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# **ORIGINAL PAPER**

# ARTICLE IN PRESS

# Effects of ensiled wheat straw with brewer's spent yeast on milk production and nutrient utilisation in dairy cows

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**KEY WORDS:** by-product, greenhouse gases, milk yield, treated feed

Received: 22 March 2025 Revised: 16 June 2025 Accepted: 23 June 2025 ABSTRACT. Brewer's spent yeast (BSY) is one of the most abundant byproducts of the brewing industry that creates environmental disposal challenges. However, its high nutritional value offers an opportunity to upgrade low-quality roughages in dairy cows' nutrition. This study evaluated the effects of ensiled wheat straw with BSY and molasses on feed intake, digestibility, milk yield, and methane production in dairy cows compared to untreated wheat straw (UWS). Wheat straw was ensiled with BSY (0.6 l/kg) and molasses (10%) for five weeks. Three mid-lactation dairy cows (3/4 Friesian × Boran) were assigned to three diets: UWS + 0.5 kg concentrate/l of milk (UWS+0.5C), ensiled wheat straw (EWS) + 0.5 kg concentrate/l of milk (EWS+0.5C), and EWS + 0.3 kg concentrate/l of milk (EWS+0.3C). EWS with BSY doubled crude protein content and reduced in vitro methane production by 28.3% (P < 0.01). EWS diets increased nutrient intake and digestibility (P < 0.01), except for neutral detergent fibre digestibility (P > 0.05). Milk yield increased by 1.13 kg/day in EWS+0.3C (P < 0.001), along with an improvement in milk total solids. Predicted methane emissions decreased by 15.7% in EWS+0.5C (P < 0.01), and net profit increased by 1.21 USD/day/cow in EWS+0.5C. These results demonstrate that ensiling wheat straw with BSY and molasses is an effective strategy to improve feed quality, milk production, sustainability emissions, and economic returns in dairy farming.

# Introduction

Global food security and climate change have become pressing concerns for animal production systems (Varzakas and Smaoui, 2024). Animal feed shortages and the rising costs of conventional roughage and concentrates have a direct effect on the quantity and quality of livestock production,

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thereby affecting food security (Makkar, 2018; Alexandre Muchanga et al., 2023). In response to these challenges, there is growing interest in utilising agro-industrial by-products as alternative feed resources (Ahmed et al., 2024; Romelle Jones et al., 2024). Brewer's spent yeast (BSY), a major residue of the brewing industry, is valued for its high protein content (30–74%), vitamins, minerals, essential amino acids, and trace elements that promote animal health, and enhance immunological function, and feed conversion efficiency (Amorim et al., 2016; Jaeger et al., 2020; Estévez et al., 2022; Zeko-Pivač et al., 2023).

In developing countries like Ethiopia, the rapid expansion of beer production generates approximately 360 758 hectolitres of BSY per year (Yadessa et al., 2023). If not effectively utilised, this byproduct may contribute to environmental pollution (Gokulakrishnan et al., 2023). However, emerging research suggests that incorporating BSY into animal diets can increase milk production and reduce enteric methane emissions (Bryant et al., 2021). This effect is attributed to the high content of  $\beta$ -glucans and mannan-oligosaccharides in BSY, which selectively stimulate the proliferation of beneficial ruminal microbial populations, improving fibre digestion and overall nutrient utilisation (Pszczolkowski et al., 2016; Avramia and Amariei, 2021; Ciobanu et al., 2024; Wei et al., 2024).

Methane (CH<sub>4</sub>) is the second most important greenhouse gas contributing to climate change, following carbon dioxide (Króliczewska et al., 2023), necessitating dietary interventions that modulate rumen fermentation to reduce emissions (Gonzalez-Ronquillo and Toro-Mujica, 2023; Lileikis et al., 2023). While prior studies have supplemented BSY directly in concentrates (Geberemariyam et al., 2024), tested BSY straw mixtures only in vitro (Bryant et al., 2021), or evaluated BSY silages in small ruminants rather than dairy cattle (Oancea et al., 2023), no study has yet examined BSY-ensiled wheat straw fed to lactating cows under varying concentrate allowance. This study addresses that gap through a  $3 \times 3$  Latin square design, testing BSY- and molasses-treated wheat straw under two concentrate-to-milk ratios (0.5 and 0.3 kg/l, respectively), reflecting feeding strategies common in East Africa. Using a recently validated nutrient intake-based methane prediction model (Wang et al., 2024), we present the first in vivo evidence that BSY-ensiled wheat straw can reduce dietary concentrate requirements, maintain or increase milk yield, lower enteric methane intensity, and improve margin over feed cost. These findings advance earlier work on forage-based methane mitigation, offering a scalable, circular-economy solution for brewing regions in Africa and similar agroecosystems.

This study tested the hypothesis that incorporating BSY-ensiled wheat straw, combined with varying levels of concentrate supplementation, would improve feed intake, nutrient digestibility, milk production and composition, while also offering economic and environmental benefits, including reduced methane production. The objective was to assess the effects of BSY- and molasses-treated ensiled wheat straw on feed intake, digestibility, milk yield, and methane production compared to untreated wheat straw under different concentrate supplementation regimes.

#### Material and methods

#### Ethics approval and consent to participate

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the National Animal Feeds and Nutrition Research of Ethiopia. The study was conducted in accordance with the ARRIVE guidelines and the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

#### **Experimental feed**

The wheat variety (Danda'a) was cultivated following recommended agronomic practices, with fertiliser applied at rates of 158 kg/ha for nitrogen (N), phosphorus (P), and sulphur (S) (NPS) and 85 kg/ha for urea. The crop was harvested at physiological maturity (130 days) at the Holeta Agricultural Research Center (HARC). Wheat straw (WS) and grain were separated, straw was dried, baled, and stored under shaded conditions at the centre's dairy farm for three months.

The commercial concentrate was purchased from a feed processing factory in Addis Ababa, Ethiopia. Its nutritional composition (g/kg dry matter (DM)) was as follows: DM (923), organic matter (OM, 920), crude protein (CP, 172), neutral detergent fibre (NDF, 427), and acid detergent fibre (ADF, 273). Molasses was purchased from Wonji Sugar Factory in Ethiopia, transported to the experimental site and stored in barrels. Autolysed liquid was obtained from the Heineken Brewery Factory in Addis Ababa, Ethiopia. The BSY was subjected to heating at a temperature above 80 °C and cooled for 12 h before being mixed with the feed. The nutritional values of molasses and BSY (g/kg DM) were as follows: molasses – DM (720), OM (860),

CP (43), NDF (3.45), ADF (1.25); BSY – DM (121), OM (908), CP (422), NDF (2.01), and ADF (1.11).

WS was chopped into 5–10 cm pieces. BSY was then mixed at a rate of 0.6 l/kg of chopped WS, and molasses was added at 10% of the total weight of the mixture. The mixture was compacted with heavy weights and ensiled in three above-ground concrete silos (2 m length × 1 m width × 1.2 m depth) for five weeks of anaerobic fermentation. To ensure sufficient silage supply, two silos were kept in constant refill rotation. After daily silage collection, each silo was promptly resealed and covered to maintain anaerobic conditions throughout the experimental period.

# Experimental design and feeding trial

The feeding trial was conducted at HARC, Ethiopia using three mid-lactation dairy cows  $(83.2 \pm 6.2 \text{ days in milk, mean} \pm \text{SEM})$  of highgrade genetics (3/4 Friesian × Boran), with an average body weight of  $395.6 \pm 5.14$  kg and an average milk yield of  $8.0 \pm 0.16$  kg/day. The study employed a 3 × 3 Latin square design with 25-day periods (15-day adaptation, 10-day data collection). Cows (primiparous and multiparous) were randomly assigned to three dietary treatments: (1) untreated wheat straw (UWS) ad libitum plus 0.5 kg concentrate per 1 of milk (UWS+0.5C), (2) ensiled wheat straw (EWS) ad libitum plus 0.5 kg concentrate per 1 (EWS+0.5C), and (3) EWS ad libitum plus 0.3 kg concentrate per 1 (EWS+0.3C). The 0.5C concentrate level represents current East African peri-urban dairy practice, while 0.3C reflects the minimum feeding level during feed price fluctuations. The experimental design addressed two key objectives: evaluating BSY-treated straw performance against conventional UWS at standard concentrate levels (0.5C) and assessing EWS potential to reduce concentrate requirements. A UWS+0.3C treatment was omitted due to ethical constraints limiting animal numbers to three cows, with the Latin square design optimising statistical power within these limitations while enabling isolation of both BSY ensiling effects and concentrate-sparing capacity.

The body weight of cows was measured fortnightly for two consecutive days just before morning feeding. Concentrate was divided equally and offered twice daily at 5:00 and 17:00 during milking sessions. Feed intake was measured daily by recording amounts offered and refused, with *ad libitum* provision maintained at 120% of expected intake. Prior to the trial, all cows were treated for external parasites (external treatments and Albendazole 500 mg deworming) and were housed in individual stalls in a ventilated barn with sloped concrete flooring and drainage. Daily management included 45 min of outdoor exercise and continuous access to fresh water. Methane emissions were estimated using multiple regression equations based on dry matter intake (DMI) and neutral detergent fibre intake (NDFI). The following equation was applied: CH<sub>4</sub> (MJ/day) = 0.3989 ( $\pm$  1.1073) + 0.8685 ( $\pm$  0.1585) × DMI + 0.6675 ( $\pm$  0.4264) × NDFI. This model, derived from Thornton (2010), demonstrates strong predictive accuracy (R² = 0.88), for dairy cattle under similar feeding regimes.

# Feed fermentation parameters and gas production

Upon opening the silos, temperature was immediately recorded using a thermometer inserted into the silage mass. For pH determination, a 20 g silage sample was homogenised with 100 ml distilled water in a glass beaker and stirred continuously for 1 min using a glass rod. The mixture was left to equilibrate for 60 min at ambient temperature before measurement. The pH values were obtained using a Hanna Benchtop pH meter calibrated daily with standard buffer solutions (pH 4.0 and 7.0).

In vitro gas production was measured using the established protocol of Menke and Steingas (1988). Ruminal fluid was collected before morning feeding from three local breed rams (19  $\pm$  1.0 kg) using an oesophageal suction tube. The fluid was pooled in a pre-warmed thermally isolated containers and strained through double-layered cheesecloth prior to use. For incubation, 200 mg of dried feed sample (1 mm particle size) was added in duplicate to 120 ml glass syringes pre-warmed to 39 °C. Each syringe received 30 ml of incubation medium consisting of 10 ml ruminal fluid and 20 ml McDougall's buffer solution. Control syringes contained only buffered ruminal fluid. The syringes were gently shaken 30 min after incubation began and hourly for the first 10 h, while maintained at 39 °C in a water bath. After 24 h of incubation, 4 ml of 1 N NaOH was added to each syringe to determine methane production, which was calculated using the following equation:

$$\frac{GP = (Vt - V0 - GP0)}{Ws}$$
(Menke and Steingass, 1988),

where: GP – gas production (ml/g DM), V0 – volume at 0 h (ml), Vt – volume at 24 h (ml), and GP0 – the mean blank value at 0 h (ml), and Ws – weight of dried sample in mg.

## *In vitro* digestibility

The in vitro DM digestibility was determined using the two-stage Tilley and Terry (1963) method. Ruminal fluid was collected from three cannulated Boran-Friesian steers (540 kg, 72 months old) before morning feeding. The steers were maintained on a diet of natural pasture hay (5.3% CP, DM basis) fed ad libitum plus 2 kg concentrate (16.9% CP, DM basis) per head. The collected ruminal fluid was transported in pre-warmed isolated flasks and maintained at 39 °C. For the assay, 0.5 g samples were incubated in duplicate with 10 ml ruminal fluid and 50 ml buffer solution at 39 °C for 48 h. This was followed by a second 48-h digestion phase with the addition of 5 ml of acid pepsin solution per tube. Blank and standard samples were incubated in duplicate with buffered ruminal fluid for quality control. After incubation, residues were dried and ashed to determine digestible organic matter in dry matter (DOMD). Metabolizable energy (ME) was calculated using the following formula:

 $ME = 0.16 \times g/kg$  DOMD (McDonald et al., 2002).

#### Milk yield and composition

Cows were milked manually twice daily at 5:00 and 17:00, with milk yields recorded using graduated cylinders. During the final five days of each experimental period, composite milk samples (100 ml) were collected at both milking sessions into sterile plastic containers. Samples were immediately refrigerated at 4 °C until analysis. Milk composition was determined using a Lacto Scan ultrasonic milk analyser (INDI, 2018; Milkotronic Ltd., Nova Zagora, Bulgaria). Fat corrected milk yield (FCMY, kg/day) and milk production efficiency (MPE) were calculated as follows:

FCMY (kg/day) =  $0.4 \times \text{daily milk yield (kg/day)} +$  $15 \times \text{ fat yield (kg/day)};$ MPE = daily FCMY (1)/daily DM intake (kg) (NRC, 2001; McDonald et al., 2002).

#### In vivo digestibility

The digestibility of the feeds was determined by total faecal collection method over seven consecutive days during each experimental period. Attendants collected all faeces from the concrete floor immediately after defecation, using dedicated buckets. The collection area was cleaned with high-pressure water following each urination event to prevent contamination. Collected faeces from individual cows were homogenised thoroughly, and a 1% representative subsample was obtained daily. These subsamples were stored in polyethylene bags at -20 °C until processing.

For analysis, samples were thawed, oven-dried at 65 °C for 72 h, ground to pass through a 1 mm sieve, and analysed using identical procedures to those employed for feed analysis. The digestibility coefficients were calculated using the following equation:

Apparent DM/nutrient digestibility (%) DM/nutrient intake - faecal DM/nutrient excreted × 100 DM/nutrient intake

## Cost-benefit analysis

The prices of feeds and milk were obtained from local market in Holeta city, Ethiopia, and converted to US dollars (USD). The cost-benefit ratio was calculated as the total revenue from milk sales divided by the total feed costs (Sarma et al., 2014).

# Feed chemical analysis

Samples from each silo were collected twice and analysed in duplicate at the Holeta Agricultural Research Center's Animal Feed and Nutrition Research Laboratory. Fresh samples were dried in an oven at 6 °C for 72 h, then ground to pass through a 1 mm sieve. DM content was determined by oven-drying 1 g of sample at 105 °C for 24 h (AOAC method 934.01; AOAC International, 2007). Ash content was measured by incinerating dried samples in a muffle furnace at 550 °C for 2.5 h (AOAC, method 942.05; AOAC method 934.01; AOAC International, 2007). Crude protein (CP) was calculated as nitrogen (N)  $\times$  6.25, with N quantified using the Kjeldahl method (AOAC, method 990.03; AOAC method 934.01; AOAC International, 2007). NDF, ADF, and lignin were analysed sequentially using the Van Soest et al. (1991) procedure with heat-stable amylase for NDF.

#### Data analysis

All statistical calculations were performed using R software (version 4.4.1, R Core Team, 2023; Vienna, Austria) (Shah, 2013). The chemical composition and in vitro analysis of feed samples were evaluated using the following model:

$$\boldsymbol{Y}_{ij} = \boldsymbol{\mu} + \boldsymbol{S}_i + \boldsymbol{E}_{ij,}$$

where:  $Y_{ij}$  – nutrient composition,  $\mu$  – overall mean,  $S_i$  – effect of silo, and  $E_{ij}$  – experimental error. The feeding trial data were analysed using a Latin square design with the model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + E_{ijk},$$

$$\begin{split} Y_{ijk} &= \mu + C_i + P_j + T_k + E_{ijk}, \\ where: \ Y_{ijk} &- \ dependent \ variable \ (intake \ and \ digestibility, \ milk \ yield, \ and \ composition; \end{split}$$
methane production),  $\mu-\text{overall}$  mean,  $\boldsymbol{C}_{_{i}}-\text{parity}$ effect (i = 1 to 3),  $P_j$  – period effect (j = 1 to 3),  $T_k$  – effect of diet (k = 1 to 3),  $E_{ijk}$  – experimental

error. Mean comparisons were performed using Duncan's multiple range test at  $P \le 0.05$  significance level. Assumptions of normality and homogeneity of variance were verified prior to analysis.

#### Results

# Chemical composition and fermentation quality

DM, NDF, and total gases were affected by (P < 0.05) individual silo treatments, whereas temperature and pH remain unaffected (P > 0.05). Compared to UWS, EWS had lower DM, NDF, ADF, total gases, and CH<sub>4</sub> production (P < 0.001) and higher CP and OM digestibility (P < 0.001). Ensiling WS with BSY and molasses significantly reduced (P < 0.01) NDF, ADF, CH<sub>4</sub>, and pH by 26.4, 11.4, 28.33, and 32.6%, respectively, compared to UWS. OM digestibility in EWS increased by 77 g/kg DM compared to UWS (Table 1).

#### Dry matter and nutrient intake

EWS+0.5C significantly increased (P < 0.01) DM, OM, and CP intakes by 4.13, 3.6, and 16.1%, respectively, compared to UWS+0.5C, while NDF and ADF intakes were lower than in other treatment diets (Table 2).

# Apparent feed digestibility

The digestibility of DM, OM, CP, and ADF in EWS+0.5C was significantly improved (P < 0.001) in the EWS+0.5C group by 21.6, 18.1, 14.6, and 39.2%, respectively, compared to UWS+0.5C. However, NDF digestibility and body weight were not affected (P > 0.05) by treatments (Table 3).

#### Milk and methane production

Milk yield and fat-corrected milk yield were significantly increased (P < 0.001) in EWS+0.5C by 1.13 and 1.33 (kg/day/cow), respectively, compared to UWS+0.5C. Similarly, milk production efficiency, fat, protein, and lactose yields were also

Table 1. Chemical composition, fermentation quality, and gas production of ensiled wheat straw (EWS) and untreated wheat straw (UWS)

Item	LIMO	EWS						
	UWS	Silo1	Silo2	Silo3	Mean	SEM	P-value	
DM, g/kg as basis	931	385ª	384ª	382 <sup>b</sup>	384	0.39	0.01	
OM, g/kg DM	895	896	896	896	896	0.95	0.95	
CP, g/kg DM	37.2	69.6	71.3	70.5	70.5	0.80	0.39	
NDF, g/kg DM	753	560ª	548 <sup>b</sup>	555°	554	0.15	0.01	
ADF, g/kg DM	564	503	493	503	500	0.94	0.96	
pmL, g/kg DM	93.1	94.1	94.4	94.4	94.3	0.93	0.96	
DOMD, g/kg DM	422	498	498	500	499	0.67	0.65	
ME, MJ <sup>-1</sup> kg DM	6.75	7.97	7.98	8.00	7.98	0.03	0.65	
Fermentation parameters								
temperature, °C	-	16.3	16.3	16.3	16.3	0.28	0.98	
pН	6.45	4.38	4.36	4.31	4.35	0.05	0.59	
total gases, ml <sup>-1</sup> g DM	195	168.5ª	164 <sup>b</sup>	163⁵	165	4.4	0.01	
CH, ml-1 g DM	35	26.9	24.6	23.6	25.05	1.6	0.36	

UWS – untreated wheat straw, EWS – ensiled wheat straw, DM – dry matter, OM – organic matter, CP – crude protein, NDF – neutral detergent fibre, ADF – acid detergent fibre, pmL – permanganate lignin, DOMD – digestibility of organic matter in dry matter, ME – metabolizable energy, SEM – standard error of the mean,  $^{abc}$  – means within a row with different superscripts are significantly different at P < 0.05; silo 1–3 – replicate ensiled samples of EWS

Table 2. Feed and nutrient intake (kg/day, dry matter basis) of dairy cows fed different experimental diets

Intake	UWS+0.5C	EWS+0.5C	EWS+0.3C	SEM	P-value	
UWS	7.36					
EWS	-	7.45	9.39			
Concentrate mix	4.72	5.16	3.06			
Dry matter	12.1°	12.6ª	12.4 <sup>b</sup>	0.05	<0.01	
Organic matter	10.9 <sup>b</sup>	11.3ª	11.4ª	0.06	<0.01	
Crude protein	1.43°	1.66ª	1.52⁵	0.03	<0.01	
Neutral detergent fibre	9.26ª	4.80°	5.75 <sup>b</sup>	0.04	0.01	
Acid detergent fibre	4.75ª	4.35⁵	4.59ª	0.07	<0.01	

UWS – untreated wheat straw, EWS – ensiled wheat straw, SE – standard error of the mean, UWS+0.5C – wheat straw *ad libitum* with 0.5 kg of commercial concentrate (CC) per I of milk, EWS+0.5C – ensiled wheat straw (EWS) *ad libitum* with 0.5 kg of CC per I of milk, EWS+0.3C – EWS *ad libitum* with 0.3 kg of CC per I of milk, about a row with different superscripts are significantly different at *P* < 0.05

Table 3. Feed and nutrient digestibility (%) and body weight of dairy cows fed different experimental diets

Parameters	UWS+0.5C	EWS+0.5C	EWS+0.3C	SEM	<i>P</i> -value
Digestibility					
dry matter	52.6 <sup>b</sup>	64.0ª	64.4ª	0.85	<0.01
organic matter	58.0 <sup>b</sup>	68.5ª	66.4ª	0.63	<0.01
crude protein	61.1 <sup>b</sup>	70.0a	69.1ª	1.10	<0.01
neutral detergent fibre	54.1	58.4	55.8	3.94	0.65
acid detergent fibre	41.1°	57.2ª	52.1 <sup>b</sup>	0.93	0.01
Body weight, kg	398	400	398	11.0	0.28

SE – standard error of the mean, UWS+0.5C – wheat straw *ad libitum* with 0.5 kg of commercial concentrate (CC) per I of milk, EWS+0.5C – ensiled wheat straw (EWS) *ad libitum* with 0.5 kg of CC per I of milk, EWS+0.3C – EWS *ad libitum* with 0.3 kg of CC per I of milk, abc – means within a row with different superscripts are significantly different at *P* < 0.05

Table 4. Milk production and predicted methane emissions in dairy cows fed different experimental diets

	UWS+0.5C	EWS+0.5C	EWS+0.3C	SEM	P-value
Milk yield, kg/day	9.27 <sup>b</sup>	10.4ª	10.0ª	0.21	0.01
FCMY, kg/day	8.67 <sup>b</sup>	10.0ª	9.49ª	0.21	<0.01
Fat yield, g/day	330°	388ª	363 <sup>b</sup>	7.3	0.01
Protein yield, g/day	297 <sup>b</sup>	348ª	330 <sup>b</sup>	6.5	0.01
Lactose yield, g/day	437 <sup>b</sup>	498ª	473ª	3.11	0.01
Milk composition, g/kg					
fat	35.6°	37.3ª	36.6 <sup>b</sup>	0.11	<0.01
protein	32.2°	33.4ª	33.0ª	0.12	0.01
lactose	46.8°	47.8a	47.3b	0.10	<0.01
ash	64.1	62.1	65.1	0.20	0.06
solid not fat	85.3⁵	87.0ª	86.3ª	0.02	<0.01
MPE (FCMY:DMI)	0.71 <sup>b</sup>	0.80a	0.76 <sup>ab</sup>	0.02	<0.01
CH₄, MJ/day	17.2ª	14.5°	15.1⁵	0.06	<0.01
CH, /kg of milk	1.84ª	1.39°	1.49°	0.04	0.01

UWS+0.5C – wheat straw *ad libitum* with 0.5 kg of commercial concentrate (CC) per I of milk, EWS+0.5C – ensiled wheat straw (EWS) *ad libitum* with 0.5 kg of CC per I of milk, EWS+0.3C – EWS *ad libitum* with 0.3 kg of CC per I of milk, DMI – daily dry matter intake, MPE – meat production efficiency, FCMY – fatcorrected milk yield, MPE – milk production efficiency, SEM – standard error of the mean; abc – means within a row with different superscripts are significantly different at *P* < 0.05

higher (P < 0.01) by 12.9, 18.2, 16.7, and 13.6%, respectively. However, milk ash content was not significantly affected (P > 0.05). Predicted  $\mathrm{CH_4}$  emission (MJ/day) was significantly reduced (P < 0.01) by 15.7% in EWS+0.5C compared to UWS+0.5C, with the lowest (P < 0.01)  $\mathrm{CH_4}$  yield per kg of milk observed in the EWS+0.5C group (Table 4).

#### **Cost-benefit analysis**

The total production cost of the EWS+0.5C diet was higher than that of the control and the EWS+0.3C diets. However, ensiling WS with BSY and molasses improved farm profitability, increasing net profit by 27% with EWS+0.3C and by 11.5% with EWS+0.5C compared to UWS+0.5C. Additionally, treated WS reduced concentrate feed requirements in dairy cows without affecting milk production and quality (Table 5).

Table 5. Partial costbenefit analysis of experimental diets

Costs, USD/kg	UWS+ 0.5C	EWS+ 0.5C	EWS+ 0.3C	
Feed cost				
UWS	0.71	0.00	0.00	
EWS	0.00	0.73	0.97	
CC	3.99	4.24	2.59	
Labour and material costs, USD				
chopping only	0.67	0.64	0.82	
silage preparation	0.00	0.45	0.56	
total cost (TC), USD-1day-1 cow	5.37	6.03	4.93	
Total return (TR), USD				
milk price	9.83	11.0	10.6	
net profit, USD-1day-1 cow	4.46	4.87	5.67	
cost:benefit ratio (TR:TC)	1.83	1.82	2.16	

UWS+0.5C — wheat straw *ad libitum* with 0.5 kg of commercial concentrate (CC) per I of milk, EWS+0.5C — ensiled wheat straw (EWS) *ad libitum* with 0.5 kg of CC per I of milk, EWS+0.3C — EWS *ad libitum* with 0.3 kg of CC per I of milk, USD — United States dollar

# **Discussion**

# Chemical composition and fermentation quality

The inclusion of BSY (0.6 l/kg wheat straw) and molasses (0.1 g/kg wheat straw) in EWS significantly altered its chemical composition compared to UWS. DM content decreased (931 vs. 383 g/kg DM), while CP concentration nearly doubled (37.2 vs. 70.4 g/kg DM). The decrease in DM content can be attributed to moisture introduced by molasses and BSY, as well as fermentation losses during the ensiling process. The increase in CP content in EWS was likely due to the addition of CP-rich BSY (Terefe et al., 2023). Changes in NDF and ADF composition during ensiling may improve digestibility and reduce methane production. Variability in DM, NDF, and total gas production between silos could result from differences in compaction and sampling methods. These findings are consistent with previous studies showing that the addition of BSY (30-50% DM basis) and molasses to rice or wheat straw improves CP, while reducing NDF, ADF, and gas production (Zhao et al., 2019; Terefe et al., 2023). Additionally, BSY and brewer's spent grain have been reported to reduce CH<sub>4</sub> emissions due to the hop-derived compounds absorbed by yeast during brewing, which may inhibit methanogenic microbes (Bayat et al., 2015).

#### **Nutrient intake and digestibility**

The improved DM and nutrient intakes observed in cows fed EWS suggested that the addition of BSY and molasses increased roughage palatability. EWS intake was particularly higher in the EWS+0.3C group, likely to meet milk the nutrient requirements for milk production. In contrast, intake remained relatively stable in the EWS+0.5C group, presumably due to the higher concentrate level adequately meeting nutritional demands. The significant reduction in NDF and ADF intake in EWS+0.5C implied that BSY and molasses improved fibre digestibility, reducing the need for high fibre intake while still ensuring sufficient energy supply. This finding aligns with studies by Van Wyngaard et al. (2018) and Yadessa et al. (2024), who also reported increased feed intake in dairy cows following BSY supplementation.

In the present study, DM, OM, CP, and ADF digestibility were improved by 21.6, 18.1, 14.6, and 39.2%, respectively. These findings are consistent with previous research of Oliveira

et al. (2016) and Yadessa et al. (2024), who reported that BSY supplementation increased DM (4.5%), OM (10.5%), CP (6.5%), and ADF (6.5%) digestibility in beef and dairy cattle, and sheep, likely due to stimulation of cellulolytic bacteria and more efficient ruminal fermentation. However, no significant changes in dairy cow body weight were observed across dietary treatments, which is consistent with previous research indicating that BSY supplementation does not markedly affect body weight in dairy cows (Yadessa et al., 2024).

### Milk production and quality

Increased CP intake and nutrient digestibility contributed to significantly higher milk production, with gains of 1.13 and 0.73 kg/day observed in the EWS+0.5C and EWS+0.3C dietary groups, respectively. Milk quality also improved, as indicated by increased daily fat production of 58 g/day in EWS+0.5C and 33 g/day in EWS+0.3C. Similarly, McDonald et al. (2002) demonstrated that ensiling roughages enhanced digestibility by hydrolysing bonds between lignin and other fibre fractions, thereby improving fermentation, and milk yields. The benefits of BSY supplementation extend beyond basic nutrition, as it contains probiotics, prebiotics and mannan-oligosaccharides), (β-glucans well as postbiotics that promote the development of beneficial ruminal and intestinal microbiota, leading to more efficient nutrient utilisation (Pszczolkowski et al., 2016; Avramia and Amariei, 2021; Ciobanu et al., 2024; Wei et al., 2024). Supplementation with brewer's spent grain and yeast has also been shown to improve milk production and milk composition, including increases in protein, fat, and total solids, as well as milk production efficiency (Bayat et al., 2015; Yadessa et al., 2024). However, these benefits are not always consistent and depend on whether diets are formulated to be isonitrogenic and isoenergetic (Oancea et al., 2023).

## Methane production

The present study found that the improved nutritional value of the EWS may explain the observed decrease in methane production. Similarly, the inclusion of BSY appears to contribute to this effect, supporting earlier findings by Pszczolkowski et al. (2016). Gastelen et al. (2019) also reported a positive correlation between NDF content and CH<sub>4</sub> generation, while Van Wyngaard et al. (2018) demonstrated that higher-quality feeds led to lower methane emissions per unit of milk produced.

Conversely, Benaouda et al. (2024) observed that increasing dietary NDF content from 40.2 to 50.5% led to lower CH<sub>4</sub> generation per kg of DMI (from 32.1 to 21.3 1 CH<sub>4</sub>/kg), due to reduced nutrient digestibility in the rumen.

#### **Economic viability**

The present study demonstrates that EWS supplementation enhances both feed efficiency and farm profitability. Despite higher silage production costs, the increased milk yield generated additional revenue, resulting in a net profit gain of 1.21 USD/ day/cow for EWS+0.5C and 0.51 USD/day/cow for EWS+0.3C compared to the control diet. Importantly, reducing concentrate supplementation from 0.5 to 0.3 kg per l of milk maintained near-equivalent milk yields (10.4 vs. 10.0 kg/day) while increasing profitability by 1.21 USD/day/cow. This feeding strategy suggests a potential reduction in the reliance on cereal grains, minimising competition with human cereal demand without compromising productivity. The current findings align with earlier studies demonstrating that ensiling roughages improves feed efficiency and economic viability in dairy production (Krasniqi et al., 2018, Ntakyo et al., 2020).

#### **Conclusions**

This study demonstrated that ensiled wheat straw (EWS) with brewer's spent yeast (BSY) and molasses is an effective solution to increase crude protein content, reduce fibre fractions, improve in vitro digestibility, and lower in vitro methane production – particularly relevant for dairy production systems in developing countries. The use of EWS improved milk yield and composition, regardless of concentrate levels. Financially, the optimal strategy was the use of 0.3 kg of concentrate per 1 of milk, while the highest environmental benefits, including greater reductions in methane emissions, were observed with 0.5 kg of concentrate per 1 of milk, likely due to reduced neutral detergent fibre (NDF) intake. These results suggest that incorporating BSY and molasses into low-quality feeds optimises both economic returns and environmental sustainability by utilising agro-industrial by-products, and improving resource efficiency in dairy farming.

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# **Conflict of interest**

The Authors declare that there is no conflicts of interest.

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