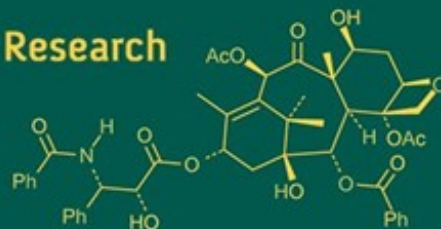


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Enteric methane emission, nutrient intake and utilization of lactating crossbred Karan Fries cows fed rations varying in total digestible nutrients

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ABSTRACT

Enteric methane is a loss of dietary energy from ruminants and a contributor to global warming, and feeding of digestible nutrients on commercial farms often departs from requirements. This study evaluated feeding total digestible nutrients 15% below or above the Indian Council of Agricultural Research (2013) requirement on enteric methane emission, nutrient intake, digestibility and blood metabolites of lactating crossbred Karan Fries cows. Eighteen cows were assigned to three groups of six according to body weight, milk yield, parity and days in milk: control (fed to requirement), below-requirement and above-requirement. The ration comprised green berseem, wheat straw and concentrate mixture. A 45-day feeding trial was followed by a 7-day digestion trial, and methane was measured for five days using the sulphur hexafluoride tracer technique. Intake of total digestible nutrients was 102.9, 86.7 and 115.1% of requirement in the three groups, respectively. Dry matter intake was higher ($P < 0.05$) in the above-requirement group (14.74 kg d⁻¹) than in the control (12.21) and below-requirement (11.22) groups. Nutrient digestibility and milk yield did not differ among groups, and daily methane emission (72.8, 114.5 and 101.7 g d⁻¹) was not significantly different. However, methane per kg of dry matter intake was lower ($P < 0.05$) in the above-requirement group (7.29 g) than in the below-requirement group (13.78 g), with the control intermediate (8.63 g), and the share of gross energy intake lost as methane was higher ($P < 0.05$) in the below-requirement group (10.24%) than in the control (9.39%) and above-requirement (9.03%) groups. Blood urea nitrogen was lower in the above-requirement group than in the control, and plasma glucose was lower in the below-requirement group. Feeding digestible nutrients below requirement increased methane loss per unit of feed intake, supporting requirement-based feeding as a practical mitigation measure.

Keywords: Enteric methane, Total digestible nutrients, Karan Fries cows, Sulphur hexafluoride tracer, Nutrient digestibility, Energy intake

1. Introduction

Livestock make a substantial contribution to anthropogenic greenhouse gas emissions. The Food and Agriculture Organization attributed about 18% of human-induced emissions to the sector, including roughly 9% of carbon dioxide, 37% of methane and 65% of nitrous oxide emissions (Steinfeld *et al.*, 2006) [16], and a later assessment placed the sector's share at 14.5%, with enteric fermentation as the largest single source (Gerber *et al.*, 2013) [6]. Methane is produced in the rumen by methanogenic archaea as a by-product of microbial fermentation of feed, and Indian livestock are estimated to emit about 9.1 Tg of enteric methane each year (Singh *et al.*, 2012) [15]. Besides its warming effect, enteric methane represents a loss of feed energy; across a wide range of diets, 2–12% of gross energy intake is lost in this form (Johnson *et al.*, 1995) [10].

The quantity of methane produced by a cow depends on dry matter intake, the chemical composition and digestibility of the diet, and the level of feeding (Moe *et al.*, 1979; Johnson *et al.*, 1995) [12, 10]. Methane yield per unit of feed generally declines as the level of feeding rises, because faster ruminal passage and less extensive fermentation lower the methane formed per unit of intake (Blaxter *et al.*, 1965; Waghorn *et al.*, 2011) [3, 19]. Nutritional management that improves feed conversion efficiency is therefore regarded as one of the most practical ways of reducing methane intensity without compromising production (Beauchemin *et al.*, 2008; Waghorn *et al.*, 2011; Hristov *et al.*, 2013) [2, 19, 7].

On Indian dairy farms, rations are often not formulated to meet the requirements of individual animals, and cows are commonly offered energy and protein either more than, or below, their requirement. Field studies on ration balancing in lactating crossbred cows have reported reductions in daily methane emission of the order of 10–15% (Kannan *et al.*, 2009; Mohini *et al.*, 2010; Garg *et al.*, 2013) [11, 5, 13], and cows kept on an imbalanced plane of nutrition lost a larger share of gross energy as methane than cows fed to requirement

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(Mohini and Singh, 2010; Garg *et al.*, 2013) [5]. Most of these studies, however, compared animals before and after balancing under field conditions. Information from controlled trials that deliberately vary the supply of total digestible nutrients (TDN) around the standard requirement is scarce, particularly for Karan Fries, the Holstein Friesian × Tharparkar crossbred developed at the ICAR–National Dairy Research Institute, Karnal.

The present study was therefore designed to quantify the effect of feeding TDN 15% below and 15% above the Indian Council of Agricultural Research (ICAR) requirement, levels simulated from feeding patterns observed on organized dairy farms of Punjab and Haryana, on nutrient intake, digestibility, enteric methane emission measured by the sulphur hexafluoride (SF₆) tracer technique, and selected blood metabolites in lactating Karan Fries cows fed a typical ration of green berseem, wheat straw and concentrate mixture.

2. Materials and Methods

Location, animals and experimental design

The study was carried out in the experimental cattle shed of the Livestock Research Centre, ICAR–National Dairy Research Institute, Karnal, Haryana, India (29°42' N, 76°58' E; altitude 227 m above sea level). The area receives about 700 mm of rain annually, and ambient temperature ranges from near freezing in winter to 45 °C in summer. The feeding trial began on 5 April 2019. The experimental protocol, including handling and management of the animals, was approved by the Institutional Animal Ethics Committee.

Eighteen lactating Karan Fries cows were allotted to three groups of six based on body weight, 4% fat-corrected milk yield, parity and days in milk. Group averages were 424.3, 411.4 and 415.0 kg for body weight, 16.0, 15.6 and 15.9 kg d⁻¹ for 4% fat-corrected milk yield, 2 for parity in each group, and 87.2, 87.8 and 86.7 days in milk. Cows in the control group (*T*₁) were fed TDN according to the ICAR requirement (ICAR, 2013); cows in groups *T*₂ and *T*₃ were fed TDN 15% below and 15% above the *T*₁ level, respectively. The two levels were chosen as the most prevailing feeding levels, simulated from a preceding survey of 45 organized dairy farms in Punjab and Haryana.

Housing, feeding and records

Cows were tied individually in a well-ventilated shed with arrangements for individual feeding. During a 10-day adaptation period, all animals were dewormed with fenbendazole (10 mg kg⁻¹ body weight) and offered the control ration to establish dry matter intake. Cows were allowed to wallow once weekly and were splashed with cold water three times daily to relieve heat stress, and clean tap water was available free of choice three times daily. The ration consisted of green berseem (*Trifolium alexandrinum*) fodder, wheat straw and a concentrate mixture supplied by the Farm Maintenance Section and the feed store of the institute. The feeding trial lasted 45 days, including an adaptation period of 21 days. Dry matter intake was recorded daily as the difference between dry matter offered and residue left. Body weight was recorded fortnightly on two consecutive mornings, before feeding and watering, on a computerized weighing platform. Milk yield was recorded at each milking with a spring balance (capacity 10 kg; accuracy ±0.05 kg), and milk fat was measured weekly with an automatic milk analyser (Lactostar, Funke Gerber, Berlin).

Chemical analysis of feeds

Feeds offered and residues were analysed fortnightly for dry matter, organic matter, crude protein (Kjeldahl nitrogen × 6.25) and ether extract by standard methods (AOAC, 2005) [1], and for neutral detergent fibre (NDF; assayed with amylase), acid detergent fibre (ADF) and acid detergent lignin by the procedures of Van Soest *et al.* (1991) [18]. Nitrogen in the neutral and acid detergent residues was determined by the Kjeldahl method to obtain neutral and acid detergent insoluble crude protein (NDICP and ADICP). The dry matter content of berseem and wheat straw was also determined twice weekly.

Digestion trial and energy values

After the adaptation period, a seven-day digestion trial was conducted with the cows housed individually in metabolic cages that allowed quantitative collection of faeces, following two days of adaptation to the cages. Feed offered, residues and total faeces voided in 24 h were weighed daily. A faecal aliquot (1/200, in duplicate) was dried at 65 °C for 48 h for dry matter determination, and the dried material was pooled, ground to pass a 1 mm sieve and analysed as described above. Apparent digestibility was calculated as

$$\text{Digestibility (\%)} = \frac{\text{Nutrient intake} - \text{Faecal excretion}}{\text{Nutrient intake}} \times 100 \quad (1)$$

The TDN content of feeds was estimated from truly digestible fractions according to NRC (2001): truly digestible non-fibre carbohydrate (*tdNFC*) = $0.98 \times [100 - ((\text{NDF} - \text{NDICP}) + \text{CP} + \text{EE} + \text{ash})] \times \text{PAF}$, where PAF is the processing adjustment factor (1 for fodders); truly digestible crude protein (*tdCP*) = $\text{CP} \times \exp[-1.2 \times (\text{ADICP}/\text{CP})]$ for forages and $[1 - 0.4 \times (\text{ADICP}/\text{CP})] \times \text{CP}$ for concentrates; truly

digestible fatty acids (*tdFA*) = $\text{EE} - 1$ (zero when $\text{EE} < 1\%$); and truly digestible NDF (*tdNDF*) = $0.75 \times (\text{NDF}_n - \text{L}) \times [1 - (\text{L}/\text{NDF}_n)^{0.667}]$, where $\text{NDF}_n = \text{NDF} - \text{NDICP}$ and L = lignin. All values were expressed as % of dry matter. Because TDN is based on apparent digestibility, metabolic faecal TDN (7 units) was subtracted (Weiss *et al.*, 1992) [20]:

$$\text{TDN (\%)} = \text{tdNFC} + \text{tdCP} + (\text{tdFA} \times 2.25) + \text{tdNDF} - 7 \quad (2)$$

Digestible energy (DE, Mcal kg⁻¹) was calculated as $0.04409 \times \text{TDN (\%)} + 1.01 \times \text{DE} - 0.45 (+ 0.0046 \times (\text{EE} - 3))$ for feeds with more than 3% EE (NRC, 2001); 1 Mcal = 4.184 MJ. Gross, digestible and metabolizable energy intakes (GEI, DEI and MEI) were computed from the daily intakes of the feeds. TDN intake of each group was compared with its requirement according to ICAR (2013).

Blood metabolites

Blood samples were collected on days 0, 23 and 45 of the feeding trial for plasma glucose, blood urea nitrogen (BUN) and non-esterified fatty acids (NEFA). Glucose was determined by the glucose oxidase–peroxidase method (Trinder, 1969) [17] with a commercial kit (Liquimax glucose, Avecon Healthcare, India; endpoint reading at 505 nm). Plasma urea was determined by the modified Berthelot (urease) method (Chaney *et al.*, 1962) [4] with a commercial kit

(Liquimax urea, Avecon Healthcare, India; 578 nm), and BUN was calculated as $\text{urea} \times 0.467$. NEFA were measured colorimetrically at 570 nm with a free fatty acid quantification kit (K612-100, BioVision, USA) using palmitic acid as the standard.

Enteric methane measurement

Enteric methane emission was measured for five successful days using the SF₆ tracer technique (Johnson *et al.*, 1994) [9]. Permeation tubes containing SF₆, prepared at the Environmental Laboratory of the institute, were calibrated by weekly weighing over the preceding 40 days to determine the release rate, and one tube was placed in the rumen of each cow about two days before measurement. Each cow wore a halter fitted with a capillary tube connected to an evacuated canister designed to half-fill over 24 h, thereby sampling air around the nose and mouth. On each day a background sample was collected with an identical sampling kit placed at a strategic location in the nat

urally ventilated shed, and animal concentrations were corrected for background. After sampling, canisters were pressurized with nitrogen. SF₆ was analysed by gas chromatography (Nucon 5700, Nucon Engineers, New Delhi) with an electron capture detector (250 °C), and CH₄ with a flame ionization detector (100 °C) and a Porapak-Q column (1.5 m; 3.2 mm o.d.; 80–100 mesh); injector and column tem-

peratures were 40 and 50 °C, and nitrogen was the carrier gas. Samples were analysed in duplicate and standards in triplicate, with standards run at the beginning and end of each day and a medium standard after every ten samples. Methane emission rate was calculated as

$$Q_{CH_4} = Q_{SF_6} \times \left[\frac{(S_{CH_4} - B_{CH_4})}{(S_{SF_6} - B_{SF_6})} \right] \times \left(\frac{M_{CH_4}}{M_{SF_6}} \right) \times 10^6 \quad (3)$$

where *S* and *B* denote sample and background concentrations (CH₄ in ppm, SF₆ in ppt), *M* is molecular weight and Q_{SF₆} is the SF₆ release rate (mg d⁻¹); the factor 10⁶ converts the ratio of mixing ratios (ppm to ppt). Methane was expressed per day (g and MJ), per unit of intake of dry matter (DMI), digestible dry matter (DDMI), organic matter (OMI), digestible organic matter (DOMI), crude protein (CPI), digestible crude protein (DCPI), NDF (NDFI), digestible NDF (DNDFI), ADF (ADFI) and digestible ADF (DADFI), and as a percentage of GEI, DEI and MEI.

Statistical analysis

Data were analysed by one-way analysis of variance using SPSS software, and blood metabolite data (group × sampling day) by two-way analysis of variance. Differences were considered significant at *P* < 0.05. Results are presented as mean ± standard error.

3. Results

Feed composition and plane of nutrition

The chemical composition of the feeds offered during the digestion trial is given in Table 1. Berseem contained 16.21% crude protein and 43.12% NDF on a dry matter basis, the concentrate mixture 20.33% crude protein and 28.68% NDF, and wheat straw 3.80% crude protein and 62.83% NDF. Estimated TDN contents were 64.43, 75.58 and 50.19%, and ME contents 2.42, 2.92 and 1.78 Mcal kg⁻¹ dry matter, for berseem, concentrate mixture and wheat straw, respectively. The composition of the concentrate mixture and wheat straw was stable across fortnights, whereas the quality of berseem declined in the third fortnight (crude protein from 17.13 to 12.12%, NDF from 44.35 to 56.95% and TDN from 64.70 to 54.61%).

The intended contrast in supply of digestible nutrients was achieved (Table 2). During the digestion trial, TDN intake was 102.9, 86.7 and 115.1% of the ICAR requirement in T₁, T₂ and T₃, respectively, while dry matter intake was 78.4, 72.9 and 83.5% and crude protein intake 97.3, 92.6 and 106.8% of the respective requirements.

Feed intake, milk yield and body weight

Over the feeding trial, mean dry matter intake was 12.21, 11.22 and 14.74 kg d⁻¹ in T₁, T₂ and T₃, respectively (Table 3); intake in T₃ was higher (*P* < 0.05) than in T₁ and T₂, which did not differ. Intake did not differ among groups in the first fortnight (12.18, 11.26 and 14.36 kg d⁻¹); from the second fortnight onward, it was higher in T₃ (15.36 and 14.49 kg d⁻¹ in the second and third fortnights) than in T₂ (11.52 and 10.88 kg d⁻¹). Dry matter intake expressed as % of body weight and per unit of metabolic body weight (kg^{0.75}) was higher (*P* < 0.05) in T₃ than in T₂, with T₁ intermediate. TDN intake averaged 8.28, 7.53 and 9.86 kg d⁻¹; it was higher in T₃ than in T₂ (*P* < 0.05), with T₁ intermediate, and in the third fortnight T₃ (9.73 kg d⁻¹) exceeded both T₁ (7.98 kg d⁻¹) and T₂ (7.24 kg d⁻¹). Mean milk yield (13.81, 10.90 and 12.33 kg d⁻¹) and body weight (431.1, 426.3 and 433.4 kg) did not differ among groups.

Nutrient intake and digestibility

During the digestion trial, intakes of dry matter, organic matter, crude protein and ether extract were higher (*P* < 0.05) in T₃ than in T₂, with T₁ intermediate (Table 4). Intake of dry matter was 3.87 kg d⁻¹ (35.5%) higher in T₃ than in T₂. Intakes of NDF (4.61, 4.41 and

5.58 kg d⁻¹) and ADF (3.93, 3.72 and 4.21 kg d⁻¹) did not differ significantly.

Apparent digestibility of dry matter, organic matter, crude protein, ether extract, NDF and ADF did not differ significantly among the groups (Table 4). Numerically, digestibility of dry matter, organic matter, crude protein and NDF was highest in T₃ (62.06, 64.63, 68.51 and 63.20%) and lowest in T₂ (55.24, 57.97, 64.57 and 55.50%) for the first three, whereas ether extract digestibility was highest in T₁ (73.54%).

Enteric methane emission and energy loss

Gross, digestible and metabolizable energy intakes were higher (*P* < 0.05) in T₃ than in T₂, with T₁ intermediate (Table 5). Relative to T₂, GEI, DEI and MEI in T₃ were higher by 14.55 Mcal d⁻¹ (28.9%), 11.33 Mcal d⁻¹ (33.6%) and 8.65 Mcal d⁻¹ (31.8%), respectively.

Daily methane emission (72.80, 114.47 and 101.69 g d⁻¹ in T₁, T₂ and T₃) and methane energy (5.63, 7.69 and 5.67 MJ d⁻¹) did not differ significantly among groups (Table 5). Methane per unit of intake differed (*P* < 0.05) between T₂ and T₃ for dry matter (13.78 vs 7.29 g kg⁻¹), digestible dry matter (23.18 vs 11.28 g kg⁻¹), digestible organic matter (26.95 vs 12.25 g kg⁻¹), crude protein (81.81 vs 40.46 g kg⁻¹) and NDF (33.34 vs 19.21 g kg⁻¹), with T₁ intermediate and not different from either group. Compared with T₂, methane per kg DMI was 47.1% lower in T₃. Methane per unit of OMI, DCPI, DNDFI, ADFI and DADFI did not differ significantly.

Methane energy loss as a percentage of GEI was higher (*P* < 0.05) in T₂ (10.24%) than in T₁ (9.39%) and T₃ (9.03%), which did not differ. The same pattern was found for loss as a percentage of DEI (25.86% in T₂ vs 13.79% in T₁ and 13.03% in T₃) and of MEI (31.85% vs 17.17% and 16.34%).

Blood metabolites

Plasma glucose averaged 53.91, 49.94 and 54.70 mg dl⁻¹ in T₁, T₂ and T₃ (Table 6;). It was lower (*P* < 0.05) in T₂ than in T₁ and T₃ (7.4% and 8.7% lower, respectively), a difference that was evident at the end of the trial (47.34 vs 57.46 and 58.40 mg dl⁻¹). BUN averaged 14.97, 12.53 and 10.25 mg dl⁻¹; it was 31.5% lower (*P* < 0.05) in T₃ than in T₁, with T₂ intermediate. Concentration of NEFA (159.78, 161.03 and 158.05 μmol l⁻¹) did not differ among groups.

4. Discussion

The feeding strategy produced clear differences in the supply of digestible nutrients, with T₃ receiving about 33% more TDN than T₂. Dry matter intake rose with energy supply, and the difference between T₃ and T₂ was significant from the second fortnight of the trial. Intake in all groups remained below the dry matter requirement (73–84%), probably because the high moisture content of berseem (about 18% dry matter) limited the amount of feed that could be consumed. Milk yield and body weight did not differ significantly among groups, although mean milk yield in T₂ was numerically 21% lower than in T₁; with six cows per group and a high between-animal variation (standard error of 2.7–3.1 kg d⁻¹), the trial had limited power to detect differences of this size.

Apparent digestibility of nutrients was not significantly affected by the plane of nutrition. Digestibility of dry matter, organic matter, crude protein and NDF was numerically higher in T₃, by 3–8 percentage units over the other groups, but the variation among animals was large (for example, the standard error of NDF digestibility was 4.25 in

T_1). Digestibility commonly falls as intake rises because passage from the rumen is faster (Blaxter *et al.*, 1965; Waghorn *et al.*, 2011) [3, 19]; no such depression was seen in T_3 , suggesting that the level of intake remained compatible with efficient fermentation.

Daily methane emission was not significantly affected by treatment. Methane output is normally positively related to dry matter intake (Moe *et al.*, 1979; Johnson *et al.*, 1995) [12, 10], yet T_2 , which had the lowest intake, emitted numerically the most methane (114.5 g d⁻¹) and T_1 the least (72.8 g d⁻¹). The coefficient of variation, calculated from the standard errors with six animals per group, was about 9% in T_1 compared with 45% in T_2 and 38% in T_3 , so the ranking of the groups for daily emission is probably explained by between-animal variation, which is inherent in the SF₆ technique, rather than by the diets. The daily values observed here were lower than the 190–223 g d⁻¹ reported for crossbred cows in field studies on ration balancing (Mohini *et al.*, 2010; Garg *et al.*, 2013) [13, 5].

Expressed per unit of intake, however, methane was clearly higher on the below-requirement ration. Methane per kg of dry matter intake was 47% higher in T_2 than in T_3 (13.78 vs 7.29 g), and the same pattern held for digestible dry matter, digestible organic matter, crude protein and NDF intake. Methane yield is known to be higher when feed intake is low, because slower passage allows more extensive ruminal fermentation per unit of feed (Blaxter *et al.*, 1965; Waghorn *et al.*, 2011) [3, 19]; the numerically lowest digestibility coefficients in T_2 , although not significant, point in the same direction. Similarly, methane energy loss as a share of gross energy was higher in T_2 (10.24%) than in T_1 and T_3 (9.39 and 9.03%). The direction of this response agrees with reports that underfed cows lost more gross energy as methane (9.15%) than cows fed according to requirement (7.09%) (Mohini *et al.*, 2010) [13], that balancing of the ration lowered the share of gross energy lost as methane (8.81 to 8.26%) and daily emission by 10.1% (Garg *et al.*, 2013) [5], and that balanced feeding reduced methane emission by 10–15% (Kannan *et al.*, 2009) [11]. In the present study the difference between the below-requirement and the other groups was about 0.9–1.2 percentage units of gross energy, which is smaller than that reported by Mohini *et al.* (2010) [13]. All

the values lay within the 2–12% range for energy lost as methane (Johnson *et al.*, 1995) [10]. When methane energy was related to DEI and MEI, the loss in T_2 was about twice that in the other groups; given the wide variation among animals in T_2 , the magnitude of this difference should be interpreted with caution, although its direction is consistent with that obtained with gross energy.

Feeding TDN 15% above the requirement lowered methane per kg of dry matter intake by 1.34 g (15.5%) and per kg of digestible dry matter intake by 2.28 g (16.8%) relative to the control, but these differences were not significant. Feeding above requirement also increased intake and total daily methane numerically, and it raises feed cost and the excretion of surplus nutrients. On environmental grounds alone, therefore, the practical message from this trial is to avoid feeding below requirement rather than to feed above it, in line with the view that improving feed conversion efficiency, rather than simply increasing intake, is the most effective route to lower methane intensity (Beauchemin *et al.*, 2008; Waghorn *et al.*, 2011; Hristov *et al.*, 2013) [2, 19, 7].

The blood metabolites reflected the plane of nutrition. Plasma glucose was lowest in T_2 and the difference became evident only at the end of the trial, which agrees with a gradual effect of a lower energy supply. Lower BUN in T_3 than in T_1 is consistent with better capture of ruminal ammonia in microbial protein when more fermentable energy is available, and Garg *et al.* (2013) [5] gave a similar interpretation of the relation between energy supply and BUN in balanced rations. The similar and low NEFA concentrations, together with stable body weight, indicate that the energy shortfall in T_2 was not severe enough to cause marked mobilization of body fat during the 45-day trial.

The findings should be viewed considering several limitations. Only six cows were studied per group, the trial lasted 45 days with methane measured on five days, methane per kg of milk was not evaluated, and rumen fermentation and microbial variables were not measured, so the mechanisms proposed above remain to be tested. The results apply to cows in mid lactation fed a berseem–wheat straw–concentrate ration, and larger studies, including different forages and stages of lactation, are needed to confirm them.

Table 1. Chemical composition of feeds offered during the digestion trial (% of dry matter unless stated)

Parameter	Berseem	Concentrate mixture	Wheat straw
Dry matter (% as fed)	17.74	90.56	91.19
Organic matter	88.67	90.48	89.88
Crude protein	16.21	20.33	3.80
Ether extract	2.25	4.77	1.70
Neutral detergent fibre	43.12	28.68	62.83
Acid detergent fibre	46.09	12.25	48.76
Acid detergent lignin	3.98	2.41	6.20
Total digestible nutrients	64.43	75.58	50.19
Digestible energy (Mcal kg ⁻¹)	2.84	3.33	2.21
Metabolizable energy (Mcal kg ⁻¹)	2.42	2.92	1.78

Table 2. Plane of nutrition of the experimental groups during the digestion trial

Parameter	T ₁ (control)	T ₂ (below)	T ₃ (above)
Body weight (kg)	431.28	424.00	434.03
Milk yield (kg d ⁻¹)	13.01	12.90	17.71
Milk fat (%)	4.02	3.67	4.11
Dry matter requirement (kg d ⁻¹)	15.30	14.96	17.59
Dry matter intake (% of requirement)	78.38	72.91	83.53
Crude protein requirement (kg d ⁻¹)	2.13	1.97	2.51
Crude protein intake (% of requirement)	97.32	92.57	106.77
TDN requirement (kg d ⁻¹)	8.15	8.64	8.97
TDN intake (kg d ⁻¹)	8.39	7.49	10.33
TDN intake (% of requirement)	102.94	86.66	115.14

Table 3. Effect of feeding total digestible nutrients (TDN) below and above requirement on feed intake, milk yield and body weight over the 45-day feeding trial

Parameter	T ₁ (control)	T ₂ (below)	T ₃ (above)
Dry matter intake (DMI)			
DMI (kg d ⁻¹)	12.21 ^a ± 0.79	11.22 ^a ± 0.80	14.74 ^b ± 0.83
DMI (% of body weight)	2.91 ^{ab} ± 0.29	2.65 ^a ± 0.11	3.41 ^b ± 0.21
DMI (g kg ^{-0.75})	131.54 ^{ab} ± 11.83	120.04 ^a ± 4.92	155.48 ^b ± 9.24
TDN intake (TDNI)			
TDNI (kg d ⁻¹)	8.28 ^{ab} ± 0.52	7.53 ^a ± 0.55	9.86 ^b ± 0.57
TDNI (% of body weight)	1.97 ^{ab} ± 0.20	1.78 ^a ± 0.08	2.28 ^b ± 0.14
TDNI (g kg ^{-0.75})	89.29 ^{ab} ± 7.95	80.56 ^a ± 3.43	104.04 ^b ± 6.33
Milk yield and body weight			
Milk yield (kg d ⁻¹)	13.81 ± 3.06	10.90 ± 2.65	12.33 ± 3.21
Body weight (kg)	431.09 ± 28.25	426.34 ± 33.69	433.39 ± 10.76

Values are mean ± standard error ($n = 6$). a,b Means bearing different superscripts in a row differ significantly ($P < 0.05$). T₁, fed to ICAR requirement; T₂, 15% less TDN than T₁; T₃, 15% more TDN than T₁; kg^{0.75}, metabolic body weight.

Table 4. Effect of feeding total digestible nutrients below and above requirement on nutrient intake and apparent digestibility during the digestion trial

Parameter	T ₁ (control)	T ₂ (below)	T ₃ (above)
Nutrient intake (kg d⁻¹)			
Dry matter	11.99 ^{ab} ± 0.59	10.90 ^a ± 1.11	14.77 ^b ± 1.48
Organic matter	10.72 ^{ab} ± 0.52	9.73 ^a ± 0.97	13.18 ^b ± 1.31
Crude protein	2.12 ^{ab} ± 0.12	1.87 ^a ± 0.21	2.68 ^b ± 0.28
Ether extract	0.42 ^{ab} ± 0.03	0.34 ^a ± 0.04	0.52 ^b ± 0.06
Neutral detergent fibre	4.61 ± 0.29	4.41 ± 0.42	5.58 ± 0.52
Acid detergent fibre	3.93 ± 0.14	3.72 ± 0.23	4.21 ± 0.31
Apparent digestibility (%)			
Dry matter	58.32 ± 2.40	55.24 ± 4.05	62.06 ± 2.76
Organic matter	60.98 ± 2.42	57.97 ± 4.07	64.63 ± 2.52
Crude protein	65.39 ± 0.84	64.57 ± 2.19	68.51 ± 1.10
Ether extract	73.54 ± 2.63	68.15 ± 3.20	71.48 ± 5.74
Neutral detergent fibre	55.66 ± 4.25	55.50 ± 0.84	63.20 ± 1.67
Acid detergent fibre	34.87 ± 2.17	40.73 ± 0.97	40.71 ± 4.67

Values are mean ± standard error ($n = 6$). a,b Means bearing different superscripts in a row differ significantly ($P < 0.05$). T₁, fed to ICAR requirement; T₂, 15% less TDN than T₁; T₃, 15% more TDN than T₁.

Table 5. Effect of feeding total digestible nutrients below and above requirement on energy intake, enteric methane emission and methane energy loss

Parameter	T ₁ (control)	T ₂ (below)	T ₃ (above)
Energy intake (Mcal d⁻¹)			
Gross energy	59.88 ^{ab} ± 3.02	50.27 ^a ± 4.98	64.82 ^b ± 4.80
Digestible energy	41.46 ^{ab} ± 2.43	33.69 ^a ± 3.62	45.02 ^b ± 3.66
Metabolizable energy	33.07 ^{ab} ± 1.83	27.20 ^a ± 2.83	35.85 ^b ± 2.78
Methane emission			
CH ₄ (g d ⁻¹)	72.80 ± 2.64	114.47 ± 20.80	101.69 ± 15.97
CH ₄ (MJ d ⁻¹)	5.63 ± 0.88	7.69 ± 1.09	5.67 ± 0.89
Methane per unit of intake (g kg⁻¹)			
Dry matter	8.63 ^{ab} ± 1.57	13.78 ^b ± 2.52	7.29 ^a ± 1.21
Digestible dry matter	13.56 ^{ab} ± 2.60	23.18 ^b ± 4.74	11.28 ^a ± 1.76
Organic matter	14.82 ± 8.24	13.25 ± 3.55	8.15 ± 1.34
Digestible organic matter	16.64 ^{ab} ± 3.66	26.95 ^b ± 6.24	12.25 ^a ± 1.92
Crude protein	50.05 ^{ab} ± 9.29	81.81 ^b ± 14.55	40.46 ^a ± 6.95
Digestible crude protein	64.54 ± 13.36	99.30 ± 18.81	60.29 ± 14.32
Neutral detergent fibre	21.90 ^{ab} ± 3.52	33.34 ^b ± 6.00	19.21 ^a ± 3.19
Digestible neutral detergent fibre	60.44 ± 12.92	113.10 ± 36.40	49.76 ± 9.46
Acid detergent fibre	25.62 ± 4.12	39.06 ± 7.29	25.09 ± 4.30
Digestible acid detergent fibre	48.87 ± 9.27	75.12 ± 19.19	49.97 ± 11.25
Methane energy loss (% of energy intake)			
Gross energy	9.39 ^a ± 1.51	10.24 ^b ± 1.48	9.03 ^a ± 1.35
Digestible energy	13.79 ^a ± 2.30	25.86 ^b ± 4.71	13.03 ^a ± 1.89
Metabolizable energy	17.17 ^a ± 2.83	31.85 ^b ± 5.79	16.34 ^a ± 2.40

Values are mean ± standard error ($n = 6$). a,b Means bearing different superscripts in a row differ significantly ($P < 0.05$). T₁, fed to ICAR requirement; T₂, 15% less TDN than T₁; T₃, 15% more TDN than T₁.

(continued on next page)

Table 6. Effect of feeding total digestible nutrients below and above requirement on plasma glucose, blood urea nitrogen (BUN) and non-esterified fatty acids (NEFA)

Day of trial	T ₁ (control)	T ₂ (below)	T ₃ (above)
<i>Plasma glucose (mg dL⁻¹)</i>			
0	51.55 ± 1.92	49.68 ± 2.66	50.37 ± 1.38
23	52.73 ± 4.09	52.79 ± 1.58	55.34 ± 1.56
45	57.46 ^b ± 1.52	47.34 ^a ± 2.30	58.40 ^b ± 4.39
Mean	53.91 ^b ± 1.84	49.94 ^a ± 1.91	54.70 ^b ± 1.09
<i>BUN (mg dL⁻¹)</i>			
0	15.73 ± 1.92	11.56 ± 1.22	15.10 ± 1.85
23	18.00 ± 2.30	15.88 ± 3.14	7.92 ± 1.38
45	11.18 ± 2.48	10.16 ± 0.96	7.72 ± 0.57
Mean	14.97 ^b ± 1.40	12.53 ^{ab} ± 1.24	10.25 ^a ± 1.11
<i>NEFA (μmol L⁻¹)</i>			
0	144.81 ± 13.18	158.61 ± 5.78	158.33 ± 5.94
23	171.37 ± 2.30	165.11 ± 5.17	170.68 ± 2.95
45	163.15 ± 4.18	159.37 ± 10.96	145.13 ± 7.71
Mean	159.78 ± 6.55	161.03 ± 7.30	158.05 ± 5.53

Values are mean ± standard error. a,b Means bearing different superscripts in a row differ significantly ($P < 0.05$). T₁, fed to ICAR requirement; T₂, 15% less TDN than T₁; T₃, 15% more TDN than T₁.

5. Conclusion

Feeding total digestible nutrients 15% below the ICAR requirement increased methane emitted per unit of feed intake and the proportion of dietary energy lost as methane in lactating Karan Fries cows, whereas daily methane emission, nutrient digestibility and milk yield did not differ significantly among groups. Feeding 15% above the requirement increased intake but did not significantly reduce methane intensity relative to feeding according to requirement. Under-feeding of energy on commercial farms is therefore likely to raise methane per unit of feed, and meeting the requirement is a practical, low-cost step towards lower enteric methane intensity in crossbred dairy cattle. Confirmation in larger trials is warranted.

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