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Lactational performance and enteric methane emissions in dairy cows fed high-oil oats, cold-pressed rapeseed cake, and 3-nitrooxypropanol in a grass silage–based diet

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ABSTRACT

The objective of this study was to evaluate the effects of high-oil oats and cold-pressed rapeseed cake (RSC) as dietary ingredients, along with supplementation of 3-nitrooxypropanol (3-NOP), on apparent total-tract digestibility, milk production, and enteric CH_4 emissions in dairy cows fed a grass silage–based diet. Twenty-four lactating Nordic Red cows were grouped into 3 blocks. The experiment was conducted as a cyclic change-over where each treatment had 3 observations per period. The experiment consisted of 4 periods of 28 d each, including 18 d of diet adaptation and 10 d of data and sample collection. The 2 × 2 × 2 factorial design included 2 energy sources (barley or high-oil oats), 2 protein supplements (rapeseed meal [RSM] or RSC), and 3-NOP supplementation at 2 levels (0 or 68 mg/kg of DM), resulting in 8 dietary treatments. The basal diet consisted of 60% grass silage (on a DM basis). Inclusion rates of the experimental concentrates were 27% to 29% and 29% to 31% for barley and high-oil oats, respectively, and 8% to 10% and 10% to 12% for RSM and RSC, respectively. Diets were offered ad libitum as a TMR. Daily DMI, milk yield, BW, and gas emissions were recorded throughout the experiment. Gas emissions were measured using the GreenFeed system. Ether extract (EE) content across experimental diets ranged from 2.9% to 6.1% of DM. Total DMI tended to increase with high-oil oats compared with barley and decrease with RSC compared with RSM. Digestibility of DM, OM, NDF, and EE decreased with high-oil oats versus barley, and EE digestibility increased with RSC versus RSM. Despite lower nutrient digestibility, milk and ECM yield increased by 2.4 kg/d with high-oil oats compared with barley. Milk yield decreased by 1.1 kg/d and ECM yield tended to decrease by 1.0 kg/d with 3-NOP supplementation. Milk protein concentration decreased with high-oil oats versus barley and with RSC versus RSM. Feed efficiency increased with high-oil oats compared with barley. Daily CH_4 emissions (g/d), CH_4 yield (g/kg of DMI), and CH_4 intensity (g/kg of ECM) decreased by 11.2%, 14.2%, and 15.3%, respectively, when barley was replaced with high-oil oats in combination with RSM but were not affected in combination with RSC. Daily CH_4 emissions, CH_4 yield, and CH_4 intensity decreased by 12.5%, 10.6%, and 12.7%, respectively, when RSM was replaced with RSC in combination with barley but not in combination with high-oil oats. Daily CH_4 emissions, CH_4 yield, and CH_4 intensity decreased by 33.5%, 30.9%, and 31.2%, respectively, with 3-NOP supplementation, with slightly greater efficacy on CH_4 intensity when barley was used as the energy source. Urinary urea concentration was greater with high-oil oats than with barley, and lower with RSC than with RSM, but only in combination with high-oil oats. In conclusion, both high-oil oats and RSC are practical dietary ingredients for reducing enteric CH_4 emissions and CH_4 intensity. High-oil oats may additionally improve feed efficiency and production performance in dairy cows. Supplementation with 3-NOP reduced enteric CH_4 emissions by more than 30% with additive effects when combined with RSC, but negatively affected production performance.
Key words: sustainability, alternative feed sources, blood metabolites, urinary minerals

INTRODUCTION

Ruminants contribute to anthropogenic CH_4 emissions arising from enteric fermentation in their digestive tract. Mitigation strategies include increasing the dietary fat content by feeding oil seeds or plant oils (Brask et al., 2013; Bayat et al., 2018), feeding additives such as the red macro algae *Asparagopsis* spp. (Stefenoni et al., 2021), nitrates (Olijhoek et al., 2016), or the synthetic

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CH_4 inhibitor 3-nitrooxypropanol (**3-NOP**; Hristov et al., 2015a). In addition to reducing enteric CH_4 emissions, mitigation strategies should be applicable under practical feeding conditions and should not compromise production performance or animal health. On the farm level, replacing one dietary ingredient with another ingredient could provide a practical strategy for CH_4 mitigation.

Replacing barley grain (*Hordeum vulgare*) with oat grain (*Avena sativa*) has been shown to reduce enteric CH_4 emissions (g/d) by 4.6% and CH_4 intensity (g/kg of ECM) by 4.8% to 5.7%, without negative effects on milk or ECM yield in dairy cows (Fant et al., 2021; Ramin et al., 2021). Most of the CH_4-mitigating effect of oats has previously been attributed to their lower digestibility, whereas a smaller portion has been attributed to their greater fat content compared with barley (4.9% vs. 2.8% of DM; Fant et al., 2020; Fant et al., 2021). In previous studies, the crude fat content in oats ranged between 4.5% and 5.4% of DM, although cultivars with a crude fat content up to 18% have been developed (Peterson and Wood, 1997). For example, the oat cultivar Fatima, developed by Lantmännen Cerealia AB (Malmö, Sweden), is considered a high-oil oat cultivar and is reported to have an average crude fat content of 9.4% of DM (Hagman et al., 2016). Few studies have evaluated high-oil oat cultivars as a feed for dairy cows. Ekern et al. (2003) compared regular (5.6% crude fat of DM) and high-oil oats (7.2% crude fat of DM) and reported greater milk and ECM yields when cows were fed high-oil oats. However, to the best of our knowledge, no in vivo study has investigated the effects of high-oil oats on diet digestibility or enteric CH_4 emissions and CH_4 intensity. Rapeseed meal (**RSM**) is a common protein supplement for dairy cows in temperate regions. An alternative to RSM is cold-pressed rapeseed cake (**RSC**), which has a greater crude fat content (10%–20% of DM) than RSM. Replacing RSM with RSC decreases enteric CH_4 emissions by ~6.8% and CH_4 intensity by 6.9% to 12.4%, without negative effects on milk or ECM yield (Brask et al., 2013; Bayat et al., 2022). Furthermore, Räisänen et al. (2024) reported reduced enteric CH_4 emissions and CH_4 intensity by 9.4% and 11.7%, respectively, when feeding a combination of regular oats and RSC compared with feeding a combination of barley and RSM.

It has been well established that 3-NOP supplementation decreases enteric CH_4 emissions and CH_4 intensity in dairy cows by ~28% to 32% (Kebreab et al., 2023; Martins et al., 2024). The effects of 3-NOP on lactational performance have been variable. Although some studies have reported no effect on milk or ECM yield at 3-NOP doses ranging from 40 to 80 mg/kg of DM (Hristov et al., 2015a) or specifically at 60 mg/kg DM (van Gastelen et al., 2022; Maigaard et al., 2025), others have observed reductions in milk yield at a dose of 80 mg/kg of DM (van Gastelen et al., 2022; Maigaard et al., 2025). These variable findings appear to be influenced by both the 3-NOP dose and the chemical composition of the diet (Martins et al., 2025). Currently, 3-NOP is marketed as Bovaer and used on commercial dairy farms in several Nordic countries. Few studies have evaluated the effects of combining multiple dietary CH_4 mitigation strategies. Zhang et al. (2021) reported an additive effect on enteric CH_4 emissions when combining 3-NOP with rapeseed oil in the diet of beef cows, whereas Maigaard et al. (2024) found that the combination of 3-NOP and whole cracked rapeseed did not result in a greater CH_4 reduction than feeding 3-NOP alone to dairy cows.

Therefore, the objective of this study was to evaluate the individual and combined effects of high-oil oats, RSC, and 3-NOP on apparent total-tract digestibility, lactational performance, and enteric CH_4 emissions in dairy cows fed a grass silage–based diet. We hypothesized that CH_4 emissions and CH_4 intensity would be reduced by substituting barley with high-oil oats, substituting RSM with RSC, and by supplementing the diet with 3-NOP, without adversely affecting milk or ECM yield. The expected CH_4 reductions from high-oil oats were based on the mechanisms described here for regular oats, and the CH_4 reductions from RSC were based on its greater fat content relative to RSM. Furthermore, we hypothesized that feeding a combination of high-oil oats, RSC, and 3-NOP would result in a greater reduction in CH_4 emissions and CH_4 intensity than feeding each of these components individually.

MATERIALS AND METHODS

The experiment was conducted at the Röbäcksdalen dairy research facility, Swedish University of Agricultural Sciences in Umeå, Sweden (63°45′N; 20°17′E) in fall 2023. All experimental procedures were approved by the Swedish Ethics Committee on Animal Research (Dnr A 6-2021 Umeå, Sweden) and in accordance with Swedish legislation and the European Union Directive 2010/63/EU (as amended) on the protection of animals used for scientific purposes.

Experimental Design, Animals, and Diets

Twenty-four lactating Nordic Red dairy cows were enrolled in a cyclic change-over design with 4 periods (Davis and Hall, 1969). Each period consisted of 18 d of diet adaptation and 10 d of data collection and sampling. Cows were divided into 3 blocks based on DIM, parity, and milk yield. The cows were at 70 ± 33.7 DIM, weighed 602 ± 63.7 kg, and produced 38.7 ± 7.06 kg of milk/d at the start of the experiment. Within each block, cows were randomly assigned to treatment sequences.

Table 1. Chemical composition of dietary ingredients[1]

Item	Grass silage	Barley	High-oil oats	RSM	RSC	GreenFeed concentrate
DM, %	31.5	90.5	91.3	92.4	93.0	90.2
In DM, %						
Ash	8.98	2.53	3.33	7.66	6.61	7.09
CP	20.2	9.50	12.1	36.8	29.7	21.1
NDF	41.7	15.2	27.3	32.1	24.0	22.3
iNDF[2]	3.09	2.85	17.0	12.2	9.22	6.99
pdNDF[3]	38.6	12.3	10.3	19.9	14.8	15.3
Starch	0.96	61.3	43.1	2.24	1.14	32.0
Ether extract	3.56	1.71	7.19	2.41	18.8	4.83

[1]Grass silage concentrations were as follows: ammonia N (5.38% of N), lactic acid (10.8% of DM), acetic acid (1.89% of DM), propionic acid (0.41% of DM), and butyric acid (<0.001% of DM). pH = 3.79. RSM = rapeseed meal, RSC = cold-pressed rapeseed cake. GreenFeed concentrate was a commercial concentrate obtained from Lantmännen Lantbruk AB (Malmö, Sweden).

[2]iNDF = indigestible NDF.

[3]pdNDF = potentially digestible NDF, calculated as NDF − iNDF.

Dietary treatments followed a 2 × 2 × 2 factorial design (8 treatments in total). This resulted in 3 observations per treatment per period, and a total of 12 observations per treatment over the entire experiment.

The dietary treatments included 2 energy sources, barley (27%–29% of diet DM) or high-oil oats (29%–31% of diet DM; Fatima; Lantmännen, Malmö, Sweden), 2 protein supplements, RSM (8%–10% of diet DM) or RSC (10%–12% of diet DM), and 3-NOP (Bovaer 10; DSM-Firmenich, Kaiseraugst, Switzerland) supplemented at 0 mg/kg of DM or 68 mg/kg of DM. The target dose of 3-NOP was 60 mg/kg of diet DM, based on manufacturer recommendations and prior studies. However, post-trial analysis revealed an actual average dose of 68 mg/kg of DM, which is reported as the analyzed dose throughout the manuscript. The basal diet consisted of grass silage made from primary-growth perennial leys mainly composed of timothy (*Phleum pratense*), with 10% red clover (*Trifolium pratense*).

The chemical composition of feed ingredients is presented in Table 1. Diets were fed as a TMR offered ad libitum and aimed to include grass silage at 60%, experimental grain at 27% to 31%, experimental protein supplement at 8% to 12%, and minerals at 1% of diet DM. The ingredient composition and analyzed chemical composition of the 8 dietary treatments are presented in Table 2. The CP concentration was similar among diets (18.3%–18.8% of diet DM), whereas dietary concentrations of NDF and starch varied. The concentration of ether extract (**EE**) ranged from 2.9% to 6.1% of diet DM, with the largest difference (3.2 percentage points) observed between the barley + RSM and high-oil oats + RSC diets.

Throughout the experiment, the animals were housed in an insulated, loose-housed barn with unrestricted access to water and salt blocks. They were milked twice daily in a milking parlor at 0600 and 1630. The TMR, prepared by an automatic mixer (Nolan A/S, Viborg, Denmark), was delivered to feed bins 2 times per day by automated feeding wagons (Mullerup Smart Feeder M2000, Ullerslev, Denmark). Each dietary treatment was assigned to 2 feed bins, accessible only to the 3 cows on that treatment via automatic ear tag identification. The 3-NOP was mixed in powder form into the TMR in both morning and evening delivery. To achieve the target 3-NOP dose of 60 mg/kg of DMI, we adjusted for an estimated intake of 1.0 kg of DM/cow per day from the bait feed provided in the GreenFeed (**GF**) units (C-Lock Inc., Rapid City, SD). The bait feed was not supplemented with 3-NOP. The estimated total DMI, including both the TMR and the GF bait, was 24.0 kg of DM/cow per day. Consequently, the formulated 3-NOP concentration in the TMR was slightly increased (+3 mg of 3-NOP/kg of DM) to ensure an overall dietary inclusion level of 60 mg 3-NOP/kg of DMI. To minimize the risk of cross-contamination, the 4 control diets (without 3-NOP) were mixed first, followed by the preparation of the 4 diets containing 3-NOP. Subsequently, 4 rinsing diets were mixed, which were not fed to the cows in the experiment.

Data Collection and Sampling

Individual daily feed intake was measured throughout the experiment using Roughage Intake Control feeders (Hokofarm Group B.V., Marknesse, Netherlands), while individual daily milk yield was recorded using gravimetric milk recorders (SAC, S.A. Christensen and Co. Ltd., Kolding, Denmark). For statistical analysis, only data from the final 10 d of each period were included. The BW of the cows was recorded during 3 consecutive days before the start of the experiment and on the last 3 d of each period using a walk-through weighing system (Hokofarm Group B.V., Marknesse, Netherlands) as the cows exited the milking parlor after morning milking.

Methane emissions, CO_2 emissions, H_2 emissions, and O_2 consumption were measured by the GF system (C-

Table 2. Ingredient and chemical composition of the experimental diets fed to dairy cows

	Diet[1]							
	Barley				High-oil oats			
	RSM		RSC		RSM		RSC	
Item	CON	3-NOP	CON	3-NOP	CON	3-NOP	CON	3-NOP
Dietary composition, % of DM								
Grass silage	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Barley	29.0	29.0	27.0	27.0	0	0	0	0
High-oil oats	0	0	0	0	31.0	31.0	29.0	29.0
RSM	10.0	10.0	0	0	8.0	8.0	0	0
RSC	0	0	12.0	12.0	0	0	10.0	10.0
Mineral mix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Bovaer (3-NOP)	0	0.068	0	0.068	0	0.068	0	0.068
Chemical composition, % of DM								
Ash	6.89	6.89	6.87	6.87	7.04	7.04	7.02	7.02
CP	18.6	18.6	18.3	18.3	18.8	18.8	18.6	18.6
NDF	32.6	32.6	32.0	32.0	36.0	36.0	35.3	35.3
iNDF[2]	3.89	3.89	3.73	3.73	8.10	8.10	7.71	7.71
pdNDF[3]	28.7	28.7	28.3	28.3	27.9	27.9	27.6	27.6
Starch	18.6	18.6	17.3	17.3	14.1	14.1	13.2	13.2
Ether extract	2.87	2.87	4.85	4.85	4.56	4.56	6.10	6.10
ME, MJ/kg DM[4]	12.0	12.0	12.3	12.3	11.7	11.7	11.9	11.9

[1]Protein supplement: RSM = rapeseed meal; RSC = cold-pressed rapeseed cake; CON = control diet, 0 mg 3-NOP/kg of DM; 3-NOP = 68 mg 3-NOP/kg of DM. 3-NOP = 3-nitrooxypropanol.

[2]iNDF = indigestible NDF.

[3]pdNDF = potentially digestible NDF, calculated as NDF − iNDF.

[4]Calculated based on analyzed values and values obtained from Finnish national feed tables (LUKE, 2025).

Lock Inc., Rapid City, SD) as described by Hristov et al. (2015b). Data were recorded throughout the experiment, but only data recorded during the final 10 d of each period were used for statistical analysis. All 24 cows had unrestricted access to 2 GF units, except for an interval of a minimum of 5 h between visits. To encourage visits, cows received 8 drops of bait feed (50 g each) every 40 s during their time in the GF system. To ensure accurate concentrate dispensing, a mass pellet drop test was conducted on both GF units, yielding an average drop weight of 50.5 ± 1.81 g (n = 10). The bait feed was a commercial concentrate (Komplett Xtra 200; Lantmännen, Malmö, Sweden). The amount of concentrate consumed by each cow in the GF system was accounted for in the calculation of total DMI. Calibrations were performed weekly using span gas (a mixture of CO_2, CH_4, and O_2) and zero gas (N_2). Recovery tests for CO_2 (99.4% ± 3.73%; n = 12) were conducted once before the experiment and subsequently every month. Air flow was monitored daily, and the air filter was replaced whenever airflow dropped below 30 L/s.

Milk samples (~20 mL) were collected during 4 consecutive milking times in each period, starting from evening milking on d 26 until morning milking on d 28. Collected samples were immediately preserved with 2-bromo-2-nitropropane-1 (Eurofins Milk Testing Sweden AB, Jönköping, Sweden) and refrigerated at 4°C. Samples were shipped to the laboratory of Eurofins Milk Testing Sweden AB within 2 d after sampling.

Fecal grab samples (300 mL) were collected parallel to 6 consecutive milking sessions (1 sample per milking) in each period, starting at morning milking on d 26 until evening milking on d 28 of each period. Fecal samples were collected from 16 cows (2 blocks). The 6 fecal samples collected from each cow were pooled by cow at the end of each period, dried in a forced-air oven at 60°C for 48 h, and ground into 2 different particle sizes. For chemical composition analysis, the samples were ground using a cutter mill (SM300, Retsch GmbH, Haan, Germany) and passed through a 1-mm sieve. For indigestible NDF (**iNDF**) determination, the samples were ground manually with a pestle and mortar and passed through a 2-mm sieve.

Urine spot samples were collected from the same 16 cows (2 blocks) after 4 consecutive milking times during each period, starting from d 26 after evening milking until d 28 after morning milking. Midstream urine samples were collected after massaging the area below the vulva. Immediately after collection, 2 mL of 50% H_2SO_4 was added to acidify the samples to pH <2. Daily pooled samples were frozen and stored at −20°C. Subsequently, the urine samples were shipped on dry ice to the Clinical

Pathology Laboratory of the Veterinary Teaching Hospital (OVUD) at the University of Parma (Parma, Italy), where analyses were performed within 3 mo of collection. Before analysis, samples were thawed at 4°C and gently mixed. The urine was then centrifuged at 400 × *g* for 5 min at 4°C using a refrigerated centrifuge, and the supernatant was used for chemical analysis. All procedures were conducted in accordance with the American Society for Veterinary Clinical Pathology quality assurance guidelines for urinalysis and clinical chemistry (Gunn-Christie et al., 2012).

Blood samples were collected once per period, on d 23 after morning milking from the 16 cows (2 blocks) used for fecal and urine sampling. Samples were collected from the coccygeal vein into 2 evacuated 10-mL tubes containing Li-heparin as an anticoagulant (BD Vacutainer plasma tubes, VWR International AB, Stockholm, Sweden). After collection, tubes were gently mixed and placed on ice until centrifugation. Samples were centrifuged within 2 h from collection at 2,900 × *g* for 10 min at room temperature. Separated plasma was pipetted in aliquots of 1.5 mL into 12 Eppendorf vials and stored at −80°C until analysis.

Silage samples were collected 3 times during each sampling period; on d 23, 26, and 28. A subsample (~200 g) was frozen and stored at −20°C until fermentation quality analysis. For analysis of chemical composition, another subsample (2 × 200 g) was dried in a forced-air oven at 60°C for 48 h and stored in a dry and cool place. Concentrate samples were collected once per sampling period, on d 26, and processed similarly to the dried silage samples. Dried silage and concentrate samples were ground using a cutter mill (Retsch SM300, Retsch GmbH, Haan, Germany) and sieved through a 1-mm mesh for chemical composition analysis and a 2-mm mesh for iNDF analysis. To analyze 3-NOP concentration, TMR samples were collected on d 26 in each period. Samples were collected following delivery of fresh TMR to feed bins in the morning and in the evening. Samples were stored in plastic HPLC bottles at −20°C until shipped refrigerated to DSM-Firmenich in Kaiseraugst, Switzerland.

Chemical Analysis

Grass silage, concentrate, and fecal samples were analyzed for DM, ash, CP, EE, NDF, and iNDF concentration, whereas grass silage and concentrate samples were additionally analyzed for starch concentration. Dry matter concentration was analyzed by oven drying at 105°C for 16 h, followed by ash analysis through combustion of the dried samples at 500°C for 4 h (AOAC International, 2000). Total N concentration was analyzed using the Kjeldahl method, and CP concentration was calculated as total N multiplied by 6.25. Concentration of EE was determined through the Soxhlet method, following European Commission Regulation No. 152/2009 (European Commission, 2009) recommendations. Starch concentration in feed samples was analyzed as described by Larsson and Bengtsson (1983).

Neutral detergent fiber concentration was analyzed following Mertens (2002), with the inclusion of heat-stable α-amylase and sodium sulfite, and was expressed exclusive of residual ash. Indigestible NDF was assessed based on the method of Krizsan et al. (2015). Feed samples (2.0 g) were placed in nylon bags with an 11-μm pore size and incubated for 288 h in the rumen of 2 rumen-cannulated lactating dairy cows, with 1 replicate per cow. The diet of the cannulated cows consisted of 60% grass silage and 40% concentrate on a DM basis (Krizsan and Huhtanen, 2013). The iNDF concentration was expressed excluding residual ash.

Fermentation quality of silage was determined by measuring pH and analyzing fermentation acids and ammonia N. Before analysis, frozen silage samples were thawed and compressed to extract juice, which was then diluted 1:1 with distilled water. The diluted liquid was used to analyze lactic acid and VFA concentrations according to the method described by Ericson and André (2010). Ammonia N was determined using a Kjeltec 2100 Distillation Unit (Foss Analytical A/S, Hillerød, Denmark). The DM concentration of silage was corrected for volatile losses according to the equation by Huida et al. (1986).

Milk samples were analyzed for concentration of protein, fat, lactose, and urea by Fourier transform infrared spectroscopy with a CombiFoss 6000 analyzer (Foss Analytical A/S, Hillerød, Denmark) in the laboratory of Eurofins Milk Testing Sweden AB (Jönköping, Sweden). Blood samples were analyzed for insulin, glucose, nonesterified fatty acids (**NEFA**), and BHB concentrations. Insulin and glucose were analyzed by commercial enzymatic kits (Mercodia Bovine Insulin, Mercodia, and Enzytec Liquid D-glucose, r-Biopharm, respectively). Nonesterified fatty acids and BHB were analyzed by commercial enzymatic colorimetric assay kits (FujiFilm and Sigma Aldrich, respectively).

Urine analyte concentrations were analyzed via an automated analyzer (BT3500 Biotecnica Instruments, Rome, Italy) using dedicated methods (Biotecnica system reagent, BTR and Futurlab system reagent, FCC). The type of reaction used in the assay and BTR or FCC identification number were reported in brackets after the reported variables. The analyzed profile included urinary urea (mg/dL), determined using the urease reaction (BTR334); urinary creatinine (mg/dL), analyzed via the Jaffè method (FCC206L); calcium (Ca^{+2}, mg/dL), measured using the o-cresolphthalein complexone method (BTR432); inorganic phosphorus (P, mg/dL), determined via the molybdate reaction (BTR222); and magnesium (Mg, mg/dL), quantified using an enzymatic kinetic method based on the formation of phosphogluconic acid and NADPH (FCC260B). The analytical methods used have been previously reported (Spek et al., 2012; Løvendahl et al., 2016; Probo et al., 2022). For urinary phosphorus, urinary calcium, and urinary magnesium, the ratios with urinary creatinine were calculated and used for statistical analysis and reported results.

Calculations

The concentration of potentially digestible NDF was calculated by subtracting the concentration of iNDF from the NDF concentration. The chemical composition of each diet was calculated based on the chemical composition and the inclusion rate of each feed ingredient. The dietary ME concentration was calculated using weighted coefficients from Finnish national feed tables (LUKE, 2025) and analyzed values. Apparent total-tract digestibility of dietary components was estimated using iNDF as an internal marker according to the following equation, with OM digestibility as an example:

$$\text{OM digestibility} = 1 - [\text{iNDF in diet DM (g/kg)}/\text{iNDF in fecal DM (g/kg)}] \times [\text{OM in fecal DM (g/kg)}/\text{OM in diet DM (g/kg)}]. \quad [1]$$

Energy-corrected milk was calculated according to Sjaunja et al. (1990):

$$\text{ECM (kg/d)} = \text{milk yield (kg/d)} \times [38.3 \times \text{fat (g/kg)} + 24.2 \times \text{protein (g/kg)} + 16.54 \times \text{lactose (g/kg)} + 20.7]/3{,}140, \quad [2]$$

where fat, protein, and lactose are the concentrations of these constituents in milk. Milk N efficiency was determined by dividing milk N output (g/d) by N intake (g/d), with milk N calculated as the milk protein yield divided by a conversion factor of 6.38. Feed efficiency was expressed as the ratio of ECM (kg/d) to DMI (kg/d). The respiratory quotient was calculated as the ratio of CO_2 produced (L/d) to O_2 consumed (L/d).

Statistical Analysis

The experimental data were subjected to ANOVA using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) and analyzed as 2 × 2 × 2 factorial design according to the following statistical model:

$$Y_{ijklmn} = \mu + D_i + E_j + F_k + B_l + DE_{ij} + DF_{ik} + DB_{il} + EF_{jk} + EB_{jl} + FB_{kl} + DEF_{ijk} + P_m + BP_{lm} + C_n(B_l) + \varepsilon_{ijklmn},$$

where Y_{ijklmn} is the dependent variable; μ is the overall mean; D_i is the effect of energy source i (i = 1 to 2); E_j is the effect of protein supplement j (j = 1 to 2); F_k is the effect of 3-NOP supplementation level k (k = 1 to 2); B_l is the effect of block l (l = 1 to 3); DE_{ij}, DF_{ik}, DB_{il}, EF_{jk}, EB_{jl}, and FB_{kl} are the possible 2-way interactions between energy source, protein supplement, 3-NOP supplementation level, and block; DEF_{ijk} is the 3-way interaction between energy source, protein supplement, and 3-NOP supplementation level; P_m is the effect of period m (m = 1 to 4), BP_{lm} is the interaction between block and period, $C_n(B_l)$ is the effect of cow within block (n = number of cows in the experiment), and ε_{ijklmn} is the residual error. All terms were considered fixed, except for $C_n(B_l)$ and ε_{ijklmn}, which were considered random. Three-way interactions including block, and 4-way interactions were removed from the final model because they were not significant ($P \geq 0.11$). At the start of period 3, one cow was removed from the experiment due to an injury not related to experimental procedures or experimental diets. For period 3 and 4, this cow was replaced by a backup cow that had been receiving the barley + RSM diet with 0 mg 3-NOP/kg of DM during period 1 and 2. Presented mean values are LSM obtained by the LSMEANS statement in SAS. Differences were declared significant if $P \leq 0.05$, and a tendency toward significance was declared if $0.05 < P < 0.10$.

RESULTS

Feed Intake and Apparent Total-Tract Digestibility

Total DM intake tended to be greater ($P = 0.09$) when cows were fed high-oil oats than when fed barley (Table 3). Greater intakes of CP, NDF, and EE, but lower intake of starch ($P \leq 0.01$), were observed when cows were fed high-oil oats. A tendency for lower ($P = 0.09$) DMI and OM intake was observed in cows fed RSC compared with RSM. Moreover, lower intakes of CP, NDF, potentially digestible NDF, and starch ($P \leq 0.01$) were observed in cows fed RSC compared with RSM, whereas greater intake of EE ($P < 0.01$) was observed in the group fed RSC. An interaction was observed ($P = 0.04$) between energy source and protein supplement regarding iNDF intake, with greater iNDF intake in cows fed high-oil oats versus barley. Additionally, iNDF intake was slightly increased

Table 3. Feed intake (n = 96) and apparent total-tract digestibility (n = 64) of lactating dairy cows fed 2 different energy sources and 2 different protein supplements, with or without supplementation of 3-nitrooxypropanol (3-NOP)

	Diet[1]														
	Barley				High-oil oats										
	RSM		RSC		RSM		RSC			P-value[2]					
Item	CON	3-NOP	CON	3-NOP	CON	3-NOP	CON	3-NOP	SEM[3]	Energy	Protein	3-NOP	Energy × protein	Energy × 3-NOP	Protein × 3-NOP
Intake, kg DM/d															
Total DMI	24.4	23.8	23.8	23.6	25.7	24.5	24.3	23.7	0.48	0.09	0.08	0.11	0.35	0.50	0.46
OM	20.9	20.4	20.4	20.3	22.0	21.0	20.8	20.4	0.41	0.10	0.09	0.10	0.32	0.54	0.45
CP	4.56	4.44	4.38	4.35	4.87	4.65	4.55	4.45	0.090	0.01	0.01	0.11	0.38	0.52	0.45
NDF	7.89	7.68	7.52	7.48	9.15	8.74	8.46	8.26	0.165	<0.01	<0.01	0.12	0.28	0.51	0.49
iNDF[4]	1.00	0.98	0.92	0.92	2.10	2.01	1.89	1.85	0.031	<0.01	<0.01	0.22	0.04	0.35	0.55
pdNDF[5]	6.88	6.69	6.59	6.55	7.05	6.73	6.57	6.41	0.135	0.92	0.01	0.11	0.42	0.57	0.49
Ether extract	0.72	0.71	1.15	1.15	1.18	1.13	1.48	1.44	0.023	<0.01	<0.01	0.22	<0.01	0.34	0.79
Starch	4.78	4.63	4.39	4.36	3.92	3.71	3.49	3.43	0.079	<0.01	<0.01	0.08	0.85	0.71	0.29
Digestibility, %															
DM	77.0	78.8	77.3	77.5	67.8	68.4	66.5	68.2	0.54	<0.01	0.34	0.09	0.83	0.90	0.85
OM	78.2	79.9	78.4	78.8	69.3	69.9	67.9	69.8	0.50	<0.01	0.29	0.06	0.81	0.89	0.97
CP	69.8	71.5	71.1	71.4	69.9	72.6	71.3	70.4	1.16	0.92	0.94	0.42	0.68	0.95	0.27
NDF	70.1	72.0	70.1	72.2	56.2	55.2	53.2	55.6	0.58	<0.01	0.37	0.05	0.27	0.32	0.19
Ether extract	66.6	68.9	74.2	71.8	62.6	66.9	68.0	67.1	1.42	0.02	0.03	0.64	0.48	0.62	0.16

[1]Protein supplement: RSM = rapeseed meal, RSC = cold-pressed rapeseed cake. Supplementation of 3-NOP: CON = control, 0 mg/kg of diet DM; 3-NOP = 68 mg/kg of diet DM.

[2]The 3-way interaction (energy × protein × 3-NOP) was not significant ($P \geq 0.23$).

[3]The highest value within the variable is reported and represents the SEM associated with the LSM of main effects, which is lower than the SEM associated with the 3-way interaction (energy × protein × 3-NOP).

[4]iNDF = indigestible NDF.

[5]pdNDF = potentially digestible NDF, calculated as NDF − iNDF.

by feeding RSM, but only in combination with high-oil oats. Total DMI and intake of chemical constituents were not affected by 3-NOP supplementation ($P \geq 0.10$), with the exception of a tendency toward reduced starch intake ($P = 0.08$).

Apparent total-tract digestibility of DM and OM was lower ($P < 0.01$) in cows fed high-oil oats than in cows fed barley and tended to be greater ($P \leq 0.09$) in cows supplemented with 3-NOP (Table 3). Lower digestibility of NDF ($P < 0.01$) was observed in cows fed high-oil oats versus barley, whereas NDF digestibility was increased ($P = 0.05$) by 3-NOP supplementation. Digestibility of EE was lower ($P = 0.02$) in cows fed high-oil oats compared with barley, and was greater ($P = 0.03$) in cows fed RSC compared with RSM.

Milk Production and Composition

Milk and ECM yields were greater ($P < 0.01$) when high-oil oats were fed compared with barley, but were not affected by protein supplement ($P = 0.78$; Table 4). Milk yield was reduced ($P = 0.02$), and ECM yield tended to be reduced ($P = 0.07$), by 3-NOP supplementation. Milk protein concentration was lower ($P < 0.01$) when high-oil oats were fed compared with barley, and when RSC was fed compared with RSM, whereas milk fat concentration was not affected by any of the dietary treatments ($P \geq 0.35$). A 3-way interaction ($P = 0.04$) was observed for MUN concentration, with increased MUN levels in response to 3-NOP supplementation, except when barley was fed in combination with RSC or high-oil oats were fed in combination with RSM. Feed efficiency was greater ($P = 0.04$) when high-oil oats were fed compared with barley, but was not affected by protein supplement ($P = 0.10$) or by 3-NOP supplementation ($P = 0.93$).

Gas Emissions

Dry matter intake of bait feed in the GF units was slightly greater ($P = 0.01$) when RSC was fed compared with RSM (Table 5). An interaction ($P = 0.03$) between energy source and protein supplement was observed for CH_4 emissions (g/d), CH_4 yield (g/kg of DMI), and CH_4 intensity (g/kg of ECM). Methane emissions were reduced by replacing barley with high-oil oats when combined with RSM, but were not affected when combined with RSC. Similarly, CH_4 emissions were not affected by replacing RSM with RSC when high-oil oats were used as the energy source, but emissions were reduced when barley was used. Similar patterns were observed for CH_4 yield and CH_4 intensity. When CH_4 was expressed per kilogram of OM digested, a tendency for an interaction ($P = 0.06$) between energy source and protein supplement was observed. Methane per kilogram of OM digested was similar (22.3–22.9 g/kg) for the barley + RSM, high-oil oats + RSM, and high-oil oats + RSC diets, whereas lower emissions (19.7 g/kg of OM digested) were observed for the barley + RSC diet compared with each of the other diets.

Methane emissions, CH_4 yield, and CH_4 intensity were reduced ($P < 0.01$), and H_2 emissions, H_2 yield, and H_2 intensity were increased ($P < 0.01$) by 3-NOP supplementation. A tendency for an interaction ($P = 0.06$) between energy source and 3-NOP supplementation was observed, with a greater reduction in CH_4 emissions and CH_4 yield occurring in diets containing barley compared with high-oil oats. For CH_4 intensity, greater efficacy of 3-NOP was observed when barley was fed instead of high-oil oats ($P = 0.05$). The respiratory quotient was slightly lower when high-oil oats were fed instead of barley ($P < 0.01$), and when RSC was fed instead of RSM ($P = 0.05$).

Blood Plasma and Urine Parameters

Increased plasma concentrations of NEFA ($P = 0.04$), along with a tendency for slightly increased glucose concentrations ($P = 0.07$), were detected in cows fed high-oil oats compared with barley (Table 6). Blood parameters were not affected by 3-NOP supplementation ($P \geq 0.26$). A 3-way interaction ($P < 0.01$) was observed between energy source, protein supplement, and 3-NOP supplementation regarding urinary urea concentration. An increase in urinary urea was observed in response to 3-NOP supplementation only with the high-oil oats + RSC diet (Table 7). Additionally, urinary urea concentration was greater with high-oil oats compared with barley ($P < 0.01$), and lower with RSC than with RSM ($P < 0.01$), but only in combination with high-oil oats. Urinary phosphorus, expressed as the ratio to urinary creatinine, was greater ($P = 0.02$) when high-oil oats were fed compared with barley, and was lower ($P = 0.04$) when RSC was fed compared with RSM. An increase ($P < 0.01$) in the urinary calcium-to-creatinine ratio was observed with 3-NOP supplementation.

DISCUSSION

The objective of this study was to evaluate apparent total-tract digestibility, lactational performance, and enteric CH_4 emissions of dairy cows fed 2 different energy sources (barley vs. high-oil oats) and 2 different protein supplements (RSM vs. RSC), with or without 3-NOP supplementation. Diets were formulated to be isonitrogenous but varied in starch, NDF, iNDF, and EE content due to inherent differences in the feed ingredients. It was hypothesized that replacing barley with high-oil oats, due to their lower digestibility and greater EE content, would reduce enteric CH_4 emissions without

Table 4. Lactation performance of dairy cows (n = 96) fed 2 different energy sources and 2 different protein supplements, with or without supplementation of 3-nitrooxypropanol (3-NOP)

	Diet[1]															
	Barley				High-oil oats					*P*-value						
	RSM		RSC		RSM		RSC									
Item	CON	3-NOP	CON	3-NOP	CON	3-NOP	CON	3-NOP	SEM[2]	Energy	Protein	3-NOP	Energy × protein	Energy × 3-NOP	Protein × 3-NOP	Energy × protein × 3-NOP
Yield, kg/d																
Milk	34.4	33.6	34.7	33.2	36.7	35.6	37.0	36.1	0.85	<0.01	0.78	0.02	0.59	0.82	0.78	0.61
ECM	39.4	38.9	39.7	37.5	41.6	40.7	41.6	41.3	0.98	<0.01	0.84	0.07	0.40	0.52	0.57	0.28
Protein	1.37	1.35	1.34	1.30	1.43	1.39	1.42	1.39	0.033	<0.01	0.25	0.11	0.38	0.99	0.77	0.78
Fat	1.64	1.64	1.67	1.56	1.74	1.71	1.74	1.75	0.049	<0.01	0.91	0.23	0.40	0.39	0.53	0.20
Lactose	1.62	1.57	1.66	1.58	1.75	1.68	1.76	1.71	0.042	<0.01	0.26	0.01	0.90	0.75	0.83	0.57
Concentration, g/kg																
Milk protein	40.1	40.7	38.9	39.5	39.1	39.3	38.6	38.6	0.50	<0.01	<0.01	0.10	0.10	0.13	0.84	0.75
Milk fat	48.1	49.4	48.4	47.6	47.7	48.5	47.4	48.6	0.93	0.64	0.56	0.35	0.65	0.54	0.56	0.38
Milk lactose	46.9	46.7	48.0	47.7	47.5	47.4	47.7	47.7	0.28	0.05	<0.01	0.15	<0.01	0.41	0.83	0.66
MUN, mg/dL	11.8	12.9	10.9	11.5	13.5	13.5	11.5	12.6	0.262	<0.01	<0.01	<0.01	0.40	0.46	0.43	0.04
Milk N efficiency, g/kg	294	297	302	293	287	293	307	309	6.2	0.63	0.06	0.98	0.10	0.48	0.43	0.73
FE[3]	1.61	1.63	1.68	1.59	1.62	1.67	1.73	1.75	0.038	0.04	0.10	0.93	0.22	0.22	0.30	0.56
BW, kg	641	634	632	630	635	634	629	635	11.7	0.71	0.11	0.63	0.38	0.17	0.17	0.78

[1]Protein supplement: RSM = rapeseed meal, RSC = cold-pressed rapeseed cake. Supplementation of 3-NOP: CON = control, 0 mg/kg of diet DM; 3-NOP = 68 mg/kg of diet DM.

[2]The highest value within the variable is reported and represents the SEM associated with the LSM of main effects, which is lower than the SEM associated with the 3-way interaction (energy × protein × 3-NOP).

[3]FE = feed efficiency, calculated as the ratio of ECM (kg/d) to total DMI (kg/d).

Table 5. Enteric gas emissions of lactating dairy cows (n = 96) fed 2 different energy sources and 2 different protein supplements, with or without supplementation of 3-nitrooxypropanol (3-NOP)

	Diet[1]														
	Barley				High-oil oats										
	RSM		RSC		RSM		RSC			*P*-value[2]					
Item	CON	3-NOP	CON	3-NOP	CON	3-NOP	CON	3-NOP	SEM[3]	Energy	Protein	3-NOP	Energy × protein	Energy × 3-NOP	Protein × 3-NOP
Concentrate intake from GreenFeed, kg/d	0.97	0.94	1.06	1.10	1.09	0.95	1.07	1.12	0.031	0.29	0.01	0.60	0.51	0.61	0.09
CH_4, g/d	437	292	392	246	384	263	368	251	8.8	<0.01	<0.01	<0.01	0.03	0.06	0.95
CH_4, g/kg of DMI	18.0	12.3	16.4	10.7	15.0	11.0	15.4	10.8	0.41	<0.01	0.05	<0.01	0.03	0.06	0.70
CH_4, g/kg of OM digested[4]	27.3	18.7	23.6	15.9	25.7	19.9	25.7	18.9	0.84	0.08	0.02	<0.01	0.06	0.17	0.95
CH_4, g/kg of ECM	11.3	7.6	9.8	6.7	9.4	6.6	8.9	6.2	0.25	<0.01	<0.01	<0.01	0.03	0.05	0.38
CO_2, kg/d	14.8	14.9	14.4	14.2	14.0	14.4	13.7	14.2	0.25	0.01	0.02	0.28	0.39	0.16	0.78
CO_2, g/kg of DMI	610	627	605	605	548	595	574	606	11.9	<0.01	0.83	0.02	0.12	0.12	0.42
CO_2, g/kg of ECM	381	389	366	383	342	362	334	350	9.2	<0.01	0.12	0.02	0.98	0.69	0.83
H_2, g/d	2.33	6.11	2.40	5.81	2.27	5.77	2.03	5.93	0.177	0.38	0.70	<0.01	0.84	0.78	0.96
H_2, g/kg of DMI	0.096	0.262	0.101	0.247	0.087	0.241	0.086	0.253	0.0085	0.31	0.98	<0.01	0.58	0.83	0.83
H_2, g/kg of ECM	0.059	0.165	0.062	0.156	0.054	0.149	0.050	0.148	0.0058	0.09	0.70	<0.01	0.96	0.74	0.73
O_2, kg/d	9.92	10.09	9.86	9.75	9.74	9.88	9.62	9.78	0.171	0.12	0.14	0.36	0.63	0.52	0.54
RQ[5]	1.083	1.071	1.061	1.056	1.045	1.055	1.038	1.053	0.0063	<0.01	0.05	0.73	0.22	0.06	0.58

[1]Protein supplement: RSM = rapeseed meal, RSC = cold-pressed rapeseed cake. Supplementation of 3-NOP: CON = control, 0 mg/kg of diet DM; 3-NOP = 68 mg/kg of diet DM.

[2]The 3-way interaction (energy × protein × 3-NOP) was not significant ($P \geq 0.31$).

[3]The highest value within the variable is reported and represents the SEM associated with the LSM of main effects, which is lower than the SEM associated with the 3-way interaction (energy × protein × 3-NOP).

[4]CH_4, g/kg of OM digested, is based on 64 observations.

[5]RQ = respiratory quotient calculated as the ratio of CO_2 produced (L/d) to O_2 consumed (L/d).

Table 6. Blood metabolites of lactating dairy cows (n = 64) fed 2 different energy sources and 2 different protein supplements, with or without supplementation of 3-nitrooxypropanol (3-NOP)

	Diet[1]									*P*-value[2]						
	Barley				High-fat oats											
	RSM		RSC		RSM		RSC									
Item	CON	3-NOP	CON	3-NOP	CON	3-NOP	CON	3-NOP	SEM[3]	Energy	Protein	3-NOP	Energy × protein	Energy × 3-NOP	Protein × 3-NOP
Insulin, µg/L	0.328	0.418	0.263	0.33	0.373	0.419	0.327	0.346	0.0343	0.52	0.17	0.26	0.87	0.64	0.80
Glucose, mg/dL	65.6	69.2	65	65.6	69.7	68.7	66.8	69.3	1.05	0.07	0.22	0.26	0.70	0.60	0.90
NEFA,[4] µEq/L	157	161	224	192	214	227	309	208	23.0	0.04	0.12	0.28	0.84	0.57	0.16
BHB, m*M*	1.23	1.12	1.12	1.13	1.11	1.17	0.99	1.02	0.061	0.21	0.16	0.96	0.48	0.46	0.75

[1]Protein supplement: RSM = rapeseed meal, RSC = cold-pressed rapeseed cake. Supplementation of 3-NOP: CON = 0 mg/kg of diet DM, 3-NOP = 68 mg/kg of diet DM.

[2]The 3-way interaction (energy × protein × 3-NOP) was not significant ($P \geq 0.20$).

[3]The highest value within the variable is reported and represents the SEM associated with the LSM of main effects, which is lower than the SEM associated with the 3-way interaction (energy × protein × 3-NOP).

[4]NEFA = nonesterified fatty acids.

Table 7. Urea and mineral concentration (reported as ratio to urinary creatinine) in urine of lactating dairy cows (n = 64) fed 2 different energy sources and 2 different protein supplements, with or without supplementation of 3-nitrooxypropanol (3-NOP)

	Diet[1]									*P*-value							
	Barley				High-oil oats												
	RSM		RSC		RSM		RSC										
Item	CON	3-NOP	CON	3-NOP	CON	3-NOP	CON	3-NOP	SEM[2]	Energy	Protein	3-NOP	Energy × protein	Energy × 3-NOP	Protein × 3-NOP	Energy × protein × 3-NOP
Urea, mg/dL	13.6	15.1	14.2	13.4	21.0	19.1	16.1	18.7	0.56	<0.01	0.01	0.51	0.06	0.99	0.33	<0.01
Calcium	0.076	0.152	0.097	0.217	0.114	0.151	0.108	0.167	0.0150	0.96	0.26	<0.01	0.35	0.23	0.43	0.78
Magnesium	0.181	0.199	0.219	0.178	0.187	0.217	0.184	0.226	0.0114	0.49	0.67	0.36	0.82	0.07	0.36	0.18
Phosphorus	0.022	0.022	0.019	0.020	0.027	0.036	0.022	0.023	0.0020	0.02	0.04	0.30	0.16	0.42	0.46	0.36

[1]RSM = rapeseed meal, RSC = cold-pressed rapeseed cake. Supplementation of 3-NOP: CON = control, 0 mg/kg of diet DM; 3-NOP = 68 mg/kg of diet DM.

[2]The highest value within the variable is reported and represents the SEM associated with the LSM of main effects, which is lower than the SEM associated with the 3-way interaction (energy × protein × 3-NOP).

negatively affecting lactational performance. Similarly, it was hypothesized that replacing RSM with RSC, due to its greater EE content, would lower CH_4 emissions without negatively affecting lactational performance. An additional objective of the study was to assess potential interactions between energy source, protein supplement, and 3-NOP supplementation. The target dose of 3-NOP was 60 mg/kg of diet DM. However, post-trial analysis showed that the actual average dose was 68 mg/kg of DM. Therefore, the findings are discussed in the context of the analyzed 3-NOP concentration.

Feed Intake and Apparent Total-Tract Digestibility

The reported effects of 3-NOP on DMI in dairy cows are variable. In agreement with our results, Hristov et al. (2015a) reported no effect on DMI at doses of 40 to 80 mg 3-NOP/kg of DM. In contrast, Maigaard et al. (2024) and Kjeldsen et al. (2024) observed reductions of 11% to 13% at a dose of 80 mg/kg of DM. Similarly, Maigaard et al. (2025) evaluated 3-NOP at 60 and 80 mg/kg of DM and reported a 9% reduction in DMI at the greater dose (averaged across 2 different basal diets), but no effect at the lower dose. A recent meta-analysis by Martins et al. (2025) found that 3-NOP supplementation reduced DMI by an average of 0.8 kg/d compared with controls, with a negative association between 3-NOP dose and DMI. Moreover, greater dietary forage-to-concentrate ratio and CP content each lowered the negative effect of 3-NOP on DMI. Therefore, similar DMI between 3-NOP and control cows in our study may be explained by a combination of factors, including the relatively low 3-NOP dose (68 mg/kg of DM), a 60:40 forage-to-concentrate ratio, and a dietary CP content (18.5% of diet DM) above the average (16.3% of diet DM) reported by Martins et al. (2025).

Replacing barley with regular oats has previously reduced total-tract OM digestibility by 2.8% to 3.8% and NDF digestibility by 6.5% to 10.0% (Vanhatalo et al., 2006; Ramin et al., 2021), which is consistent with the findings of this study with high-oil oats, although reductions in this study were greater (12.2% for OM and 22.6% for NDF digestibility). The reduced total-tract OM digestibility observed with high-oil oats can primarily be attributed to the dietary substitution of starch, a readily digestible carbohydrate, with NDF, which is a less digestible carbohydrate. Most of the variation in OM digestibility observed when cows are fed grass silage–based diets can be explained by the amount and digestibility of NDF (Huhtanen et al., 2009). In our study, lower NDF digestibility can be partly attributed to the greater ratio of iNDF to NDF in high-oil oats compared with barley. Additionally, due to a tendency for increased total DMI with high-oil oats, iNDF intake increased by 105%. Furthermore, the increased NDF-to-starch ratio resulting from the replacement of barley with high-oil oats may have contributed to the observed reduction in NDF digestibility. Zhao et al. (2016) reported decreases in both OM and NDF digestibility as the dietary NDF-to-starch ratio increased from 0.86 to 2.34; however, in their study the change was driven by forage NDF rather than concentrate NDF, as in our study. In contrast, Beckman and Weiss (2005) found that, when confounding factors such as inherent NDF digestibility were minimized, increasing the NDF-to-starch ratio from 0.74 to 1.27 reduced OM digestibility but had no effect on NDF digestibility. In the present study, the NDF-to-starch ratio increased from 1.80 to 2.61. However, because this change was accompanied by other changes in dietary composition, specific mechanisms cannot be elucidated.

Although dietary fat can impair fiber digestion, particularly through inhibition of ruminal cellulolytic bacteria (Jenkins, 1993), reductions in ruminal NDF digestibility may be partly compensated by postruminal digestion, resulting in no adverse effect on total-tract digestibility (Harvatine and Allen, 2006). Negative effects of dietary fat on total-tract NDF digestibility have been reported mainly for SFA containing 12 or 14 carbon atoms (Weld and Armentano, 2017). In contrast, fats composed mainly of long-chain fatty acids with 16 or 18 carbon atoms, such as those present in oat oil (Fant et al., 2023), have been shown to have only minor effects on total-tract NDF digestibility (Weld and Armentano, 2017). Therefore, it is unlikely that the increased EE content of the high-oil oat diet explains the observed reduction in NDF digestibility in the present study.

The reported effect of 3-NOP on apparent total-tract digestibility of OM and NDF have been variable. Melgar et al. (2020a) found no effect of 3-NOP on NDF digestibility at a dose of 60 mg/kg of DM. In contrast, Maigaard et al. (2024) reported substantial increases in total-tract OM and NDF digestibilities with 3-NOP supplementation at 80 mg/kg of DM, which was associated with reduced DMI and, consequently, decreased passage rate. In our study, the observed increase in total-tract NDF digestibility with 3-NOP supplementation was minor (2.1%) and likely of low biological relevance. This may be explained by the fact that DMI was not affected by 3-NOP supplementation, similar to the findings of Melgar et al. (2020a).

Milk Production and Composition

To the best of our knowledge, no previous study has directly compared the lactational performance of cows fed high-oil oats versus barley. However, several studies have reported increased milk yields of 1.1 to 2.7 kg/day when barley was replaced with regular oats (Vanhatalo et al., 2006; Tosta et al., 2019; Fant et al., 2021), along with increases in ECM yield of 0.8 to 0.9 kg/d (Vanhatalo et al., 2006; Fant et al., 2021). In contrast, some studies have found no significant differences in milk or ECM yield between diets with barley and regular oats (Ramin et al., 2021). Furthermore, Ekern et al. (2003) reported that replacing regular oats with high-oil oats increased both milk and ECM yield by 1.2 kg/d. In our study, the observed increases in both milk and ECM yield by 2.4 kg/d with high-oil oats, despite greater dietary NDF content and lower total-tract OM digestibility, deserves further attention. Increasing fat content of dairy cow diets is known to raise dietary energy density and increase milk yield, up to 6.0% fat of diet DM (Palmquist and Jenkins, 1980; Weiss and Pinos-Rodríguez, 2009; Patra, 2013). In our study, replacing barley with high-oil oats led to a shift from dietary starch to NDF and EE. Starch intake decreased by 20%, and NDF and EE intakes increased by 13% and 40%, respectively, in cows fed high-oil oats. Despite the reduction in starch intake and OM digestibility, the increased energy contribution from fat may have compensated for this, providing sufficient energy for milk synthesis. Greater dietary fat intake increases the supply and uptake of long-chain fatty acids, which can be directly incorporated into milk fat in the mammary gland (Chilliard, 1993). This reduces the need for de novo fatty acid synthesis, which requires glucose as a precursor, potentially sparing glucose for lactose synthesis and thereby supporting greater milk volume (Rigout et al., 2002; Hammon et al., 2008).

As previously discussed, increasing dietary fat content has been associated with greater milk yields (Patra, 2013). Therefore, replacing RSM with RSC, which increased the dietary EE content, could be expected to increase milk yield. However, in the present study, milk yield remained unchanged. In contrast, earlier studies reported increased milk yields when RSM was replaced with RSC as a strategy to raise dietary fat content. Brask et al. (2013) observed numerical increases in milk and ECM yields of 3.8 and 3.0 kg/d, respectively, while Bayat et al. (2022) reported significant increases of 4.0 and 2.6 kg/d. In those studies, dietary fat content (on a DM basis) increased by 1.6 to 2.0 percentage points, similar to the 1.8 percentage points in our study. This discrepancy may be partly explained by differences in DMI. In our study, replacing RSM with RSC tended to reduce DMI by 0.8 kg/d, while DMI was unaffected in the previous studies.

In a study by Räisänen et al. (2024), replacing barley and RSM with regular oats and RSC reduced DMI but tended to increase milk yield by 1.1 kg/d. In our study, milk yield increased by 2.6 kg/d with the high-oil oats + RSC diet compared with the barley + RSM diet, at similar DMI. Additionally, milk yield was 2.2 kg/d greater with the high-oil oats + RSM diet than with the barley + RSC diet, despite similar dietary EE content and intake. This indicates that factors beyond increased dietary fat content contributed to the variation in milk yield. Notably, DMI was 1.4 kg/d greater for cows fed the high-oil oats + RSM diet than for those fed the barley + RSC diet, while DMI did not differ across the other treatment combinations (including energy source and protein supplement). This represents a 6.5% increase in DMI, which closely mirrors the 5.9% increase in milk yield.

In line with the findings of our study, previous studies have reported reduced milk protein concentrations following replacement of barley with regular oats (Ekern et al., 2003; Vanhatalo et al., 2006; Fant et al., 2021). This effect has previously been attributed to a dilution phenomenon, where increases in milk yield are not proportionally matched by increases in protein synthesis, leading to a reduced milk protein concentration. In this study, milk yield increased by 7.0%, whereas milk protein concentration decreased by 2.3%, supporting the presence of a dilution effect. The reduction in milk protein concentration observed in this study following the replacement of RSM with RSC cannot be attributed to a dilution effect. The numerical increase in milk yield was only 0.6%, whereas the decrease in milk protein concentration was 2.3%, which exceeds the reduction expected from dilution alone. Instead, the lower milk protein concentration may be explained by reduced intakes of CP and starch in cows fed the RSC diet, decreasing the MP supply and thereby limiting protein synthesis in the mammary gland (Daniel et al., 2016).

Previous studies have generally reported no significant effects of 3-NOP supplementation at 60 mg/kg of DM on milk or ECM yield (Hristov et al., 2015a; Melgar et al., 2020a; Maigaard et al., 2025). At greater inclusion rates (e.g., 80 mg/kg of DM), some studies have observed reductions in milk yield of 4.5% to 11.7% (Maigaard et al., 2024; Maigaard et al., 2025) or a trend toward a 7.2% reduction (Kjeldsen et al., 2024), while others found no effect at the same dose (Hristov et al., 2015a; Melgar et al., 2020b). van Gastelen et al. (2022) also reported a 4.4% decrease in milk yield at 80 mg/kg of DM, but no effect at 60 mg/kg of DM. Given these findings, the 3.0% reduction in milk yield observed in this study is consistent with what might be expected at the actual 3-NOP dose of 68 mg/kg of DM. Although the original hypothesis assumed no effect on lactational performance at the target dose of 60 mg/kg of DM, the slightly greater actual dose means the hypothesis cannot be clearly confirmed or rejected.

The 3.0% reduction in milk yield and the tendency for a 2.4% reduction in ECM due to 3-NOP supplementation most likely resulted from the numerical reduction in DMI (2.6%) and are consistent with the literature (Martins et al., 2025). The smaller decline in ECM may be explained by the numerical increases in milk protein and milk fat concentrations (0.9% and 1.3%, respectively, averaged across all diets). Previous findings on the effect of 3-NOP on milk fat concentration are mixed. In line with our results, several studies reported no effect of 3-NOP across doses of 40 to 80 mg/kg of DM and varying basal diets (Hristov et al., 2015a; Melgar et al., 2020a; Maigaard et al., 2025). In contrast, other studies observed increased milk fat concentrations with 3-NOP (Melgar et al., 2021; van Gastelen et al., 2020; Maigaard et al., 2024). This increase has been attributed to shifts in rumen fermentation, specifically an increase in butyrate concentration, which is a precursor for de novo synthesis of milk fatty acids (Hristov et al., 2022).

Because the diets were isonitrogenous, the greater MUN concentrations observed with high-oil oats compared with barley may be explained by the lower dietary starch content. This likely altered the balance between available N in the rumen and readily fermentable energy required for efficient microbial protein synthesis (Huntington, 1997). The elevated MUN concentrations were also consistent with increased urinary urea concentrations. In this study, MUN concentrations decreased when RSM was replaced by RSC in diets without 3-NOP, which is consistent with the findings of Bayat et al. (2022). Supplementation with 3-NOP increased MUN in some diets (barley + RSM and high-oil oats + RSC), aligning with mixed responses reported in earlier studies (Hristov et al., 2015a; Melgar et al., 2021; Maigaard et al., 2025).

Gas Emissions

Because interactions in relation to CH_4 emissions were observed between energy source and protein supplement, as well as a tendency for interaction between energy source and 3-NOP supplementation, the results will be discussed within the context of these interactions. Our previous work (Ramin et al., 2021) showed that replacing barley with regular oats, combined with RSM as the protein supplement, reduced daily CH_4 emissions and CH_4 yield by 4.7% and 4.4%, respectively. In the current study, using high-oil oats with RSM led to greater reductions in daily CH_4 emissions (11.2%) and CH_4 yield (14.2%). Previous studies (Fant et al., 2020; Ramin et al., 2021; Fant et al., 2021) suggest that the lower digestibility of oats compared with barley is a primary driver of reduced CH_4 emissions. A positive association between digestibility and enteric CH_4 emissions has been well established (Blaxter and Clapperton, 1965; Ramin and Huhtanen, 2013). In the present study, OM digestibility was 12.0% lower for the high-oil oats + RSM diet compared with the barley + RSM diet, whereas the difference was only 3.8% in our earlier study (Ramin et al., 2021). This greater reduction in digestibility likely contributed to the larger decrease in CH_4 emissions observed. We also hypothesized that the greater dietary fat content of the high-oil oat diet would contribute to the mitigation effect because dietary fats are known to reduce enteric CH_4 production (Patra, 2013; Martins et al., 2024). In the previous study (Ramin et al., 2021), the difference in dietary fat content between the barley and oat diets was 0.9 percentage points (on a DM basis), whereas in the current study it was approximately double (1.7 percentage points), potentially explaining the greater reduction in CH_4 emissions. However, CH_4 emissions per kilogram of OM digested were similar between the barley + RSM and high-oil oats + RSM diets, indicating that the reduction in CH_4 yield was mainly due to lower OM digestibility and thus a reduced supply of fermentable carbohydrates (dietary starch concentrations 18.6% vs. 14.1% of DM), rather than a direct inhibition of methanogenesis by dietary fat.

Several studies have reported reductions in CH_4 emissions and CH_4 yield following the replacement of RSM with RSC in dairy cow diets. Brask et al. (2013) and Bayat et al. (2022) reported a 4.6% decrease in CH_4 yield per percentage unit increase in dietary fat from RSC, which is smaller than the reduction observed in the present study (5.3%) when barley was used as the energy source. Consistent with our findings, Bayat et al. (2022) also observed a decrease in CH_4 expressed per kilogram of OM digested with RSC, and Brask et al. (2013) observed a tendency for reduced CH_4 emissions per kilogram of digested OM with different forms of rapeseed fat. Räisänen et al. (2024) compared a diet with barley and RSM to a diet with regular oats and RSC and reported a 9.4% reduction in daily CH_4 emissions with the latter diet, which is slightly lower than the reduction in this study (15.1%). However, part of the reduction observed by Räisänen et al. (2024) was explained by decreased DMI, and no effect on CH_4 yield was observed.

Interestingly, no effect of RSC on daily CH_4 emissions or CH_4 yield was observed when high-oil oats were used as energy source in the present study. This suggests that, under the conditions of the current experiment, replacing barley with high-oil oats or replacing RSM with RSC are both viable strategies for reducing enteric CH_4 emissions, but combining these approaches does not provide additional mitigation benefits. The lack of an additive effect remains unclear, but could be explained by differences in nutrient composition between barley and high-oil oats. Patra (2013) found that the CH_4 mitigating effect of dietary fat increased with greater levels of nonfibrous carbohydrates in the diet. Similarly, a meta-analysis by Martins et al. (2024) concluded that the CH_4-mitigating effect of fat supplementation increased with greater dietary starch content and decreased with greater dietary acid detergent fiber content. Therefore, the lower starch and greater fiber content in the high-oil oat diet may have reduced the CH_4-mitigating effect of fat in RSC.

In the present study, average reductions in daily CH_4 emissions, CH_4 yield, and CH_4 intensity were 33.5%, 30.9%, and 31.2%, respectively, across all diets supplemented with 3-NOP. These reductions are in line with previous findings using maize silage (Hristov et al., 2015a; Melgar et al., 2020b; Melgar et al., 2021), and are comparable to or slightly greater than those reported in studies using grass silage (van Gastelen et al., 2022; Johansen et al., 2025). Moreover, a meta-analysis by Kebreab et al. (2023) reported reductions of 32.7%, 30.9%, and 32.6%, respectively, at an average 3-NOP inclusion rate of 70.5 mg/kg of DM, which closely aligns with the present study's results, given the comparable dose of 68 mg/kg of DM. Although the observed trend suggesting greater efficacy of 3-NOP when combined with barley compared with high-oil oats is modest, it is a noteworthy finding for further discussion. Previous studies have shown that the nutritional composition of the diet can influence the efficacy of 3-NOP (van Gastelen et al., 2022, 2024). In the meta-analysis by Kebreab et al. (2023), increasing dietary concentrations of NDF and crude fat were associated with decreased 3-NOP efficacy. In our study, diets with high-oil oats had greater levels of both NDF and EE, with NDF increasing from 32.3% to 35.7% of diet DM. According to Kebreab et al. (2023), a 1% DM increase in dietary NDF content reduced the effect of 3-NOP on daily CH_4 emissions and CH_4 yield by 0.633% and 0.647%, respectively. In our study, the efficacy of 3-NOP in reducing daily CH_4 emissions and CH_4 yield tended to decline by 2.93% and 4.26%, respectively, for every 1% DM increase in dietary NDF content, calculated relative to the reduction observed in the control diet. It should be noted that the EE content also increased (from 3.9% to 5.3% of diet DM) in these diets, which could have contributed to the observed trend for reduction in 3-NOP efficacy.

In our study, the tendency for reduced CH_4 mitigation efficacy of 3-NOP when high-oil oats were used as the energy source, alongside the apparent additive effect of RSC and 3-NOP, presents a contradictory finding. This is particularly unexpected if the CH_4-mitigating effect of high-oil oats is attributed solely to their greater fat content compared with barley, similar to the case of RSC versus RSM. However, as previously discussed, part of the CH_4-reducing effect of oats has also been linked to their lower total-tract digestibility (Fant et al., 2020, 2021). Furthermore, high-oil oats and RSC represent 2 distinct fat sources, which may interact differently within the rumen environment. Thus, the observed discrepancy may reflect differences in fat type and potential interactions with other dietary components (Kebreab et al., 2023).

The observed additive effect of 3-NOP and RSC on CH_4 emissions, yield, and intensity deserves further discussion. An additive effect of dietary fat and 3-NOP was also reported by Zhang et al. (2021), where supplementation of 3-NOP in beef cattle diets, using rapeseed oil as the fat source, led to a synergistic reduction in CH_4 emissions. In contrast, Maigaard et al. (2024) found no such additive effect when 3-NOP was supplemented in dairy cow diets and whole cracked rapeseed was used as the fat source, suggesting that the form of fat source may influence the interaction with 3-NOP. As previously noted, the meta-analysis by Kebreab et al. (2023) showed that increasing dietary crude fat content may reduce the efficacy of 3-NOP. However, in the present study, no such reduction in efficacy was observed when dietary fat content was increased by replacing RSM with RSC. It is unlikely that the relatively small reduction in NDF content (from 34.3% to 33.7% of diet DM) with RSC was sufficient to offset a potential negative effect of increased fat, especially considering that dietary starch content also decreased (from 16.4% to 15.3% of diet DM), which has been associated with reduced 3-NOP efficacy (Kebreab et al., 2023). It is important to note that the models used by Kebreab et al. (2023) did not distinguish between different types or forms of fat sources, and missing nutritional data were estimated using standard feed tables. These limitations, along with the differences between our findings and those of previous studies, highlight the need for more focused studies to investigate how the type and form of dietary fat influence the effectiveness of 3-NOP in reducing CH_4 emissions from ruminants.

Urine Parameters

Urinary mineral analyses were included to complement the nutritional and environmental assessment of dietary treatments. Urea concentration in urine was measured as a complement to MUN. Because creatinine is excreted in urine at a relatively constant rate, analyte concentrations were normalized to urinary creatinine to reduce the effect of hydration status and fluctuations in urine specific gravity (Scott and Stockham, 2025). Accordingly, mineral excretion results are reported exclusively as normalized values.

Because 50% to 90% of N in urine is excreted as urea, the greater urinary urea concentrations observed with high-oil oats compared with barley may indicate increased potential for N losses to the environment (Dijkstra et al., 2013). This response is likely related to a reduced supply of readily fermentable energy to rumen microbes when high-oil oats replaced barley. The increased P excretion in the urine of cows consuming high-oil oats is mainly associated with the slightly greater P content in this feed type (Mayer et al., 2023). However, only about

1% of ingested P is excreted in urine, and mainly following excessive P feeding (Morse et al., 1992). The increase in urine Ca+ concentration with supplementation of 3-NOP represents an interesting result. Although the increase in urinary Ca+ was significant ($P < 0.01$), the absolute difference was modest, and its biological relevance remains unclear. It might suggest subtle changes in calcium handling or homeostasis, but further studies would be needed to clarify the underlying mechanisms and implications. Thiel et al. (2019) demonstrated that 3-NOP undergoes partial absorption and is excreted in urine as metabolites, such as 3-nitrooxypropionic acid. Nevertheless, to the best of our knowledge, no studies have directly established a connection between 3-NOP and alterations in urinary mineral excretion.

The limitations of the method used to assess urinary mineral excretion should be acknowledged. The spot urine sampling method offers a practical and feasible alternative for estimating urinary excretion when total urine collection is not possible (Chizzotti et al., 2008). However, this method may introduce bias due to diurnal and day-to-day variation in urine composition. Accuracy may be improved by the collection of multiple spot samples throughout the day, as recommended by Lee et al. (2019). In the present study, this approach was not feasible. Although sampling time points were selected based on practicality and were applied consistently across all cows, they may not have fully captured the daily variation in urinary excretion. This limitation should be considered when interpreting the findings.

CONCLUSIONS

Replacing barley with high-oil oats increased milk yield, ECM yield, and feed efficiency, whereas replacing RSM with RSC had no effect. Supplementation with 3-NOP (68 mg/kg DM) reduced milk yield and tended to reduce ECM yield. Compared with barley, high-oil oats reduced daily CH_4 emissions, yield, and intensity by 11% to 15% when combined with RSM. Similarly, replacing RSM with RSC lowered daily CH_4 emissions, yield, and intensity by 11% to 13% when combined with barley. These results suggest that either substitution is a practical strategy for CH_4 mitigation but combining them does not offer additional mitigation benefits. Overall, high-oil oats are a promising alternative to barley for improving lactational performance while reducing enteric CH_4 emissions. However, potential increases in N emissions should be considered. Although the combination of high-oil oats and RSC did not improve lactational performance or CH_4 mitigation, it may offer advantages for N efficiency and reduced N losses relative to the high-oil oats + RSM combination. Supplementation with 3-NOP reduced CH_4 emissions, yield, and intensity by 31% to 34%, averaged across all diets. The effects of RSC and 3-NOP on CH_4 were additive under the experimental conditions of the present study.

NOTES

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Nonstandard abbreviations used: 3-nitrooxypropanol = 3-NOP; CON = control diet, 0 mg of 3-NOP; EE = ether extract; GF = GreenFeed; iNDF = indigestible NDF; NEFA = nonesterified fatty acids; pdNDF = potentially digestible NDF; RQ = respiratory quotient; RSC = cold-pressed rapeseed cake; RSM = rapeseed meal.

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