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Defatted black soldier fly larvae meal as a substitute of soybean meal in dairy cow diets

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The replacement of soybean meal (**SBM**) with insect meal, e.g. black soldier fly larvae meal (**BSFLM**), as an alternative protein source in livestock feed is gaining traction globally. To date, no *in vivo* studies have been conducted to test the impact of BSFLM on the milk production of dairy cows. This study quantified the effects of replacing SBM with defatted black soldier fly larvae meal (**DBSFLM**) in diets of lactating dairy cows on feed intake, milk production and composition, apparent total-tract digestibility, feed- and nitrogen use efficiency. Twelve lactating Holstein-Friesian cows were used in a replicated 3 × 3 Latin square design (18 d per period) with 3 protein treatments: a diet containing SBM as the main protein source (control, SBM); a diet with a 50: 50 mixture of SBM and DBSFLM (SBM: DBSFLM, 50: 50); and a diet with DBSFLM (DBSFLM). Animals were blocked per Latin square based on milk yield (**MY**), and the amount of concentrate in the diets was adjusted for MY. Diets were provided as a total mixed ration and offered *ad libitum*. Our results showed no treatment effects ($P > 0.05$) on milk yield and composition. DM and organic matter (**OM**) intake were significantly higher ($P \leq 0.003$) for cows fed SBM (17.6 and 16.2 kg/d, respectively) compared with DBSFLM (16.6 and 15.1 kg/d, respectively), while DM and OM intake of SBM: DBSFLM (17.5 and 16.0 kg/d, respectively) did not differ from SBM but were higher compared with DBSFLM. CP intake was significantly higher ($P = 0.003$) for cows fed SBM (2.68 kg/d) compared with both DBSFLM (2.50 kg/d) and SBM: DBSFLM (2.54 kg/d), while CP intake for SBM: DBSFLM did not differ from DBSFLM. Apparent total-tract digestibility of NDF and ADF were significantly lower ($P \leq 0.024$) for cows fed SBM (46.7 and 45.6%, respectively) compared with DBSFLM (54.0 and 52.0%, respectively), with SBM: DBSFLM (51.0 and 49.7%, respectively) not differing from either diet. No treatment effects were observed for DM, OM, CP and ether extract digestibility, and feed and nitrogen use efficiency. Incorporating DBSFLM into dairy cow diets did not significantly compromise the milk yield and composition, suggesting its potential to reduce the reliance on SBM and contribute to circular food systems.

Implications

Black soldier fly larvae can convert organic waste into high-quality animal feed, offering a sustainable alternative protein source. This study used defatted black soldier fly larvae meal as a substitute for soybean meal in dairy cow diets. The results showed that replacing soybean meal with defatted black soldier fly larvae meal did not affect milk yield or composition. These findings suggest that this alternative protein source can reduce reliance on imported soybean meal, contributing to more circular and sustainable livestock systems. Further research should explore long-term effects and the mechanisms of digestion involved.

Introduction

Circularity is a pathway towards sustainable food production. This approach ensures that natural resources are managed within their regenerative and absorptive capacities, prioritises the use of agricultural land for crops intended for direct human consumption, and systematically reintegrates by-products and waste generated across food production, processing, and consumption stages back into the food system (de Boer and van Ittersum, 2018; Muscat et al., 2021; van Zanten et al., 2019). In sub-Saharan Africa, current

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livestock systems show a high level of circularity, but with the shift towards intensified farming circularity tends to diminish. Intensification is often accompanied by the use of high-quality feed ingredients including soybean meal (**SBM**) to boost animal productivity (Oosting et al., 2022). East African countries are not self-sufficient in the production of SBM, leading to relatively high imports of soybeans and SBM (van Ittersum et al., 2016; Vernooij and Veldkamp, 2018). This import of soybeans and SBM has disrupted the nutrient cycles by transferring nutrients in feed-producing regions and amassing them in livestock areas, ultimately resulting in soil degradation and overfertilisation, respectively (Oita et al., 2016; Rezende et al., 2023; van Selm et al., 2023; Wang et al., 2022). Additionally, soybean production is driven by the demand for SBM (Garnett, 2009; Wilkinson and Lee, 2018), creating a competition for arable land with the cultivation of food crops intended for human consumption (Mottet et al., 2017; van Hal et al., 2019). Therefore, increasing circularity in East African food systems will increase demand for alternative local protein sources.

Insects are an alternative and circular feed resource because they can convert bio-waste into high–quality feed ingredients, with limited requirements of arable land compared to crop production (Abro et al., 2020; Bosch et al., 2019; Salomone et al., 2017). For instance, black soldier fly larvae (**BSFL**; *Hermetia illucens* L.) can grow on a wide range of organic waste streams, potentially contributing to urban waste management solutions (Nguyen et al., 2015; De Smet et al., 2018; Surendra et al., 2020). These larvae are rich in nutrients, particularly protein, making them a suitable replacement for SBM in livestock diets (Makkar et al., 2014; Shumo et al., 2019).

Farming of BSFL has advanced significantly in Africa, particularly in Kenya with the establishment of large-scale insect production companies (Tanga and Kababu, 2023). In Kenya, the Kenya Bureau of Standards has approved the integration of dried insect products in compounded animal feeds including those for ruminants (KEBS, 2017). However, the regulatory status and application of BSFL in livestock diets differ globally, with live BSFL permitted in aquaculture, poultry, and pig feed, while only insect fat and hydrolysed insect proteins are allowed in ruminant feed in the European Union (IPIFF, 2024).

Most research on BSFL as a protein source in livestock diets has been conducted with poultry, fish, and pigs, since insects are part of their natural diet. Several studies have shown that the replacement of SBM or other protein-rich meals (e.g., fish meal) with BSFL meal (**BSFLM**) positively improved the performance of poultry (Ndotono et al., 2022; Schiavone et al., 2019), fish (Khieokhajonkhet et al., 2022; Mastoraki et al., 2022; Renna et al., 2017), and fattening pigs (Chia et al., 2021; Yu et al., 2019). However, the integration of BSFLM in ruminant diets has received limited research attention globally. Two recent studies with steers fed low-quality forage and supplemented with BSFLM or defatted BSFLM (**DBSFLM**) versus conventional supplements like whole cottonseed and SBM found no differences in feed intake or digestibility, resulting in comparable ammonia-N and total volatile fatty acid concentrations in the rumen (Fukuda et al., 2022; Tasci et al., 2024). However, another study reported lower organic matter intake with DBSFLM compared to SBM, while feed digestibility remained similar (Carrasco and Drewery, 2024). Jayanegara et al. (2017) performed an *in vitro* rumen fermentation experiment and found a rather low rumen degradability of BSFLM compared with that of SBM and this was probably attributable to the high –fat content. A high dietary fat content could be suboptimal for dry matter intake (**DMI**), as well as DM and NDF digestibility (Patra, 2013). Therefore, it is advised to defat BSFL before feeding them to ruminants.

We hypothesised that replacing SBM partly or entirely with DBSFLM in isocaloric and isonitrogenous diets would not affect the performance of lactating dairy cows. To date, no *in vivo* study has been conducted on feeding DBSFLM–enriched diets to lactating dairy cows. Therefore, the aim of this study was to investigate the effect of substituting SBM with DBSFLM in diets of lactating dairy cows on milk yield (**MY**) and composition, apparent total-tract digestibility, and feed- and nitrogen use efficiency.

Material and methods

Cows, experimental design, and experimental diets

The experiment was conducted at Mau Flora Ltd., a commercial dairy farm located in Molo, Kenya (0°15′03.6″ latitude South and 35°45′39.6″ longitude East). Twelve lactating Holstein-Friesian dairy cows (average ± SD at the beginning of the trial: days in milk, 109 ± 25.3 d; MY, 21.3 ± 3.84 kg/d; BW, 529 ± 64.2 kg) were used in a four-time replicated 3 × 3 Latin square experimental design, consisting of 3 dietary treatments and 3 periods. Cows were ranked based on MY and subsequently assigned to a Latin square. The squares will be referred to as Very Low Yielders (16.5 ± 1.96 kg/d), Low Yielders (20.2 ± 0.27 kg/d), Medium Yielders (21.9 ± 1.56 kg/d), and High Yielders (26.5 ± 0.93 kg/d). Cows in each square were randomly assigned to one of the following treatments (on a DM basis): (1) a diet containing SBM as a protein source (control, SBM), (2) a diet containing SBM and DBSFLM as protein sources (SBM: DBSFLM, 50: 50), and (3) a diet containing DBSFLM as a protein source (DBSFLM). Each experimental period lasted 18 d, with the first 14 d used for diet adaptation and the last 4 d to collect data and samples.

The BSFL used in this experiment were obtained from the Animal Rearing and Containment Unit (ARCU) of the International Centre of Insect Physiology and Ecology. The larvae were reared on locally available Irish potato peels obtained from a food processing unit in Nairobi, Kenya, following the procedure described by Shumo et al. (2019). At the 5th instar larval stage, when larvae are so-called ‘market’ ready, the larvae were harvested and washed in hot water at 84 °C for 10 min. The washed larvae were then dried using a hot air stainless-steel drying machine (CT-C-III Series, Henan Forchen Machinery Co., Ltd., Henan, China). After 2.5 h of drying at 120 °C, the dried larvae were ground to powder (larvae meal) in a stainless-steel hammer mill and sealed into bags for storage pending defatting.

During defatting, BSFLM was pressed using a single-screw press (Model No.: 6YL-100 Screw Oil Press, Changzhou Machinery & Equipment Import & Export Co., Ltd., Zhengzhou, China), equipped with a 220 mm screw, head, heating mantle, nozzle holder, and nozzles of different diameter (6, 8, and 10 mm). The press had a rotation speed of 1 500 revolutions per min. The press head was preheated at a temperature of 40 ± 10 °C before pressing and monitored continuously during pressing until the temperature of the outflowing oil was 65 ± 5°C. Temperature was measured using a type-K thermocouple connected to a digital temperature sensor (KIMO Instruments, Wilmington, NC, USA). The initial fat content before defatting was 21.5%, which was reduced to 9.3 ± 0.29% (mean ± SD) after defatting.

Cows were housed individually in tie-stalls with an individual feeding trough, a shared water bucket and a mattress as bedding material. Isocaloric and isonitrogenous diets were formulated (NRC, 2001) to meet the nutrient requirements of a lactating dairy cow averaging 125 days in milk, weighing 550 kg of BW and producing 18.6 kg/d (Very Low Yielders), 22.8 kg/d (Low Yielders), 26.9 kg/d (Medium Yielders) or 31.1 kg/d (High Yielders) of milk containing 3.7% fat and 3.2% protein. Diets were mixed manually and offered as total mixed ration (**TMR**). The TMR was offered twice daily at 0900 and 1530 h, ensuring cows had *ad libitum* access (allowing 5–10% refusals per cow each day). All cows received the same amount of roughage and molasses in the TMR (maize silage, 11 kg DM/d; barley straw, 0.9 kg DM/d; molasses, 0.8 kg DM/d). The amount of concentrate in the TMR was adjusted per square, according to the initial MY and was as follows: Very Low Yielders, 5.6 kg DM/d; Low Yielders, 6.8 kg DM/d; Medium Yielders, 8.2 kg DM/d; and High Yielders, 9.7 kg DM/d. The diets were made isocaloric by adding soybean oil to compensate for differences in fat content. As an example, the proportion of each dietary ingredient used to formulate the three diets as well as the nutrient composition of the experimental diet for the Medium Yielders are shown in Table 1. The nutrient composition of the SBM and DBSFLM are shown in Table 2.

Data collection

Feed

Samples of wheat bran, maize germ, sunflower meal, SBM and DBSFLM were collected at the start of the experiment (approximately 250 g). Fresh weight of offered feed and refusals were recorded daily. Samples of feed offered, feed refusals, maize silage and barley straw were collected on days 13–18 (approximately 500 g), and the collection of faeces and urine was on days 15–18.

Table 1
Dietary ingredients and nutrient composition (n = 3 samples per diet) of the experimental diets fed to lactating dairy cows over an 18-d feeding period*.

Item	SBM	Diet[1] SBM: DBSFLM	DBSFLM
Dietary composition, g/kg DM			
Maize silage	524	525	527
Barley straw	45	45	46
Soybean meal	87	44	0
Soybean oil	7	4	0
DBSFLM	0	45	89
Maize germ	170	170	171
Wheat bran	87	87	87
Sunflower meal	22	23	23
Urea	3	3	3
Molasses	36	36	36
Mineral supplement[2]	10	10	10
Calcium carbonate	5	5	5
Dicalcium phosphate	5	5	5
Nutrient composition, g/kg DM			
DM, g/kg as fed	355	350	351
OM	917	911	907
CP	151	152	152
EE	59.9	60.8	61.1
NDF	412	423	432
ADF	215	221	221
ADL	22.2	25.5	26.7
NFC[3]	459	453	448

Abbreviations: DBSFLM = defatted black soldier fly larvae meal; OM = organic matter; EE = ether extract; NFC = non-fibrous carbohydrates.
* Diet formulated to meet the nutrient requirements of a lactating dairy cow averaging 125 days in milk, weighing 550 kg of BW, consuming 20.9 kg of DM/d and producing 26.9 kg/d of milk containing 3.7% fat and 3.2% protein.
[1] Experimental diets: SBM = diet containing soybean meal, SBM: DBSFLM = diet containing soybean meal and defatted black soldier fly larvae meal, DBSFLM = diet containing defatted black soldier fly larvae meal.
[2] Twigalick Maziwa Max containing the following ingredients: 18% calcium, 12% phosphorus, 10% powdered fat, 5% sprayed dried molasses, 16% NaCl, 2.5% magnesium, 5 200 mg/kg zinc, 3 100 mg/kg manganese, 1 800 mg/kg copper, 310 mg/kg iodine, 85 mg/kg cobalt, 28 mg/kg selenium.
[3] NFC = 1 000 – [CP + NDF + EE + ash] (NRC, 2001).

Table 2
Chemical composition of soybean meal (SBM) and defatted black soldier fly larvae meal (DBSFLM).

Nutrient composition, g/kg DM	SBM	DBSFLM
DM, g/kg as fed	925	948
OM	937	834
CP[1]	564	570
EE	8.2	93.0
NDF	115	163
ADF	71.5	97.9
ADL	2.0	26.0
Chitin[2]	–	72.0
Nitrogen-to-protein conversion factor[3]	5.36	5.02

Abbreviations: OM = organic matter; EE = ether extract.
[1] CP = total N × 6.25.
[2] Chitin = ADF – ADL (Hahn et al., 2018).
[3] Nitrogen-to-protein conversion factor = total amino acid content (g/kg DM; Table 3) / total nitrogen content [1] (g/kg DM).

The collected samples were subsequently vacuum packed in plastic bags (material: PA/PE; thickness: 0.100 mm; Wartmann WM-1506 EL, Tilburg, The Netherlands) and stored at −18℃ until further analyses. Before analysis, samples of offered feed and refusals were thawed and pooled by cow per period proportional to quantities fed and refused.

Milk and live weight

Cows were milked 3 times a day at 0330, 1100 and 1730 h. Milk yields were recorded daily, and milk samples (approximately 0.5 L) were collected at each milking from days 15–18. At the end of the day, the three milk samples were pooled by cow proportional to MY and stored in the fridge at 7 °C. At the end of the collection period, samples of the 4 consecutive collection days (days 15–18) were pooled by cow proportional to MY to create a duplicate sample per cow. The samples were stored at −20 °C until further analyses. Cows were weighed on days 1, 7 and 14 of each period, just before the second milking (1100 h) using an animal weighing scale (Master, Ace Corporation, Maharashtra, India).

Faeces

Total faeces for each cow was collected daily by scooping from the floor using a shovel every 2 h and stored in 50 L drums from days 15–18. On day 15 at 0900 h, the drums were placed in the tie-stall for faeces collection and after every 24 h, the total faeces were weighed and consequently mixed to homogenise. A sample of 0.5% of the total faecal weight was collected into a plastic jar and stored at −20 °C until further analyses. The remaining faeces were disposed of. Faecal samples from the 3 subsequent days were stored in the same plastic jars to create a pooled sample by cow per period. Before analyses, samples were thawed and mixed to homogenise.

Urine

Total urine was collected using urine collection bags designed for cows from days 15–18. On day 15 at 0900 h, the bags were attached to the touch fastener ('Velcro') that was glued on the shaved areas around the vulva of the cows. The touch fastener allowed the bag to be easily removed and reattached to the cow. Silicon tubes attached to the base of the bags were used to direct urine into barrels prefilled with 200 mL of 20% sulphuric acid. The animals were tethered in their pens to limit movement and tampering with the urine collection bags. After every 24 h, the total volume of urine was weighed. A 300 mL sample was collected, and the pH was adjusted to between 2 and 3 by adding 20% sulphuric acid. Thereafter, the urine samples were stored at −20 °C. After the collection period, samples were thawed, and gently shaken by hand to homogenise and the samples of the last 3 consecutive days (days 16–18) were proportionally pooled by cow according to daily urine volume.

Chemical analyses of samples

All feed, milk and urine samples were submitted to the International Livestock Research Institute (ILRI; Nairobi, Kenya) for processing and laboratory analysis. Composite TMR, refusals, faeces and samples of the individual diet ingredients (e.g. SBM, DBSFLM, maize germ, etc.) were dried (55 °C in a forced-air oven for 48 h) and ground in a hammer mill (MF 10 basic, IKA, Werke GmbH & CO. KG, Staufen, Germany) to pass a 1-mm screen. Subsamples of 5 g of the ground samples were sent to Cropnuts (Limuru, Kenya), for crude fat analyses (ISO 6492, 1999a). The remaining ground samples were analysed for DM (105 °C overnight; ISO 6496, 1999b), ash (ISO 5984, 2022), nitrogen (**N**; method 990.03; AOAC, 2006), and NDF, ADF and ADL, which were analysed sequentially (Ankom200 Fiber Analyzer; Ankom Technology Corp.; van Soest et al., 1991). A conversion factor of 6.25 was used to calculate the CP content from the N content in the feed.

The fatty acid (Table 3) and amino acid composition (Table 4) of SBM, soybean oil and DBSFLM were analysed by NutriControl BV (Veghel, The Netherlands). The fatty acid composition was determined according to ISO/TS 17764-1/2 (ISO, 2002a, 2002b). In short, the fat underwent saponification and the fatty acids were esterified through the introduction of methanol and boron trifluoride. Subsequently, capillary gas chromatography, in conjunction with a flame ionisation detector, was used to analyse the resulting methyl esters (Agilent 7890B Gas Chromatograph, Agilent Technologies, Santa Clara, United States). To determine the amino acid composition, SBM and DBSFLM samples underwent oxidation and hydrolysis tailored to the specific amino acids of interest. Alternatively, tyrosine, proline and hydroxy-proline were solely hydrolysed with an acidic solvent. Subsequently, the amino acids were separated using ion chromatography (Sykam S 433 Amino Acid Analyzer, Eresing, Germany). After this separation, photometric detection occurred at two distinct wavelengths, depending on the type of amino acid being analysed.

Table 3
Fatty acid composition (g/kg of total fatty acids) of soybean meal (SBM), soybean oil (SBO) and defatted black soldier fly larvae meal (DBSFLM).

Parameter	SBM	SBO	DBSFLM
Saturated fatty acids			
Pelargonic acid (C9)	0.0	0.0	1.3
Capric acid (C10)	0.0	0.0	5.0
Lauric acid (C12)	3.1	0.0	174.6
Myristic acid (C14)	1.5	0.0	45.2
Pentadecanoic acid (C15)	0.0	0.0	6.3
Palmitic acid (C16)	155.4	116.8	207.3
Margaric acid (C17)	0.0	0.0	5.0
Stearic acid (C18)	41.5	41.3	47.7
Arachidic acid (C20)	3.1	4.0	1.3
Behenic acid (C22)	4.6	4.0	2.5
Lignoceric acid (C24)	1.5	1.0	0.0
Monosaturated fatty acids			
Pentadecenoic acid (C15:1)	0.0	0.0	2.5
Palmitoleic acid (C16:1)	1.5	2.0	60.3
Hexadecenoic acid (C16:1n9)	0.0	0.0	8.8
Heptadecenoic acid (C17:1)	0.0	0.0	2.5
Oleic acid (C18:1)	186.2	238.7	282.7
Eicosenoic acid (C20:1)	0.0	1.0	0.0
Gondoic acid (C20:1n9)	0.0	1.0	0.0
Polyunsaturated fatty acids			
Linoleic acid (C18:2n-6)	498.5	494.5	70.4
Linolenic acid (C18:3n-3)	75.4	75.5	6.3

Table 4
Amino acid concentration (g/kg DM) of soybean meal (SBM) and defatted black soldier fly larvae meal (DBSFLM).

Items	SBM	DBSFLM
Indispensable amino acids		
Arginine	38.1	25.6
Histidine	13.1	15.6
Isoleucine	23.1	20.1
Leucine	31.9	32.5
Lysine	31.9	30.7
Methionine	6.5	7.7
Phenylalanine	25.6	19.6
Threonine	18.9	17.8
Valine	24.0	27.7
Dispensable amino acids		
Alanine	22.2	32.9
Aspartic acid	57.6	43.5
Cysteine	5.8	4.1
Glutamic acid	21.7	25.5
Glycine	93.5	59.3
Proline	26.3	27.6
Serine	25.0	19.6
Tyrosine	18.7	48.1

Total N content in milk and urine was determined using micro-Kjeldahl method (method 988.05; AOAC, 1990). The CP content in milk was calculated by multiplying the total N content by 6.38 (Maubois and Lorient, 2016). The other batch of milk samples was submitted to HappyCow (Nakuru, Kenya) and analysed for milk fat and lactose content (Lactoscan S, Milkotronic Ltd., Nova Zagora, Bulgaria).

Calculations and statistical analyses

Dry matter intake was calculated as the amount of feed offered (kg/d) minus the amount of feed refused (kg/d), adjusted for moisture content (Olijhoek et al., 2023). Intake of nutrients was calculated from DMI and the respective nutrient content in the diet. Apparent total-tract digestibility was calculated as nutrient intake minus faecal excretion divided by nutrient intake (g DM/d). The average DMI of days 13–18 and faecal excretions of days 15–18 were used for the apparent total-tract digestibility calculation. The feed-efficiency was calculated by dividing the MY by DMI and the N-efficiency by dividing the nitrogen content in MY by the nitrogen content in DMI. The nitrogen-to-protein conversion factors of SBM and DBSFLM were calculated as the ratio of the total amino acid content (g/kg DM) to total nitrogen content (g/kg DM, Boulos et al., 2020).

Data on milk yield, milk composition, DMI, apparent total-tract digestibility, N-efficiency and feed-efficiency were analysed using the PROC MIXED procedure in SAS (version 9.4, SAS Institute Inc., Cary, NC) with the model:

$$Y_{ijkl} = \mu + T_i + L_j + P_k + C_l(L_j) + \varepsilon_{ijkl}$$

where variable Y_{ijkl} depends on μ as the overall experimental mean, T_i as treatment effect (i = SBM, SBM: DBSFLM, DBSFLM), L_j as Latin square effect (j = 1–4), P_k as period effect (k = 1–3), $C_l(L_j)$ as cow effects within Latin square (l = 1–12), and an Error term (ε_{ijkl}) for random residuals. Data are presented as least squares means.

To further investigate the effects of treatment, quadratic contrasts were included in the model to assess the presence of non-linear trends. These contrasts were constructed using coefficients (1, −2, 1) for the three treatment levels, which correspond to testing the second–order (quadratic) term in a polynomial contrast. The statistical significance of this contrast was determined using an F-test in the MIXED procedure. Statistical significance was declared at $P \le 0.05$. During periods 1 and 3, a few cows showed signs of illness, 1 and 2 cows, respectively. Data of these cows were excluded from the dataset, but their data were retained for the other periods.

Results

Feed intake and total-tract digestibility

Cows fed SBM or SBM: DBSFLM had higher DMI than cows fed DBSFLM ($P \le 0.02$; Table 5). In addition, organic matter (**OM**) and CP intake differed among treatments over the total collection period, with cows fed SBM or SBM: DBSFLM having higher OM intake compared with cows fed DBSFLM ($P \le 0.01$). Moreover, cows fed with SBM had higher CP intake compared with cows fed with SBM: DBSFLM or DBSFLM ($P \le 0.05$). No treatment effects for NDF and ADF intake were observed. Except for NDF and ADF, no difference in apparent total-tract digestibility was found among treatments. Cows fed SBM had a lower total tract digestibility of NDF and ADF compared with cows fed DBSFLM ($P = 0.008$ and $P = 0.024$, respectively). Dry matter intake increased over the periods from 15.9, 17.3 to 18.5 kg/d in periods 1, 2 and 3, respectively ($P \le 0.002$). Organic matter, NDF and non-fibrous carbohydrates (NFC) intake also increased over the periods ($P \le 0.002$, $P \le 0.05$ and $P < 0.001$). CP and fat intake increased between periods 1 and 2, and periods 1 and 3 ($P \le 0.05$) and ADF intake increased between periods 1 and 3 ($P = 0.001$). As expected, a square effect was observed for all nutrient intake parameters, with emphasis on a higher intake for the high yielders (square 3 and square 4) compared with the low yielders (square 1 and square 2, $P \le 0.018$). Only a square effect was observed for CP apparent total-tract digestibility with lower values for square 1 compared with squares 2 and 4 ($P \le 0.05$). No significant quadratic trends were observed for feed intake and total-tract digestibility.

Table 5
Effect of soybean meal (SBM) and defatted black soldier fly larvae meal (DBSFLM) on nutrient intake and apparent total-tract digestibility in lactating cows.

Item	Treatment			SEM	*P*-value		
	SBM	SBM: DBSFLM	DBSFLM		Treatment	Period	Square
Nutrient intake (kg/d)							
DM	17.6[a]	17.5[a]	16.6[b]	0.18	0.003	<0.001	<0.001
OM	16.2[a]	16.0[a]	15.1[b]	0.17	<0.001	<0.001	<0.001
CP	2.68[a]	2.54[b]	2.50[b]	0.03	0.003	<0.001	<0.001
EE	1.01	1.04	1.01	0.02	0.629	0.004	<0.001
NDF	7.21	7.45	7.17	0.09	0.186	<0.001	<0.001
ADF	3.78	3.89	3.68	0.05	0.078	0.001	<0.001
NFC[1]	5.27[a]	4.93[b]	4.39[c]	0.08	<0.001	<0.001	<0.001
Apparent total-tract digestibility (%)							
DM	61.8	63.4	64.1	1.22	0.422	0.555	0.120
OM	64.9	66.4	67.3	1.12	0.325	0.510	0.085
CP	67.8	66.5	67.8	1.29	0.793	0.682	0.010
EE	86.2	87.1	90.3	1.81	0.280	0.032	0.626
NDF	46.7[a]	51.0[ab]	54.0[b]	1.44	0.008	0.317	0.071
ADF	45.6[a]	49.7[ab]	52.0[b]	1.49	0.024	0.202	0.052
NFC[a]	84.6	85.2	83.5	0.97	0.5374	0.206	0.207

Abbreviations: OM = organic matter; EE = ether extract; NFC = non-fibrous carbohydrates.
[a,b,c] Within a row, means without a common superscript letter differ ($P < 0.05$).
[1] NFC = DM – [NDF + CP + EE + ash].

Table 6
Effect of soybean meal (SBM) and defatted black soldier fly larvae meal (DBSFLM) on body weight (BW), dry matter intake (DMI), milk production and composition in lactating cows.

Item	Treatment			SEM	*P*-value		
	SBM	SBM: DBSFLM	DBSFLM		Treatment	Period	Square
BW (kg)	540	539	538	1.11	0.301	<0.001	<0.001
DMI (kg/d)	17.6[a]	17.5[a]	16.6[b]	0.18	0.003	<0.001	<0.001
MY (kg/d)	23.6	23.4	22.3	0.48	0.159	0.079	<0.001
MY/DMI (kg/kg)	1.34	1.33	1.34	0.03	0.946	0.176	0.166
ECM[1] (kg/d)	23.7	24.4	23.5	0.65	0.713	0.200	<0.001
ECM/DMI (kg/kg)	1.35	1.38	1.41	0.04	0.588	0.052	0.447
Milk NE_L[2] (Mcal/d)	16.8	17.4	16.7	0.46	0.640	0.106	<0.001
Fat (g/kg)	37.1	39.6	38.9	0.84	0.154	<0.001	0.145
Fat (kg/d)	0.86	0.91	0.87	0.03	0.595	0.036	<0.001
Protein (g/kg)	30.4	30.5	32.2	0.68	0.131	0.025	0.077
Protein (kg/d)	0.71	0.71	0.72	0.02	0.983	0.007	<0.001
Lactose (g/kg)	52.1	52.8	51.6	0.46	0.271	<0.001	0.919
Lactose (kg/d)	1.19	1.19	1.12	0.02	0.054	<0.001	<0.001
Neff[3] (%)	26.3	27.3	28.3	0.70	0.186	0.431	0.057

Abbreviations: DMI = DM intake; MY = milk yield; ECM = energy corrected milk; NE_L = net energy lactation; Neff = nitrogen efficiency.
[a,b] Within a row, means without a common superscript letter differ ($P < 0.05$).
[1] ECM = [(0.3246 × MY, kg/d) + (12.86 × fat yield, kg/d) + (7.04 × protein yield, kg/d)] (Bernard, 1997).
[2] Milk NE_L, Mcal/d = MY, kg/d × [(0.0929 × fat, %) + (0.0563 × true protein, %) + (0.0395 × lactose, %)] (NRC, 2001).
[3] $N_{eff} = \frac{MY\ (kg/d) \times N\ milk\ (g/kg\ of\ milk)}{DMI\ (kg/d) \times digestible\ N\ diet\ (g/kg\ of\ DM)} \times 100\%$.

BW, milk production and –composition

Body weight and MY did not differ between treatments (Table 6). The difference in DMI and absent difference in MY did not lead to a difference in feed-efficiency. Although cows fed with SBM had a higher lactose yield compared to those fed DBSFLM ($P = 0.07$), no difference in MY parameters and milk composition parameters were found. However, some periodical effects were identified. Across all treatments, higher BW was observed in periods 2 and 3 compared with period 1 ($P < 0.001$), with increases of 21.3 and 20.8 kg, respectively. Period effects on all milk composition and yield parameters were found. This period effect was mainly observed between periods 1 and 2, and periods 1 and 3, with higher milk fat percentage and yield, lower milk protein percentage and yield, and lower lactose percentage and yield in period 1 compared with the other periods. Some square effects were observed since cows were assigned to squares based on milk production. This square effect was seen for BW, MY, energy–corrected milk, net energy lactation, fat, protein, and lactose yield, with higher outcomes for high yielders ($P \leq 0.039$). No significant quadratic trends were observed for BW, milk production and –composition.

Nitrogen metabolism

The nitrogen secretion in milk, excretion in faeces and urine, and retention did not vary among treatments (Table 7). The N-efficiency did not differ among treatments. However, between periods 1 and 2, and 1 and 3 a difference in N secretion in milk was observed ($P = 0.008$ and $P = 0.01$, respectively), with lower excretions in period 1 compared with periods 2 and 3. Higher N values in faeces were observed in period 3 compared with period 1. Some square effects were observed for all elements of the N-balance, mainly with a difference between the low yielders (squares 1 and 2) and the high yielders (squares 3 and 4). No significant quadratic trends were observed for nitrogen metabolism.

Discussion

This is the first study to examine the effect of replacing SBM with DBSFLM in dairy cow diets. Until now, studies using BSFLM or DBSFLM as ruminant feed were either done with steers (Carrasco and Drewery, 2024; Fukuda et al., 2022; Tasci et al., 2024), or sheep (Rahman et al., 2021). Our findings show that SBM can be replaced with DBSFLM, since it caused no significant differences in MY, composition, apparent total-tract digestibility, feed and N-efficiency. This is a promising finding in the search for novel feed protein sources that can contribute to circular food systems.

The composition of fatty acids and amino acids in DBSFLM can vary widely depending on factors including substrate fed to the insects, processing methods, and defatting techniques. The lauric acid content in our DBSFLM was notably lower than in studies of Meneguz et al. (2018) and Spranghers et al. (2017), in which the lauric acid content frequently exceeded 50% of the total fatty acids in BSFLM. This may be due to differences in substrate fed to the insects, which can significantly affect the fatty acid composition (Ewald et al., 2020). Additionally, it is worth noting that, as observed in other research, variations in drying methods influence the fatty acid composition (Zulkifli et al., 2022). Likewise, the method used for defatting could also result in distinct fatty acid profiles, potentially explaining the observed differences in comparison to the literature. The present study also observed lauric acid in SBM, but this has not been reported in earlier studies (Banaszkiewicz, 2011; Lee et al., 2013; Widmer et al., 2008). The fatty acid composition of SBM and soybean oil was similar and did not deviate from that presented in the literature (Lee et al., 2013; Widmer et al., 2008). Additionally, the amino acid composition of SBM aligned closely with previously documented findings (Lee et al., 2013; Widmer et al., 2008). The amino acid content of DBSFLM was slightly higher than reported by Spranghers et al. (2017), but this may be due to the lower fat content which leads to a higher protein content or due to differences in rearing substrate (Dumas et al., 2018; Schiavone et al., 2017).

Dry matter intake was higher for cows fed SBM compared with those fed DBSFLM, likely due to differences in the composition of the feeds. However, since the NDF composition of plants and insects is different, this may interfere with the interpretation of the differences in DMI between diets. Generally, when NDF originates from plant cells, a high NDF content can result in an increased rumen retention time of feed. If insect NDF has properties equal to those of plant NDF, this could explain the lower DMI in the DBSFLM treatment (Mertens, 1994). On the other hand, Warner et al. (2013) discussed that the NDF fractional passage rate varies among feed sources (e.g. NDF of concentrate vs roughage), which may also contribute to the differences in DMI. Nevertheless, it is also possible that reduced feed consumption with DBSFLM is related to lower palatability due to specific nutrients in DBSFLM,

Table 7
Effect of soybean meal (SBM) and defatted black soldier fly larvae meal (DBSFLM) on nitrogen (N) balance in lactating cows.

Item[1]	Treatment			SEM	*P*-value		
	SBM	SBM: DBSFLM	DBSFLM		Treatment	Period	Square
N intake, g/d	428[a]	407[b]	400[b]	5.05	0.003	<0.001	<0.001
Milk							
N (g/d)	111	112	112	3.15	0.983	0.007	<0.001
N (% of intake)	26.3	27.3	28.3	0.70	0.176	0.431	0.057
Faeces							
N (g/d)	135	134	127	4.63	0.470	0.012	<0.001
N (% of intake)	32.2	33.5	32.2	1.29	0.793	0.682	0.010
Urine							
N (g/d)	86.9	89.2	85.9	2.30	0.678	0.167	<0.001
N (% of intake)	20.2	22.1	21.3	0.64	0.191	0.275	<0.001
Retained							
N (g/d)	94.8	71.7	74.0	7.58	0.107	0.976	<0.001
N (% of intake)	21.3	17.1	18.1	1.68	0.255	0.821	0.026

Abbreviations: N = nitrogen.
[1] N retained = N apparently retained (N intake – milk N – urine N – faecal N) (Maubois and Lorient, 2016).
[a,b] Within a row, means without a common superscript differ ($P < 0.05$).

such as its fatty acid profile. For instance, DBSFLM had 17.5% lauric acid (C12:0) compared to only 3% in SBM. Previous studies have shown that lauric acid supplementation can decrease DMI, potentially due to reduced palatability or impaired fibre degradation in the rumen (Dohme et al., 2004; Klop et al., 2017; Külling et al., 2002). However, the concentration of lauric acid in our diets was low compared to that reported in other studies (0.14% vs ≥ 1.95% per kg DMI), making it unclear if such small quantities could explain the lower DMI. The NDF and ADF intake did not differ among treatments, possibly due to the higher NDF and ADF contents in the DBSFLM diet compared with the SBM diet (Table 2). Future studies should investigate the long-term effects of DBSFLM on feed intake.

A period effect was observed for DMI, which showed an increase over the course of the experiment. This was contrary to our expectations, we expected feed intake to decrease due to declining milk production as cows progressed further into lactation during the experiment (Koenen and Veerkamp, 1998). The increase in DMI can have several causes. First, the experiment was conducted at a commercial farm, so animals were not used to being handled and housed individually. Change in housing can lead to stress and consequently a lower feed intake (van Ackern et al., 2021). An increase in feed intake could therefore be a result of adapting to their new housing environment. The adaptation phase of 14 d prior to the first measurement week may not have been long enough for the animals to adapt to the experimental conditions. Second, the urine collection process might have also induced another stress response, given that they were never exposed to such practices prior to the experimental set-up. This initial stress response can also result in a lower feed intake. Finally, *ad libitum* access to feed can be a stimulus for gradual rumen enlargement and consequently an increase in feed intake (Niehaus, 2009).

Chitin (i.e. part of the exoskeleton of insects) forms a matrix with proteins, lipids and other compounds (e.g. minerals), and is broken down by enzymes such as chitinase, which creates chitosan (Shahidi and Abuzaytoun, 2005). It is believed that most animal species, including cows, do not produce chitinase making chitin an indigestible fibre (Dorper et al., 2021). This would also imply that the nitrogen content of BSF that is available for the animal is overestimated when using the normal nitrogen-to-protein conversion factor of 6.25. Finke (2007) expected that only a small portion of the total nitrogen would come from the nitrogen in chitin, while other researchers found that nitrogen in chitin accounted for as much as 6.89% of the total nitrogen (Diener et al., 2009; Wong et al., 2019). Janssen et al. (2017) even recommended using a conversion factor of 4.76 instead of 6.25. Our study showed a conversion factor of 5.02 (Table 2) and this would mean that we highly overestimated the true protein content of the DBSFLM, which should have resulted in a lower performance. However, no treatment effect was found for MY, milk composition, and BW gain. This could be explained by the fact that the protein meals were not analysed for all amino acids, as well as the overestimation of the conversion factor of SBM when using 6.25 instead of the measured conversion factor of 5.36. However, this lower conversion factor of SBM may also be attributed to the presence of non-protein nitrogen in soybeans (Bhatty et al., 1973). On the other hand, Kopečný et al. (1996) and Williams et al. (2020) observed chitinolytic bacteria and enzymes, respectively, in the rumen of cows. These chitinolytic enzymes likely play a role in the digestion of rumen fungi, which are known to contain chitin-rich cell membranes (Williams et al., 2020). These findings suggest that chitin can be digested by rumen fermentation, which may make N available as well. However, further research is needed to understand the digestion of insect chitin by cows.

In the realm of plant-based products, the composition of different fibre components is widely recognised. The ADF primarily consists of cellulose and lignin, while NDF includes cellulose, lignin, and hemicellulose. The method of van Soest et al. (1991) was initially designed to assess NDF and ADF content in forages but has also been adapted for measuring fibre content in insects, despite the inherent differences in fibre composition. For example, chitin is a linear biopolymer of N-acetyl-d-glucosamine (GlcNAc), which is structurally similar to cellulose, but has acetamide groups at the C2 positions of the glucose unit (Dutta et al., 2004). Since chitin is structurally similar to cellulose, chitin has been calculated by subtracting ADL from ADF. This considering that ADF in BSFLM contains chitin and catecholamines, and ADL only catecholic compounds (Hahn et al., 2018; Nafisah et al., 2019). Based on this approach, the chitin content of the DBSFLM used in our study is 7.2%. It is still unclear what the molecular structure of NDF in insects is.

The NDF apparent total-tract digestibility was higher for the DBSFML treatment compared with the SBM treatment. Mechanical processing of BSFL (i.e., drying, defatting, and milling) might have enhanced NDF digestion by breaking down the insect's physical structure, increasing its accessibility to rumen microbes, which could explain the higher apparent total-tract digestibility observed for the DBSFLM treatment. (Gomide et al., 2023). However, an *in vitro* study with cricket meals also showed an improved digestibility of NDF (Ahmed et al., 2021), demonstrating that enhanced NDF digestibility does not necessarily hinge solely on particle size but could also be due to the nutritional composition of insects (e.g. BSFL). Rumen fermentation studies with fistulated cows could give more insights into the digestion of NDF.

Apart from NDF and ADF, no treatment effect was found on the apparent total-tract digestibility of the other nutrients. Previous studies found different associations between the use of BSFLM or DBSFLM and digestibility, possibly due to differences in the fat content of the diets (Ahmed et al., 2021; Carrasco and Drewery, 2024; Fukuda et al., 2022; Jayanegara et al., 2017; Tasci et al., 2024). Our study included 9% of DBSFLM, while in an *in vitro* fermentation study, Jayanegara et al. (2017) included 40% of BSFLM in the diet as a replacement for SBM. Additionally, we used a defatted product of BSFLM (9.6% DM ether extract) instead of a full–fat BSFLM (29.1–34.8% ether extract). A high fat content in the diet lowers rumen microbial activity (Martínez et al., 2013), potentially explaining the decrease in *in vitro* rumen degradability observed by Jayanegara et al. (2017). Jayanegara et al. (2017) also hypothesised that chitin had a negative effect on ruminal fermentation and digestibility. However, no adverse effects on digestibility were found when BSFL or DBSFLM were fed to beef steers (Carrasco and Drewery, 2024; Fukuda et al., 2022; Tasci et al., 2024) or when crickets were included in *in vitro* studies with rumen fluid of non-lactating dairy cows (Ahmed et al., 2021).

High nitrogen retention was observed across all treatments, with our retention values nearly three times higher than the average estimated nitrogen retention value of 6.7% reported in the meta-analysis by Spanghero and Kowalski (2021). Despite a noticeable period effect on BW, with periods 2 and 3 showing higher BW compared to period 1, this difference in BW could not be attributed to nitrogen retention since nitrogen retention did not vary among the periods. Nitrogen volatilisation in the form of ammonia can be an explanation of the reduced N measured (Sigurdarson et al., 2018). Additionally, Spanghero and Kowalski (1997) found a decrease of 15% in measured faecal N concentration when using air drying compared to the same sample analysed without drying. Correcting for this underestimation of faecal N concentration would result in a 6% reduction in N retention.

The feed conversion rate (feed input divided by resulting net production) and feed efficiency (resulting net production divided by feed input) are often used to compare different feeds or farming systems. However, these methods do not account for the human

–edible food that are used for livestock production (i.e. feed-food competition). Livestock can convert various feed resources, including by-products from the food industry and grassland, into animal source products (Muscat et al., 2021; van Zanten et al., 2019). To evaluate the efficiency of converting feed into animal source products, the human-edible feed conversion efficiency or net food production can be used (Renna et al., 2020; Verduna et al., 2020). These metrics consider the contribution to human-edible protein by comparing the human-edible portion of milk to that of the feed. Previous studies by Wilkinson (2011) and Ertl et al. (2015) have estimated the human-edible protein proportions of various crops and by-products, including SBM with values ranging from 0.5 to 0.91. However, to date, no human edible proportion has been assigned to BSFL. Even though insects were once a traditional food source and have a great potential as food, consumer acceptance remains generally low (Alemu and Olsen, 2018; Bessa et al., 2020; Hartmann and Siegrist, 2017). As a result, BSFL could be considered to have a low human-edible proportion, potentially offering a more sustainable option in circular food systems compared to SBM.

The use of insect meal as an alternative to conventional fish and soybean meal in livestock feed is gaining global attention. However, its competitiveness with conventional feed is often questioned due to high production costs (Siva Raman et al., 2022; Veldkamp et al., 2022). Although this study did not assess the economic feasibility of replacing SBM with DBSFLM, previous research in Kenya has demonstrated the cost-effectiveness of insect-based feeds. For instance, BSFLM has been shown to be a viable alternative to fish and soybean meal in broiler and layer diets (Onsongo et al., 2018; Sumbule et al., 2021; Wamai et al., 2024). Furthermore, a hedonic pricing model identified total digestible nutrients (**TDN**) and CP as key factors influencing beef cattle feed prices (Drewery et al., 2022). Optimising BSFL rearing substrates could improve the TDN and CP content of BSFL, potentially improving its economic viability. However, processing methods play a crucial role. For example, defatting BSFLM increases CP but reduces TDN, which may negatively affect feed pricing (Drewery et al., 2022). Further research is needed to evaluate the feasibility of DBSFLM in dairy cow diets.

Conclusion

Defatted black soldier fly larvae meal can potentially be used as an alternative protein source for dairy cow diets. The inclusion of DBSFLM into dairy cow diets significantly lowered the feed intake without compromising milk production and composition. There was a considerable higher digestibility of fibre in cows receiving a diet with DBSFLM. Further research is needed to understand the mechanisms influencing the digestion of insect meals in dairy cow feeding regimes. In addition, the long-term effect of DBSFLM on dairy cow performance should be investigated. This study highlights the potential of DBSFLM to contribute to circular food systems by lowering the competition between human and animal nutrition.

Ethics approval

The experimental procedures were approved by the Institutional Animal Care and Use Committee of Kenya Agricultural and Livestock Research Organization (KALRO; approval code no. KALRO-VSRI/IACUC028/16032022).

Data and model availability statement

None of the data were deposited in an official repository. All data are available from the authors upon request.

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

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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