk allantoin + urinary allantoin + uric acid; TDN, total digestible nutrient; CP, crude protein; nitrogen balance = N intake – (N milk + N faecal + N urine); RDP, rumen degradable protein; N milk efficiency = g N milk secreted/100 g N intake; N balance = g N balance/100 g N intake.
[b]SFM replaced combinations of SBM and WM.
[c]s.e.d. = standard error of the least squares means.
[d]Probability of a significant effect linear (L) or quadratic (Q) of the SFM level.

reduction in RDP conversion to microbial protein, efficiency of N captured by mammary gland and/or conversion of N captured into milk N (Lapierre *et al.*, 2006). This may also be as a result of poor match between metabolizable amino acid profile and requirements for lysine, methionine or histidine, and/or reduced glucose flows to mammary glands (Kronfeld, 1982; Schwab and Broderick, 2017). However, efficiency of RDP conversion to microbial protein is much more likely to have caused the reduced milk N efficiency observed. Even though SFM appears to be a good source of methionine, increased RDP intake suggests duodenal supply of methionine may be inadequate. Further studies should examine how SFM inclusion affects duodenal flow of EAA, EAA in the plasma, and mammary gland metabolism of EAA.

In conclusion, replacement of SBM and WM mixture (536 and 464 g/kg of the mixture, respectively) with non-decorticated SFM in diet of lactating dairy cows does not affect DMI. However, due to reduced intake of digestible energy, efficiency of N use for milk production of dairy cows fed with SFM is depressed. It is important to note that the outcome of the current study is clearly a result of lower digestibility of fibre in non-decorticated SFM compared to that of the SBM : WM mixture. Further studies are needed to evaluate the use of decorticated SFM as a replacement for the SBM : WM mixture in diets for lactating dairy cows.

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