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Original Article

Charolais crossbred cattle: The difference in energy sources and ages on nutrient digestibility and methane emission

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Abstract

This study aims at finding out the combined Tra fish oil (TrO) and energy feed sources in the dietary combinations on feed intake, nutrient degradability, and greenhouse gas emissions of Charolais crossbred cattle. The *in vitro* gas experiment (Ex1) was arranged in a complete randomized design with two factors and four replications. Factor 1 was carbohydrate source (Es): cassava chip (Ca) and Maize (Ma), and factor 2 was Tra fish oil (TrO): with and without Tra fish oil. The *in vivo* digestion (Ex2) was arranged in a group of Latin square design with three factors. Factors 1 and 2 were similar to those in Ex1, but factor 3 was of cattle age periods, such as 13-16, 17-20, and 21-24 months. Results of Ex1 showed that the organic matter degradability was significantly distinct ($P<0.05$) at Es and TrO. Still, the interaction between Es and TrO was not variable ($P>0.05$) at 72 h incubation. Results of Ex2 showed that the methane emission (MJ/Kg DDM) was not divergent ($P>0.05$) between the Ma (169 MJ) and Ca (171 MJ). TrO (160 MJ) had lower methane emissions than NoTrO (181 MJ) by about 11.6%. The 13-16 months had higher methane emissions than 17-20 and 21-24 months (219, 161, and 131 MJ, respectively). Therefore, the Ma had better digestibility than the Ca on the beef cattle diet. Charolais crossbred cattle at 21-24 months had better intake, digestibility, and the lowest methane emission than 17-20 and 13-16 months' age periods. In addition, supplementing the diet of the Tra fish oil could reduce methane in crossbred beef cattle.

Keywords: Climate change, Local feed, Lipid, Nutrition, Combinations, Ruminants

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Introduction

In the Mekong Delta of Vietnam, beef production increasingly relies on Charolais crossbred cattle developed through artificial insemination between Zebu cows and high-performance such as Charolais, Wagyu, Angus (Vu et al., 2021). These crossbreds exhibit superior growth potential compared with local cattle but require nutritionally appropriate diets to fully express their genetic capacity. Nutrient requirements and feed utilization efficiency also vary with age, with growth performance stabilizing after post-weaning stress and improving from approximately 13 months onward (Hue, 2010). Among available crossbreds, Charolais crosses are widely adopted by farmers due to their adaptability and productivity (Quyen et al., 2021a, b).

Dietary energy source is a major driver of rumen fermentation, nutrient digestibility, and enteric methane emissions. In tropical production systems, maize and cassava chips are commonly used carbohydrate sources in beef cattle diets (Dung, 2014; Kabsuk et al., 2024), however their comparative effects on methane emission in Charolais crossbred cattle remain unclear. In addition, shifting dietary energy from carbohydrates toward lipids has been proposed as an effective methane mitigation strategy by reducing ruminal fermentation intensity (Haque, 2018). Lipid supplementation, particularly from fish oil, has been shown to suppress methanogenesis in ruminants (Beauchemin et al., 2007).

In the Mekong Delta, Tra fish oil, a by-product of Tra (Pangasius) catfish processing, is abundant and rich in unsaturated fatty acids, providing a high-energy local feed resource. This oil contains 11.0–14.0% unsaturated fatty acids, which provides 27.0 MJ/kg DM of metabolizable energy, and supplementing 3% of dry matter intake with Tra fish oil reduces *in vitro* methane emissions, which warrants further investigation in beef cattle, suggested by Thu and Dong (2021). However, its interaction with dietary carbohydrate source and cattle age under *in vivo* conditions has not been adequately evaluated. This is particularly relevant given the contribution of ruminants to global greenhouse gas emissions (Hosen et al., 2025) and the need for practical mitigation strategies in tropical systems (Liu et al., 2019).

Therefore, this study aimed to evaluate the combined effects of dietary energy source (maize versus cassava chips), Tra catfish oil supplementation, and cattle age on nutrient digestibility and methane emissions in Charolais crossbred cattle using both *in vitro* and *in vivo* approaches.

Material and Methods

Location and time

In Chau Thanh commune of An Giang province, Vietnam, the Hanh Cuong cattle farm was the location of the experiment between June 2024 and November 2024.

At Can Tho University, laboratory analyses were done at the E205 Department of Animal Science laboratory.

Experimental design and feeds

Experiment 1: *In vitro* gas

The experimental design was completely randomized (2 x 2 factors), the factor 1 was two carbohydrate sources (Es): cassava chip (Ca) and maize (Ma) and the factor 2 was with and without TrO. The experiment's chemical composition and factor parameters are presented in Tables 1 and 2.

Table-1. Chemical structure of feeds (%DM) used in the first experiment.

Feeds	DM%*	OM%	CP%	NDF%	ADF%	Ash%
Maize	90.3	96.2	8.29	13.0	3.37	3.84
Cassava chip	87.2	97.4	2.76	17.8	1.69	2.58
Broken rice	87.2	99.2	8.60	8.47	3.42	0.85
Soybean meal	93.4	93.6	46.7	8.34	5.06	6.38
Elephant grass	89.1	93.8	8.79	58.4	37.0	6.16
Rice straw	90.5	90.4	6.28	55.0	34.3	9.62

*Dry state of the sample under laboratory conditions, DM - dry matter, OM - organic matter, CP - crude protein, NDF - neutral detergent fiber, ADF - acid detergent fiber.

Table-2. Ingredient composition of experiment 1.

Elements, %DM	Ma	Ca	MaTrO	CaTrO
Maize	15.0	-	15.0	-
Cassava chip	-	15.0	-	15.0
Tra fish oil	-	-	3.00	3.00
Broken rice	15.0	15.0	15.0	15.0
Soybean meal	5.00	5.00	5.00	5.00
Elephant grass	20.0	20.0	20.0	20.0
Rice straw	44.2	44.0	41.2	41.0
Urea	0.79	1.00	0.79	1.00
Total	100	100	100	100

Ma - Maize, Ca - cassava chips, TrO - Tra fish oil.

Measurements taken

Chemical analysis

Samples were analyzed for dry matter (DM) and organic matter (OM), nitrogen (N) content of feed, refuses, feces, and urine using standard methods as described in AOAC (1990). However, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured by Van Soest's method et al. (1991).

In vitro incubation, sampling, and measurements

Rumen fluid was collected from donor cattle fed a conventional forage–concentrate diet prior to the morning feeding. The fluid was obtained via rumen cannula, filtered through four layers of cheesecloth, and maintained under continuous CO_2 flushing to preserve anaerobic conditions. Rumen fluid from all donor animals was pooled to minimize individual variability and standardized across incubations. The pooled rumen fluid was mixed with buffer solution according to the method of Menke and Steingass (1988) and immediately used for *in vitro* incubation at 39 °C. Gas production was recorded at 6, 12, 24, 48, and 72 h of incubation. At 72 h, fermentation residues were used to determine organic matter digestibility, and gas samples were analyzed for methane (CH_4) and carbon dioxide (CO_2) concentrations.

Data analysis

The data were determined using the Excel software and then analyzed for variance applying the ANOVA of the General Linear Model (GLM) of Minitab Reference Manual Release 20 by Minitab (2021). The data were analyzed using the following model: $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$ Where: μ: is the overall mean, α_i: is the effect of carbohydrate sources (Ca and Ma), β_j: is the effect of TrO (with and witout), $(\alpha\beta)_{ij}$: is the interaction between carbohydrate sources and TrO, e_{ijk}: is the residual effect, Y_{ijk}: is the observations. The Tukey test of Minitab was used to compare two treatments (P<0.05).

Experimental 2: *In vivo*

A total of 12 male Charolais crossbred cattle (Figure 1) were arranged in a Group of Latin Square Designs 3x(4x4) with three factors (21 days/period). The first factor was the ES, which included Ma and Ca, the second factor was related to TrO (Figure 2), being with or without Tra fish oil (recommended by Thu and Dong, 2021), and the third factor was three age stages of cattle (13-13-16, 17-20 and 21-24 months). The nutrition of TrO (%DM) was analyzed as 98.6%, 99.2%, and 99.8% corresponding to DM, OM, and ether extract (EE). The chemical composition of the feed is showed in Table 3. Table 4 contains the formula for 2 levels of Tra fish oil and carbohydrates.

Table-3. Nutrient composition of feeds.

Feeds	DM%	In DM%			
		OM%	CP%	NDF%	ADF%
Maize	84.9	96.8	8.62	21.8	4.22
Cassava chip	84.4	96.1	3.24	13.7	4.21
Broken rice	84.5	99.5	7.73	10.4	2.93
Soybean meal	87.1	93.4	45.0	13.6	8.39
Elephant grass	16.3	91.0	8.09	61.8	40.0
Rice straw	83.3	89.0	5.78	68.5	44.7
Urea	99.6	-	286	-	-

DM - dry matter, OM - organic matter, CP - crude protein, NDF - neutral detergent fiber, ADF - acid detergent fiber.

Table-4. Constituents of diet in the experimental cattle.

Constituents (%DM)	Ma		Ca	
	TrO	No TrO	TrO	No TrO
Maize	15.0	15.0	-	-
Cassava chip	-	-	15.0	15.0
Tra fish oil	3.00	-	3.00	-
Broken rice	15.0	15.0	15.0	15.0
Soybean meal	5.00	5.00	5.00	5.00
Elephant grass	20.0	20.0	20.0	20.0
Rice straw	40.5	43.5	40.3	43.3
Urea	0.80	0.80	1.00	1.00
Premix	0.70	0.70	0.70	0.70
Total	100	100	100	100

Ma - Maize, Ca - cassava chips, TrO - Tra fish oil.

Figure-1. The Charolais crossbred cattle in this study.

Figure-2. Tra fish oil.

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Measurements taken

Feed and nutrient consumption

Prior to feeding, all the feed was determined to weigh and provided to the second experiment. Each complement was fed two times at 7:00 h and 13:00 h. In detail, first, TrO was the drink of experimental cattle. Combining Ma and Ca with soybean meal, broken rice, urea, and premix supplements were done before feeding. The elephant grass and *ad libitum* rice straw were supplied at 8:00 h and 14:00 h. Fresh water was always *ad libitum*. Each morning of next day, refused feeds were weighed. Feed and refusals were used to determine the daily feed intake and nutrient consumption.

Chemical dissection

The feeds, refuses, and feces were examined for nutrient composition (AOAC, 1990 and Van Soest et al., 1991).

Metabolisable energy (ME) intakes and methane output

The ME in cattle diets was determined according to the proposition of Bruinenberg et al. (2002).

ME (MJ/cattle/day) = 14.2 x DOM + 5.9 x DCP (if DOM/DCP < 7)

ME (MJ/cattle /day) = 15.1 x DOM (if DOM/DCP > 7)

In detail, the digestible organic matter (DOM) and the digestible crude protein (DCP).

Mills et al. (2003) recommended the idea of greenhouse gas emissions.

The methane was determined according to the technique of Mills et al. (2003).

Methane (MJ/ cattle/day) = 1.06 + 10.27 DFP + 0.87 DMI.

In detail, DFP was dietary forage proportion.

Apparent nutrient degradability

The nutrient digestibility was experienced in the first (adaptation), second (stabilization), and third week for feces collection per experimental period by the method of McDonald et al. (2010).

Daily weight gains (DWG)

At the start and the end of the experimental period, the Charolais crossbred cattle were weighed (two Sequential days) using the electrical scale (Tru-Test, Limited Auckland, New Zealand) before morning feeding.

Data analysis

The figures were examined according to a 3-factor arrangement in a Latin square design using the ANOVA Linear Model (GLM) of Minitab Reference Manual Release 20 (Minitab, 2021). The data were analyzed using the following model: $Y_{ijkl} = \mu + \alpha_i + \beta_j + \delta_k + (\alpha\beta)_{ij} + (\alpha\delta)_{ik} + (\beta\delta)_{jk} + (\alpha\beta\delta)_{ijk} + e_{ijkl}$ Where: μ: is the overall mean, α_i: is the effect of carbohydrate sources (Ca and Ma), β_j: is the effect of TrO (with and without), δ_k: is the effect of cattle age, $(\alpha\beta)_{ij}$: is the interaction between carbohydrate sources and TrO, $(\alpha\delta)_{ik}$: is the interaction between carbohydrate sources and cattle age, $(\beta\delta)_{jk}$: is the interaction between carbohydrate sources and cattle age, $(\alpha\beta\delta)_{ijk}$: is the interaction between carbohydrate sources, TrO and cattle age, e_{ijkl}: is the residual effect, Y_{ijkl}: is the observations. The differences between treatments were determined by Tukey's pairwise comparisons ($P<0.05$).

Results and Discussion

Experiment 1: *In vitro* gas

In vitro fermentation characteristics were influenced by dietary energy source and Tra catfish oil supplementation. Cassava-based substrates exhibited greater fermentability than maize-based substrates, as indicated by higher cumulative gas production throughout incubation. At 72 h, total gas production from cassava substrates was approximately 10–15% higher than that from maize substrates, reflecting more rapid and extensive carbohydrate degradation.

Methane production followed a similar pattern. Cassava-based substrates produced higher methane per unit of incubated substrate compared with maize. Tra catfish oil supplementation consistently reduced methane production across incubation times, with reductions of approximately 10–15% relative to non-supplemented treatments. This methane-suppressing effect was observed for both energy sources, as no significant interaction between energy source and Tra catfish oil supplementation was detected.

Organic matter digestibility was slightly reduced by Tra catfish oil supplementation. Differences in digestibility between maize- and cassava-based substrates were relatively small, suggesting that the primary effect of energy source was on fermentation rate rather than overall substrate degradation. Gas production kinetics were not markedly altered by the interaction between energy source and oil supplementation, indicating that the effect of Tra catfish oil was consistent across carbohydrate types (Table 5, 6 and Figure 3).

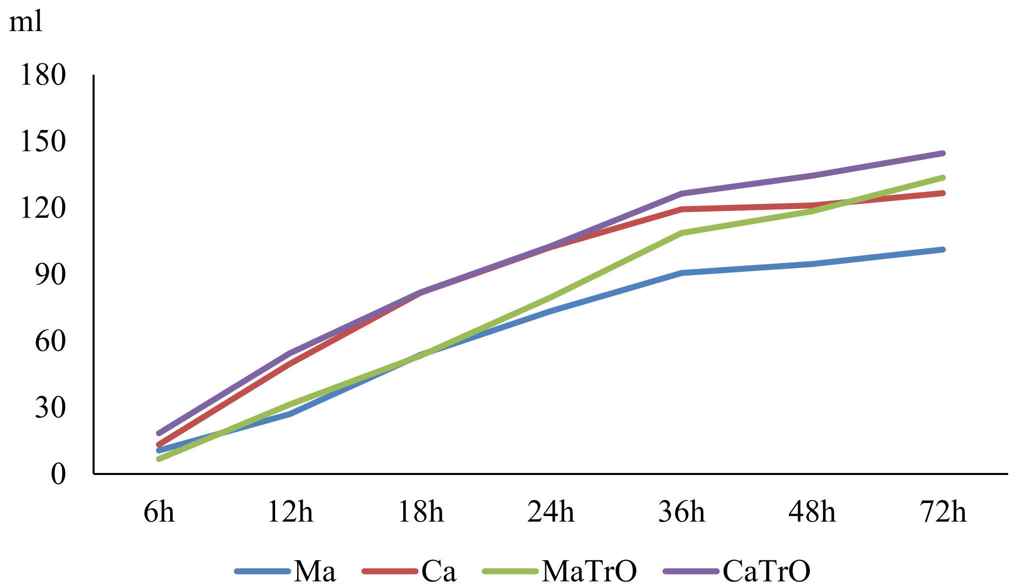

Figure-3. Total gas (ml) of Es and TrO from 6 to 72 h.

Table-5. *In vitro* OMD values (%) of treatments over incubation times.

Times, h	Es		TrO		Es*TrO				P		
	Ma	**Ca**	**TrO**	**NoTrO**	**MaTrO**	**Ma**	**CaTrO**	**Ca**	**Es**	**TrO**	**Es*TrO**
24	55,2	48,3	50,1	53,4	53,5	56,9	46,7	50,0	0,029	0,252	0,974
48	65,8	58,5	60,7	63,6	62,9	68,7	58,6	58,4	0,038	0,379	0,358
72	68,8	64,2	62,9	70,1	64,2	73,3	61,7	66,8	0,006	0,001	0,155

Ma - Maize, Ca - cassava chips, Es - energy sources, TrO - Tra fish oil, Es* TrO interaction.

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Table-6. *In vitro* organic matter digestibility (OMD) and greenhouse gas production at 72h in Exp 1.

Parameters	Es		TrO		Es*TrO				P		
	Ma	Ca	TrO	NoTrO	MaTrO	Ma	CaTrO	Ca	Es	TrO	Es*TrO
Total gas, ml	117	136	139	114	134	101	145	127	0,003	0,001	0,213
CH_4, %	14,8	16,0	17,0	13,8	16,0	13,6	17,9	14,1	0,135	0,001	0,377
CO_2, %	44,1	45,3	42,0	47,4	40,6	47,5	43,3	47,3	0,387	0,001	0,290
OMD, %	68,8	64,2	62,9	70,1	64,2	73,3	61,7	66,8	0,006	0,001	0,155
Total gas, ml/gOM	202	233	239	197	229	175	248	218	0,003	0,001	0,209
CH_4, ml/gOM	30,2	37,6	40,5	27,3	36,6	23,8	44,4	30,8	0,003	0,001	0,868
CO_2, ml/gOM	88,0	105	100	93,1	93,0	83,0	107	130	0,002	0,194	0,573
Total gas, ml/gDOM	316	342	379	280	371	261	386	298	0,088	0,001	0,464
CH_4, ml/gDOM	47,4	55,6	64,2	38,8	59,3	35,6	69,2	42,0	0,037	0,001	0,651
CO_2, ml/gDOM	137	154	159	132	151	124	167	141	0,051	0,002	0,985

Ma - Maize, Ca - cassava chips, Es - energy sources, TrO - Tra fish oil, Es* TrO interaction, OMD - organic matter digestibility, DOM - digestive organic matter.

These findings are different from the reports of Thu and Dong (2021), who reported that at 72 h, the *in vitro* DMD was substantially different (P<0.05) between the treatments, with the higher values for the TrO augmented treatments. This study found that low digestibility was due to the inhibition of protozoa when supplemented with fish oil. *In vitro* conditions, there are limitations on the continued growth of rumen microorganisms compared to that in the actual digestive system of the cow. According to Vesga et al. (2024), lipid inclusion in cattle diets can have variable effects due to capacity, adaptation time, the type of bacterial population, and lipid concentration.

Experiment 2: *In vivo*

Nutrient intakes

Total nutrient intake (Table 7) was primarily influenced by cattle age, whereas dietary energy source and Tra fish oil supplementation had no significant effect on overall intake. Dry matter intake did not differ between maize- and cassava-based diets (approximately 6.0 kg DM/animal/day) nor between diets with and without Tra fish oil. Similarly, organic matter, crude protein, fiber fractions, and metabolizable energy intakes were comparable between energy sources and oil supplementation treatments (P>0.05). The DM intake of cattle in the present study was analogous to the conclusion of Rotta et al. (2023), who described that the crossbred cattle (*Bos Taurus x Bos Indicus*) with a body weight of about 270 kg need 6.40 kg DM/day for daily weight gain of 0.80 kg/day.

In contrast, cattle age significantly affected feed and nutrient consumption (P<0.05). Dry matter intake increased progressively with age, from 4.59 kg DM/animal/day in cattle aged 13–16 months to 6.16 kg DM/animal/day in those aged 17–20 months, reaching 7.32 kg DM/animal/day in cattle aged 21–24 months. A similar age-related pattern was observed for organic matter, crude protein, neutral detergent fiber, acid detergent fiber, and metabolizable energy intake. Metabolizable energy intake increased markedly from 18.6 MJ/100 kgBW in the NoTrO group to 19.8 MJ/100 kgBW in the TrO (Figure 4). The TrO supplement was higher ME than without TrO in the experiment, which was suitable for the study of Thu and Dong (2021), who concluded that the ME of TrO was 27.0 MJ/kg DM.

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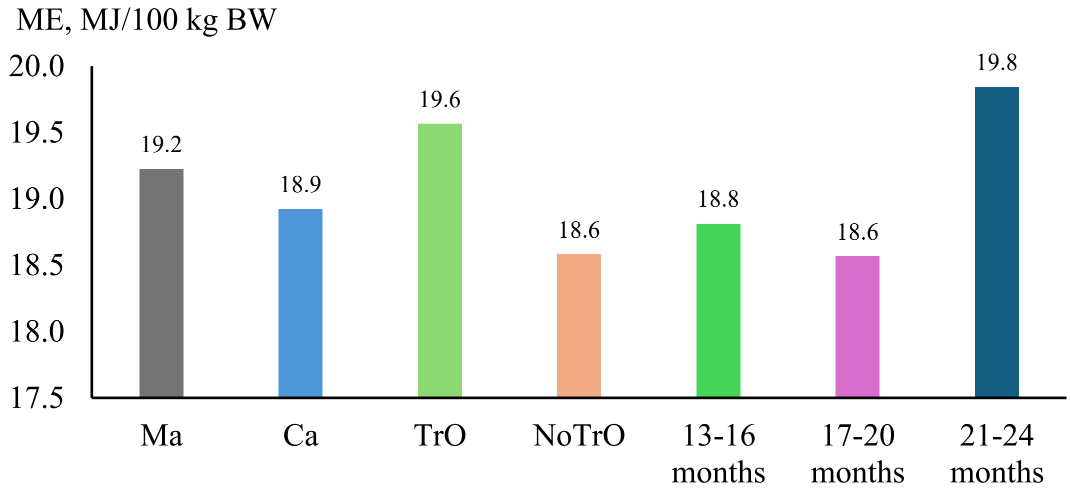

Figure-4. The ME Intake/100 kg BW from three factors of the study.

When intake was expressed relative to body weight (per 100 kg BW), no significant differences were detected among energy source, Tra fish oil supplementation, or age groups for dry matter, organic matter, crude protein, fiber fractions, or metabolizable energy (P>0.05). This indicates that differences in absolute nutrient intake among age groups were largely attributable to increased body size rather than changes in intake efficiency. Similarly, Thu and Dong (2021) showed that DM intake was not different between with and without catfish oil in an *in vivo* study on zebu cattle.

No significant two-way or three-way interactions among energy source, Tra fish oil supplementation, and cattle age were observed for nutrient intake variables, indicating consistent intake responses across dietary treatments and age classes.

Degradability and digestive nutrients

Apparent digestibility of nutrients was influenced primarily by cattle age and Tra catfish oil supplementation, whereas dietary energy source had limited effects (Table 8). Digestibility of dry matter (Figure 5) and organic matter did not differ significantly between maize- and cassava-based diets (P>0.05). The DMD in this study agreed with the result from Thu and Dong (2021), who found that DMD had higher values for the catfish oil supplemented than without catfish oil (72.4 and 64,2%, respectively) at 72 h from the *in vitro* study.

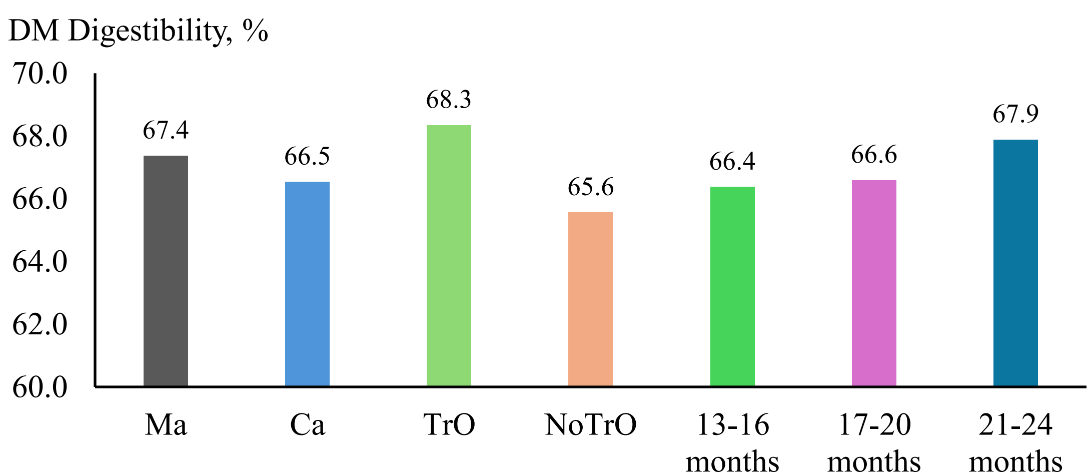

Figure-5. The DM digestibility from three factors of the study.

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In contrast, Tra catfish oil supplementation resulted in modest but consistent changes in digestibility parameters, indicating an effect on ruminal fermentation and nutrient utilization.
Cattle age significantly affected apparent fiber digestibility ($P<0.05$). Digestibility coefficients generally increased with advancing age, with older cattle exhibiting greater digestibility of dry matter, organic matter, and fiber fractions compared with younger animals. This pattern suggests improved rumen function and digestive capacity as animals matured.
No significant interactions among energy source, Tra catfish oil supplementation, and cattle age were observed for most digestibility variables, indicating that the effects of dietary treatments were consistent across age groups.

Methane emissions

Methane emissions (Table 9) were primarily affected by cattle age and Tra catfish oil supplementation, whereas dietary energy source had little influence. Tra catfish oil supplementation consistently reduced methane emissions, while absolute methane output did not differ between maize- and cassava-based diets ($P>0.05$). Methane emission intensity declined with advancing age ($P<0.05$), with younger cattle producing more methane per unit of digestible organic matter intake than older animals, indicating improved feed utilization efficiency as cattle matured (Figure 6). No significant interactions among dietary treatments and age were observed. Another study on the crossbred cattle, Thu and Dong (2021) suggested that supplementing the catfish oil at 3% in a few studies reduced CH_4 production compared to no supplement.

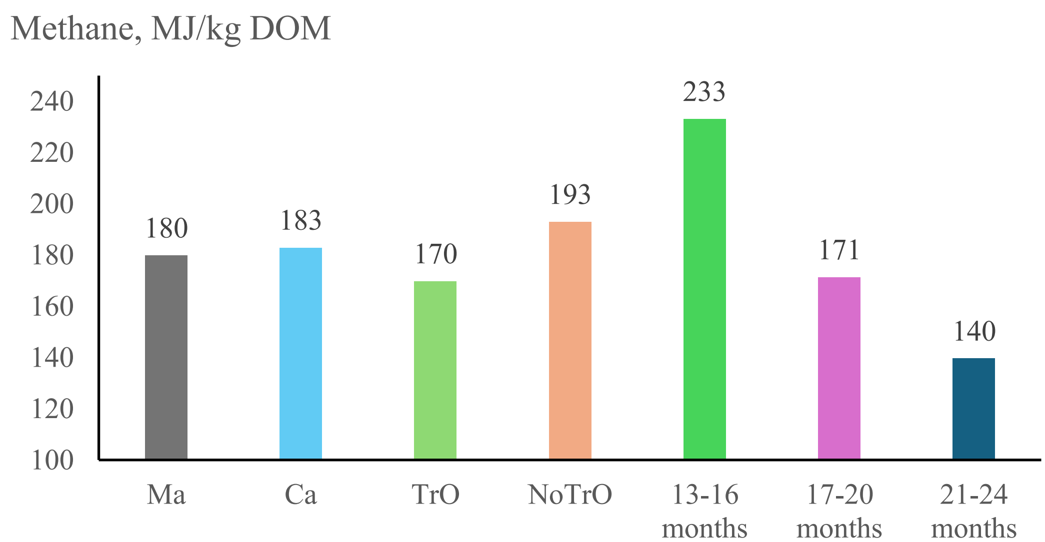

Figure-6. Methane emission from three factors of the study.

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Table-7. Total nutrient consumption of cattle crossbred by different treatments.

Parameters	Es		TrO		Mo			P						
	Ma	**Ca**	**TrO**	**NoTrO**	**13-16**	**17-20**	**21-24**	**Es**	**TrO**	**Mo**	**Es*TrO**	**Es*Mo**	**TrO*Mo**	**ES*TrO*Mo**
Feed consumption, kgDM/ animal/day														
Maize	0.905	0.000	0.452	0.453	0.343^{b}	0.459^{a}	0.556^{a}	0.001	0.987	0.001	0.987	0.001	0.937	0.937
Cassava chips	0.000	0.879	0.448	0.432	0.331^{c}	0.449^{b}	0.540^{a}	0.001	0.494	0.001	0.494	0.001	0.823	0.823
Tra fish oil	0.087	0.088	0.175	0.000	0.067^{c}	0.089^{b}	0.106^{a}	0.883	0.001	0.001	0.883	0.949	0.001	0.949
Broken rice	0.891	0.887	0.897	0.881	0.671^{c}	0.904^{b}	1.092^{a}	0.920	0.685	0.001	0.654	0.998	0.889	0.897
Soybean meal	0.305	0.304	0.307	0.301	0.230^{c}	0.309^{b}	0.374^{a}	0.919	0.689	0.001	0.657	0.997	0.887	0.897
Urea	0.046	0.059	0.054	0.051	0.040^{c}	0.054^{b}	0.064^{a}	0.001	0.262	0.001	0.865	0.592	0.924	0.883
Premix	0.043	0.043	0.043	0.042	0.032^{c}	0.043^{b}	0.052^{a}	0.917	0.688	0.001	0.064	0.996	0.897	0.906
Elephant grass	1.17	1.17	1.17	1.16	0.91^{c}	1.16^{b}	1.42^{a}	0.985	0.817	0.001	0.771	0.988	0.803	0.933
Rice straw	2.58	2.60	2.50	2.68	1.97^{c}	2.69^{b}	3.12^{a}	0.922	0.121	0.001	0.224	0.883	0.765	0.953
Nutrient consumption, kg/animal/day														
DM	6.03	6.02	6.04	6.00	4.59^{c}	6.16^{b}	7.32^{a}	0.977	0.862	0.001	0.385	0.970	0.852	0.980
OM	5.49	5.47	5.51	5.45	4.18^{c}	5.60^{b}	6.67^{a}	0.904	0.801	0.001	0.389	0.967	0.859	0.980
CP	0.658	0.644	0.652	0.650	0.494^{c}	0.664^{b}	0.794^{a}	0.605	0.938	0.001	0.555	0.974	0.842	0.946
NDF	2.85	2.78	2.76	2.87	2.15^{c}	2.89^{b}	3.40^{a}	0.547	0.309	0.001	0.312	0.910	0.747	0.993
ADF	1.72	1.72	1.68	1.76	1.32^{c}	1.77^{b}	2.08^{a}	0.953	0.272	0.001	0.299	0.945	0.750	0.990
ME, MJ/animal/day	57.7	56.7	58.4	56.1	43.2^{c}	57.9^{b}	70.6^{a}	0.644	0.277	0.001	0.238	0.861	0.302	0.742
Nutrient consumption, kg/100 kg BW														
DM	2.01	2.01	2.02	2.00	2.00	1.97	2.06	0.951	0.727	0.388	0.317	0.882	0.642	0.899
OM	1.83	1.83	1.84	1.82	1.82	1.79	1.87	0.945	0.642	0.374	0.320	0.881	0.639	0.897
CP	0.219	0.215	0.218	0.216	0.215	0.212	0.223	0.510	0.802	0.287	0.550	0.933	0.603	0.830
NDF	0.950	0.929	0.922	0.958	0.940	0.923	0.957	0.488	0.230	0.639	0.265	0.856	0.715	0.951
ADF	0.574	0.576	0.563	0.587	0.575	0.565	0.585	0.902	0.201	0.673	0.256	0.859	0.740	0.954
ME, MJ	19.2	18.9	19.6	18.6	18.8	18.6	19.8	0.580	0.078	0.141	0.200	0.774	0.052	0.492

DM - dry matter, OM - organic matter, CP - crude protein, NDF - neutral detergent fiber, ADF - acid detergent fiber, Ma - Maize, Ca - cassava chips, Es - energy sources, TrO - Tra fish oil, Mo - months, Es* TrO, Es*Mo, TrO*Mo, ES*TrO*Mo interaction, $^{a, b,c}$ Means with different letters within the same rows are different at $P<0.05$.

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Table-8. Effect of carbohydrate sources and Tra fish oil on nutrient digestibility and digestive nutrient.

Parameters	Es		TrO		Mo			P						
	Ma	Ca	TrO	NoTrO	13-16	17-20	21-24	Es	TrO	Mo	Es*TrO	Es*Mo	TrO*Mo	ES*TrO*Mo
Apparent degradability, %														
DM	67.4	66.5	68.3	65.6	66.4	66.6	67.9	0.528	0.040	0.596	0.714	0.867	0.037	0.497
OM	69.6	68.7	70.5	67.8	68.4	68.9	70.1	0.470	0.037	0.536	0.691	0.867	0.041	0.541
CP	76.5	75.8	76.6	75.6	76.0	78.3	74.1	0.662	0.527	0.110	0.776	0.860	0.188	0.727
NDF	68.5	67.0	68.7	66.7	65.6	67.7	69.8	0.289	0.180	0.066	0.769	0.779	0.190	0.669
ADF	62.5	61.8	62.9	61.1	59.9^{b}	61.6^{ab}	64.9^{a}	0.642	0.269	0.021	0.483	0.732	0.021	0.172
Digestible nutrients, kg/100 kg BW														
DM	1.35	1.34	1.38	1.31	1.33	1.31	1.40	0.693	0.089	0.157	0.207	0.788	0.042	0.453
OM	1.27	1.25	1.30	1.23	1.25	1.23	1.31	0.580	0.078	0.141	0.200	0.774	0.052	0.492
CP	0.167	0.163	0.166	0.163	0.163	0.166	0.166	0.415	0.538	0.912	0.460	0.959	0.143	0.672
NDF	0.651	0.621	0.633	0.639	0.616	0.924	0.668	0.206	0.792	0.156	0.399	0.790	0.238	0.721
ADF	0.359	0.355	0.354	0.360	0.344	0.347	0.380	0.778	0.643	0.054	0.130	0.675	0.045	0.397

DM - dry matter, OM - organic matter, CP - crude protein, NDF - neutral detergent fiber, ADF - acid detergent fiber, Ma - Maize, Ca - cassava chips, Es - energy sources, TrO - Tra fish oil, Mo - months, Es* TrO, Es*Mo, TrO*Mo, ES*TrO*Mo interaction, BW - body weight, [a, b,c] Means with different letters within the same rows are different at P<0.05.

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Table-9. Methane emissions of cattle in this study.

Parameters	Es		TrO		Mo			P						
	Ma	Ca	TrO	NoTrO	13-16	17-20	21-24	Es	TrO	Mo	Es*TrO	Es*Mo	TrO*Mo	ES*TrO*Mo
CH_4, MJ/ cattle/day	646	649	630	665	649	647	645	0.753	0.001	0.919	0.331	0.915	0.940	0.920
CH_4, MJ/100 kgBW/day	226	227	220	233	287[a]	210[b]	182[c]	0.958	0.210	0.001	0.748	0.980	0.922	0.990
CH_4, MJ/kg DMI	113	113	109	118	144[a]	107[b]	88.7[c]	0.992	0.094	0.001	0.674	0.999	0.532	0.937
CH_4, MJ/kg DDM	169	171	160	181	219[a]	161[b]	131[c]	0.792	0.010	0.001	0.602	0.979	0.059	0.732
CH_4, MJ/kg OMI	124	125	120	130	158[a]	118[b]	97.5[c]	0.943	0.082	0.001	0.680	0.999	0.525	0.938
CH_4, MJ/kg DOM	180	183	170	193	233[a]	171[b]	140[c]	0.718	0.009	0.001	0.605	0.982	0.068	0.760

Ma - Maize, Ca - cassava chips, Es - energy sources, TrO - Tra fish oil, Mo - months, Es* TrO, Es*Mo, TrO*Mo, ES*TrO*Mo interaction, DMI - dry matter intake, OMI - organic matter intake, DDM - digestive dry matter, DOM - digestive organic matter, [a, b,c] Means with different letters within the same rows are different at P<0.05.

Cattle-fed diets supplemented with fish oil (170 MJ/kg DOM) had lower CH4 emissions than diets fed without fish oil (193 MJ/kg DOM), about 11.9%. This could be because catfish oils inhibit the increase of certain methanogenic bacteria in the rumen, which produce methane during digestion. Furthermore, Tra catfish oil included high polyunsaturated fatty acid reported by to Ho and Paul (2009). Zhang et al. (2008) suggested that polyunsaturated fatty acid supplementation or fish oil reduced the Fibrobacter succinogenes population compared with the unsupplemented diet. According to Cho et al. (2024), increasing of fat affected the qualities of rumen fluid, increasing the propionate ratio in the rumen and reducing methane concentration in the breathed out gas in growth performance in Hanwoo steers.

In a previous *in vivo* study, Nhan et al. (2007) described that cattle were affected by a sudden oil supply in the 0-30 days, and there was an inclination for lower feed intake with oil drench. The trend of DM consumption was changed to a contrary direction, in which oil drench has partially stimulated feed intake (31-90 days of study). Moreover, Vesga et al. (2024) concluded that the first seven days of lipid diet inclusion are considered the most critical for ruminal adaptation, involving reductions in fibrolytic bacteria and changes in fermentation criteria. The rumen presentation signs of recovery and adaptation after 14 days. The results of Nhan et al. (2007) and Vesga et al. (2024) help the study detect a limitation. That is the lack of a 01-growth experiment in 90 days for the three factors of the experiment.

In vitro results showed that cassava based substrates were more fermentable than maize and that Tra catfish oil supplementation consistently reduced methane production, although with a slight reduction in organic matter digestibility. *In vivo*, differences between energy sources were minimal for intake, digestibility, methane emissions, and growth performance, indicating that animal-level factors moderated the fermentation differences observed *in vitro*. Across both experiments, Tra catfish oil supplementation consistently reduced methane emissions, confirming its inhibitory effect on methanogenesis under both controlled and practical feeding conditions. Cattle age exerted a strong influence *in vivo*, with older cattle showing improved nutrient utilization, lower methane emission intensity, and greater weight gain effects not captured in vitro due to the absence of animal physiological adaptation.

Although Tra catfish oil supplementation consistently reduced methane emissions, the magnitude of reduction observed in this study was moderate and was expressed mainly as a decrease in methane emission intensity (per unit of digestible intake) rather than a substantial reduction in absolute methane output. This suggests that the mitigation effect operates primarily through improved feed utilization efficiency rather than large suppression of total methane production. From a practical perspective, the level of methane reduction observed is likely to provide a limited but consistent contribution at the animal level and, if applied alone, may have a modest impact at the farm scale. Therefore, Tra catfish oil supplementation should be considered as a complementary mitigation strategy rather than a stand-alone solution for reducing enteric methane emissions.

Conclusions

In vitro fermentation showed greater gas and methane production from cassava than maize substrates, whereas *in vivo* differences between energy sources were minimal, indicating that animal-level regulation moderated substrate fermentability under practical feeding conditions. Across both experiments, Tra catfish oil supplementation consistently reduced methane emissions with only minor effects on digestibility. *In vivo*, cattle age strongly influenced intake, methane emission intensity with older cattle exhibiting improved feed utilization and lower methane emissions per unit of digestible intake.
Using the results of the present study and ongoing further exploration on Charolais crossbred cattle growth, an extensive review of TrO can be conducted to increase farmers' use of local by-product sources.

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Contribution of Authors
Truong NB & Tuan TT: Conceived idea, designed & performed experiments, data collection and article write up and editing.
Truong NB: Analyzed and interpreted data.

All authors read and approved the final draft of the manuscript.

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