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Effects of Gamma Irradiation Pretreatment and Exogenous Fibrolytic Enzyme Supplementation on the Ruminal Fermentation and Nutritional Value of *Typha latifolia*

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Abstract: Efficient bioconversion of lignocellulosic biomass into ruminant feed requires advanced strategies to enhance fiber degradation and ruminal fermentation efficiency. This study evaluates the synergistic effects of gamma irradiation (150 kGy) and exogenous fibrolytic enzyme (EFE) supplementation (4 µL/g dry matter) from *Trichoderma longibrachiatum* on the structural composition and ruminal fermentation of *Typha latifolia*. Gamma irradiation significantly reduced neutral detergent fiber (NDF) while increasing non-fiber carbohydrates (NFCs), reducing sugars (RS) and antioxidant activity. These modifications enhanced ruminal bacterial proliferation, suppressed ruminal protozoal populations, and improved ruminal fermentation efficiency by increasing gas production, dry matter degradability, and NDF degradability. Additionally, irradiation decreased ruminal NH_3-N concentrations and branched-chain volatile fatty acids (VFAs) without affecting total VFA production and ruminal pH. While EFE alone accelerated only ruminal fermentation, its combination with irradiation further reduced NDF content, enriched NFC and RS, and enhanced fermentation efficiency. This dual treatment increased total VFA production, shifted fermentation pathways toward propionate synthesis, and reduced acetate and branched-chain VFA levels. It also stimulated ruminal bacterial populations without altering ruminal pH. These findings highlight gamma irradiation as an effective pretreatment to enhance EFE hydrolysis, offering a promising strategy to improve the nutritional value of low-quality forages to integrate into ruminant diets.

Keywords: gamma irradiation; fibrolytic enzymes; ruminal fermentation; lignocellulosic biomass; *Typha latifolia*; ruminant nutrition; microbial profile

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1. Introduction

The sustainability of global livestock production is increasingly challenged by feed scarcity, with escalating costs necessitating the exploration of alternative, sustainable, and cost-effective feed resources [1]. Wetland plants such as Typha species (commonly known as cattails) have gained attention due to their high biomass yield, rapid growth, and adaptability to diverse climatic conditions [2–5]. Typha has been identified as a potential forage option for ruminants, particularly in regions where conventional forages are scarce or expensive [2]. It can replace traditional roughages, such as sorghum straw, in cattle diets, improving economic efficiency without compromising animal health, performance, or metabolic stability [4]. Additionally, its flavonoid bioactive compounds, particularly isorhamnetin-3-O-β-D-glucoside and isorhamnetin-3-O-rutinoside, which exhibit

anthelmintic properties, reduce gastrointestinal nematode infections in small ruminants and minimize the dependence on synthetic dewormers, thus contributing to sustainable livestock health management [3]. However, despite these advantages, Typha's high lignocellulosic content severely limits its ruminal fermentation and nutritional availability. In vitro studies indicate that ruminal degradation of Typha dry matter (DM) is only 38.6% at the juvenile stage, decreasing to 22.9% at maturity [6]. Previous attempts to enhance Typha's nutritional value through ensiling and chemical treatments with formic acid have yielded limited success in improving ruminal fermentation and degradability, underscoring the necessity of innovative pretreatment methods to improve its ruminal fermentation efficiency and overall feed quality [7]. Recent biotechnological advancements, particularly the application of exogenous fibrolytic enzymes (EFEs), have demonstrated significant potential in enhancing the nutritional value of lignocellulosic feedstocks, including both conventional and unconventional fibrous resources [8]. These EFEs hydrolyze structural carbohydrates in plant cell walls, breaking them down into simpler sugars before ruminal fermentation [9]. In the ruminal phase, the EFEs stimulate the proliferation of ruminal bacteria protozoa and anaerobic fungi, thereby increasing the endogenous production of microbial enzymes [10]. This enhancement accelerates and improves ruminal degradation kinetics of diets [10], leading to improved metabolizable energy of diets [11], microbial crude protein synthesis [10], and total volatile fatty acid (VFA) production [10]. In addition, some of the EFEs can escape ruminal degradation and remain active during the intestinal phase of digestion, further enhancing nutrient utilization [12] and ultimately leading to improved feed intake and improved animal performance without adverse health effects [11]. However, the efficacy of EFE action has been limited in certain feeds with high lignin content, such as extracted olive cake (containing 320 g lignin/kg DM) [13] and grape pomace (containing 268 g lignin/kg DM) [14], where the high lignin content acts as a physical and chemical barrier. This barrier restricting EFE access to structural carbohydrates reduces the hydrolysis of cellulose and hemicellulose, ultimately limiting the potential benefits of EFE supplementation [13]. Recent studies have demonstrated that physical pretreatment with microwave irradiation can enhance the activity of EFEs by breaking down lignocellulosic structures, thereby facilitating fiber hydrolysis and making the substrate more accessible to EFE. This improvement leads to increased ruminal fermentation efficiency, higher feed degradability, and greater energy availability for ruminants [15]. Previous research has also demonstrated that gamma irradiation enhances the hydrolytic efficiency of cellulase enzymes when applied to hardwood and softwood [16]. However, its specific impact on ruminal fermentation processes remains unexplored, highlighting the need for further investigation into its potential synergistic effects with EFEs in ruminant nutrition. This study hypothesizes that gamma irradiation can serve as an effective pretreatment for *Typha latifolia*, enhancing the activity of EFEs and improving its nutritional value. The combined application of gamma irradiation and EFEs is expected to improve ruminal fermentation, making *Typha latifolia* a more viable and digestible feed resource for ruminants.

Thus, the objective of this study was to assess the effects of gamma irradiation and EFEs, both individually and in combination, on the chemical composition, ruminal fermentation characteristics, and overall nutritional value of *Typha latifolia*.

2. Materials and Methods

2.1. Sample Preparation

Typha latifolia plants were collected at the early vegetative stage—characterized by rapid shoot elongation and leaf development prior to the emergence of inflorescences, measuring less than 0.5 m in height—from homogeneous wetlands in Sfax, Tunisia, during

May 2023. Sampling was conducted over three consecutive weeks (three runs) within a limited timeframe. To minimize variability, all collections were performed within the same geographic area, under similar environmental conditions, and at a similar vegetative stage. Plant material collected from each run was pooled to further reduce potential microclimatic variations.

In each run, the plants were immediately oven-dried at 40 °C for 48 h and ground through a 1 mm sieve using a Retsch mill (Retsch ZM200, Retsch GmbH, Haan, Germany). The samples were divided into four groups, with each group processed in triplicate during each run: (i) a Gamma irradiation group, where 100 g of dried, ground samples mixed with 30 mL of sterile distilled water were exposed to a total radiation dose of 150 kGy using a cobalt-60 gamma irradiator (Gamma Cell 220, Ottawa, ON, Canada) at a dose rate of 33 Gy/min, followed by stabilization at ambient temperature for 2 h according to Al-Masri and Zarkawi [17], after being oven-dried at 40 °C for 48 h. (ii) An EFE group, in which 100 g of dried, ground samples treated with 0.4 mL of EFEs composed of xylanase (2267 U/mL), endoglucanase (1161 U/mL), and exoglucanase (113 U/mL) sourced from *Trichoderma longibrachiatum* (Dyadic International Inc., Jupiter, FL, USA). The enzymes were diluted in 30 mL of sterile distilled water to ensure uniform distribution and effective substrate interaction, followed by aerobic incubation at 39 °C for 24 h, as described by Abid et al. [13], after being oven-dried at 40 °C for 48 h. (iii) A gamma irradiation + EFE group, where 100 g of dried, ground samples was first pretreated with gamma irradiation, followed by EFE supplementation as described above. (iv) A control group, where 100 g of dried, ground samples was supplemented with 30 mL of sterile distilled water but without any treatment (gamma irradiation or EFE) after being oven-dried at 40 °C for 48 h.

2.2. Chemical Composition, Bioactive Compounds, and Antioxidant Activity

Nine samples per group (three replicates per run across three runs) were analyzed for chemical composition and bioactive compounds. Ash, crude protein (CP), and ether extract (EE), which quantifies the total lipid fraction extractable in diethyl ether, were determined following the official methods of the Association of Official Analytical Chemists [18]. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were quantified using an ANKOM fiber analyzer (ANKOM Technology, Macedon, NY, USA), according to Van Soest et al. [19]. Reducing sugars (RSs) were assessed using the 3,5-dinitrosalicylic acid method [20]. Total polyphenols (TPs) and total tannins (TTs) were quantified using the Folin–Ciocalteu assay [21,22]. Antioxidant activity was evaluated using the 2,2-diphenyl-picrylhydrazl-hydrate (DPPH) radical scavenging assay, following [23]. Non-fiber carbohydrates (NFCs) were calculated using Equation (1) [24].

$$\mathrm{NFC} = 1000 - (\mathrm{NDF} + \mathrm{CP} + \mathrm{EE} + \mathrm{ash}) \quad (1)$$

where NFC, NDF, CP, EE, and ash are expressed in mg/g DM.

2.3. Ruminal Incubation

2.3.1. Rumen Inoculum Preparation

Rumen fluid was collected over three consecutive weeks (three runs) from three healthy dairy cows (5 years old, 700 kg body weight) at the same slaughterhouse in Tunis, Tunisia. The cows were fed 7 kg of oat hay and 3 kg of commercial concentrate, and had ad libitum access to water.

The ruminal fluid was transported to the laboratory within 10 min of collection in pre-warmed thermos flasks (39 °C) after CO_2 flushing. Upon arrival, the ruminal fluid was filtered through four layers of cheesecloth under continuous CO_2 flow at 39 °C, then

mixed in equal proportions and diluted (1:2) with an artificial buffer solution, also under continuous CO_2 flow at 39 °C, following the protocol of Menke and Steingass [25].

2.3.2. In Vitro Ruminal Fermentation Assay

The in vitro rumen fermentation assay was conducted using the gas production method of Theodorou et al. [26] in three runs. Three samples (200 mg DM) from each group were placed in 120 mL amber serum bottles with 30 mL of buffered rumen inoculum. Three blank bottles containing only inoculum were included per run. Bottles were sealed and incubated at 39 °C in a shaking water bath (120 rpm). Headspace gas pressure was measured at 2, 4, 6, 8, 12, 24, 48, 72, and 96 h using a pressure transducer (PX4200-0100GI, Omega Engineering, Salmon Arm, BC, Canada) connected to a data logger (Data Tracker 200, Data Track Process Instruments Ltd., Christchurch, New Zealand). Excess pressure was released at each interval to maintain microbial activity.

2.3.3. Gas Volume Calculation and Kinetics Modeling

Gas volumes were calculated using Equation (2)

$$G_{V(s)}(t) = \frac{G_{P(s)}(t) \times \left(V_{f(s)} - V_{i(s)}\right) - G_{P(b)}(t) \times \left(V_{f(b)} - V_{i(b)}\right)}{P_{atm}} \quad (2)$$

where the following hold: $G_{v\,(s)}$: gas volume produced at an incubation time t from sample bottle (mL); $G_{P\,(s)}$: gas pressure recorded at incubation time t from sample bottle (bar); $V_{f:\ (s)}$ volume of the bottle from sample bottle (mL); $Vi_{\ (s)}$: volume of inoculum added at the start of incubation from sample bottle (mL); $G_{P\,(b)}$: gas pressure recorded at incubation time t from blank bottle (bar); $V_{f\,(b)}$: volume of the bottle from blank bottle (mL); $Vi_{\ (b)}$: volume of inoculum added at the start of incubation from blank bottle; P_{atm}: atmospheric pressure (bar).

The net gas production data were fitted using Equation (3), to determine gas production kinetics following the method described by France et al. [27]:

$$Y_t = PGP \times \left(1 - e^{(-C \times (t - Lag))}\right) \quad (3)$$

where the following hold Y: cumulative volume of gas produced at an incubation time t (mL/g DM); PGP: potential gas production (mL/g DM); C: fractional rate of gas production (%/h); Lag: time at which gas production starts (h); t: time of gas measurement (h).

2.3.4. Post-Incubation Analysis

After 96 h of incubation, fermentation was terminated by immersing the serum bottles in ice water (4 °C). The pH of the rumen fluid was immediately measured using a pH meter (Orion Star A221 Portable pH Meter, Thermo Scientific, Montreal, QC, Canada). Rumen bacteria and protozoa were enumerated following the protocol described by Galyean [28]. Briefly, a 0.5 mL aliquot of rumen fluid was mixed with 4.5 mL of 10% formalin-saline solution and stored at room temperature. The mixture was thoroughly homogenized, and 0.1 mL was transferred to a Levy–Sedgewick-Rafter counting chamber (S52 glass; Pyser-SGI, Edenbridge, Kent, UK) for protozoa up to 40 µm enumeration under a light microscope at 100× magnification. An additional 0.1 mL aliquot of rumen fluid was transferred directly to a Petroff-Hausser counting chamber (Hausser Scientific®, 3900, Horsham, PA, USA) for bacterial enumeration under a light microscope at 1000× magnification.

Fermentation residues were filtered using Whatman 541 filter paper (Whatman Scientific Ltd., Maidstone, Kent, UK). An aliquot of 5 mL of the supernatant was acidified with 2 mL of 1 N H_2SO_4 and stored at −20 °C for ammonia nitrogen (NH_3-N) analysis using the

micro-Kjeldahl method [18]. Another aliquot of 1.6 mL of the supernatant was centrifuged at 11,000× *g* for 40 min at 4 °C. Subsequently, 800 µL of the resulting supernatant was injected into a Shimadzu gas chromatograph (Model 2014, Tokyo, Japan) for VFA analysis, following the method described by [29]. Residual DM and NDF content were determined according to AOAC [18] and Van Soest et al. [19], respectively. DM degradability (DMD) and NDF degradability (NDFD) were calculated as the proportion of degraded material relative to the initial amounts.

2.3.5. Energy Estimations

Metabolizable energy (ME) and net energy for lactation (NEL) were estimated according to Menke and Steingass [25] (Equations (4) and (5), respectively):

$$ME = 2.20 + 0.13570 \times GP_{24} + 0.0057 \times CP + 0.000286 \times EE^2 \quad (4)$$

$$NEL = 0.0960 \times GP_{24} + 0.0038 \times CP + 0.000173 \times EE^2 + 0.540 \quad (5)$$

where the following hold: ME: metabolizable energy (MJ/kg DM); NEL: net energy for lactation (MJ/kg DM); GP_{24}: net gas production after 24 h (mL/200 mg DM); CP: crude protein content (g/kg DM); EE: ether extract content (g/kg DM).

2.4. Statistical Analysis

The kinetic parameters of fermentation were determined using the nonlinear models procedure in SAS 9.1 (SAS Institute Inc., Cary, NC, USA). Other data were analyzed using the General Linear Model procedure in SAS 9.1 (SAS Institute Inc., Cary, NC, USA), based on the statistical model presented in Equation (6):

$$Y_{ij} = \mu + T_i + \varepsilon_{ij} \quad (6)$$

where the following hold Y_{ij}: dependent variable; µ: overall mean; T_i: effect of the ith treatment; ε_{ij}: residual experimental error.

When a significant treatment effect was observed, pairwise comparisons between treatments were conducted using Tukey's multiple comparison test. Statistical significance was established at a p value < 0.05.

3. Results

3.1. Chemical Composition and Antioxidant Activity

Gamma irradiation alone significantly altered the chemical profile of *Typha latifolia*, leading to a reduction in NDF content by 12.6% and an increase in ash by 11.1%, NFC by 49.6%, RS by 44.1%, TP by 17.3%, TT by 16.1%, and antioxidant activity by 5.7% compared to the control. EFE applied to non-irradiated plants did not induce changes in chemical composition or antioxidant activity. However, when EFE was applied to gamma irradiated plants, it further reduced NDF content by 4.4% and increased NFC by 13.1% and RS by 24.5% compared to gamma irradiation alone (Table 1).

Table 1. Effects of gamma irradiation pretreatment and exogenous fibrolytic enzymatic supplementation on chemical composition, structural carbohydrate composition, bioactive compounds, and antioxidant activity of *Typha latifolia*.

	Control	γ Irradiation	EFE	γ Irradiation + EFE	SEM	*p* Value
Chemical composition (g/kg dry matter)						
Crude protein	130	127	129	131	2.3	NS
Ether extracts	18	18	17	18	0.9	NS
	Control	**γ Irradiation**	**EFE**	**γ Irradiation + EFE**	**SEM**	***p* Value**
Ash	117 ^{b}	130 ^{a}	115 ^{b}	128 ^{a}	2.2	***
Non-fiber carbohydrates	133 ^{c}	199 ^{b}	138 ^{c}	225 ^{a}	5.3	***
Reducing sugars	34 ^{c}	49 ^{b}	37 ^{c}	61 ^{a}	3.1	***
Structural carbohydrate composition (g/kg dry matter)						
Neutral detergent fiber	602 ^{a}	526 ^{b}	595 ^{a}	503 ^{c}	11.1	***
Acid detergent fiber	342	340	339	333	7.2	NS
Acid detergent lignin	71	69	70	71	4.1	NS
Bioactive Compounds (mg gallic acid equivalents/g)						
Total polyphenols	104 ^{b}	122 ^{a}	102 ^{b}	124 ^{a}	4.2	***
Total tannins	31 ^{b}	36 ^{a}	31 ^{b}	35 ^{a}	0.7	**
Antioxidant activity (mg Terox/g)						
DPPH	299 ^{b}	316 ^{a}	298 ^{b}	317 ^{a}	5.4	***

^{a,b,c} Means in the same line with common superscripts are different at a p value < 0.05; *** p-value < 0.001; ** p value < 0.01; NS: p value > 0.05; EFE: exogenous fibrolytic enzymatic; γ irradiation: gamma irradiation; DPPH: 2,2-diphenyl-picrylhydrazl-hydrate; SEM: standard error of means.

3.2. In Vitro Gas Kinetics

Both EFEs and gamma irradiation influenced the kinetics of gas production differently (Tables 2 and 3). Gamma irradiation alone significantly increased PGP by 14.6% and cumulative gas production from 4 h to the end of incubation compared to the control. EFE supplementation alone significantly increased the fractional rate of gas production by 8.1% and cumulative gas production from 4 h to 12 h of incubation compared to the control. Applying EFEs to gamma irradiated *Typha latifolia* also significantly accelerated fermentation kinetic production by 7.2%, reduced the time at which gas production started by 24.7%, and reduced cumulative gas production from the beginning of incubation to 12 h of incubation compared to gamma irradiation alone.

Table 2. Effects of gamma irradiation pretreatment and exogenous fibrolytic enzyme supplementation on cumulative gas production in mL/g dry matter during ruminal fermentation of *Typha latifolia*.

Incubation Time	Control	γ Irradiation	EFE	γ Irradiation + EFE	SEM	*p* Value
2 h	5.1 ^{b}	5.9 ^{b}	6.2 ^{b}	8.7 ^{a}	0.73	***
4 h	14.9 ^{c}	16.9 ^{b}	17.6 ^{b}	20.4 ^{a}	0.88	***
6 h	23.4 ^{c}	26.5 ^{b}	27.4 ^{b}	31.1 ^{a}	1.01	***
8 h	31.9 ^{c}	36.1 ^{b}	35.5 ^{b}	41.6 ^{a}	1.13	***
12 h	47.8 ^{c}	52.7 ^{b}	52.9 ^{b}	61.3 ^{a}	1.22	***
24 h	83.1 ^{c}	93.2 ^{ab}	90.1 ^{bc}	103.2 ^{a}	3.09	***
48 h	122.4 ^{c}	139.1 ^{ab}	130.8 ^{bc}	151.4 ^{a}	5.09	***
72 h	142.1 ^{c}	161.9 ^{ab}	148.4 ^{bc}	172.7 ^{a}	5.22	***
96 h	149.8 ^{c}	170.6 ^{ab}	155.1 ^{bc}	182.1 ^{a}	5.41	***

^{a,b,c} Means in the same line with common superscripts are different at a p value < 0.05; *** p-value < 0.001; EFE: exogenous fibrolytic enzymatic; γ irradiation: gamma irradiation; SEM: standard error of means.

Table 3. Effects of gamma irradiation pretreatment and exogenous fibrolytic enzyme supplementation on gas production kinetics during the ruminal fermentation of *Typha latifolia*.

	Control	γ Irradiation	EFE	γ Irradiation + EFE	SEM	*p* Value
Potential gas production (mL/g dry matter)	157 ^{b}	180 ^{a}	162 ^{b}	189 ^{a}	6.9	***
Fractional rate of gas production (%/h)	3.22 ^{b}	3.19 ^{b}	3.48 ^{a}	3.42 ^{a}	0.091	*
Lag time (h)	0.98 ^{a}	0.93 ^{a}	0.90 ^{a}	0.70 ^{b}	0.077	**

^{a,b} Means in the same line with common superscripts are different at a p value < 0.05; *** p-value < 0.001; ** p value < 0.01; * p-value < 0.05; EFE: exogenous fibrolytic enzymatic; γ irradiation: gamma irradiation; SEM: standard error of means.

3.3. Fermentation Parameters

Gamma irradiation alone significantly reduced ruminal NH_3-N levels by 13.2% compared to the control and significantly decreased the proportion of branched-chain volatile fatty acids (isobutyric acid, isovaleric acid, and valeric acid by 30.7%, 22.7%, and 26.3%, respectively, compared to the control). In contrast, EFE supplementation alone did not induce significant changes in NH_3-N levels or VFA profiles. However, applying EFEs to gamma-irradiated *Typha latifolia* significantly increased VFA production by 12.2% compared to the control and by 10.3% and 11.8% compared to gamma irradiation alone and EFE supplementation alone, respectively. It increased the propionic acid proportion by 14.9% compared to the control and by 12.3% and 14.5% compared to gamma irradiation alone and EFE supplementation alone, respectively, while significantly decreasing the acetic acid proportion by 3.9% compared to the control and by 4.9% and 3.8% compared to gamma irradiation alone and EFE supplementation alone, respectively. As a result, only the dual treatment significantly lowers the acetate-to-propionate ratio—by 7.9% compared to the control and by 6.8% and 7.6% compared to gamma irradiation alone and EFE supplementation alone, respectively (Table 4).

Table 4. Effects of gamma irradiation pretreatment and exogenous fibrolytic enzyme supplementation on ruminal fermentation parameters of *Typha latifolia*.

	Control	γ Irradiation	EFE	γ Irradiation + EFE	SEM	*p* Value
Ph	6.88	6.85	6.87	6.86	0.055	NS
NH_3-N (mg/L)	243 ^{a}	211 ^{b}	245 ^{a}	214 ^{b}	7.4	***
Total volatile fatty acids (mmol/L)	29.6 ^{b}	30.2 ^{b}	29.7 ^{b}	33.2 ^{a}	1.22	***
Acetic acid (%)	60.9 ^{a}	61.5 ^{a}	60.8 ^{a}	58.5 ^{b}	0.72	*
Propionic acid (%)	25.5 ^{b}	26.1 ^{b}	25.6 ^{b}	29.3 ^{a}	0.33	***
Butyric acid (%)	8.2	8.4	8.3	8.1	0.43	NS
Isobutyric acid (%)	1.3 ^{a}	0.9 ^{b}	1.2 ^{a}	0.9 ^{b}	0.13	*
Isovaleric acid (%)	2.2 ^{a}	1.7 ^{b}	2.3 ^{a}	1.7 ^{b}	0.11	***
Valeric acid (%)	1.9 ^{a}	1.4 ^{b}	1.8 ^{a}	1.5 ^{b}	0.19	***
Acetic acid/propionic acid (%)	2.39 ^{a}	2.36 ^{a}	2.38 ^{a}	2.20 ^{b}	0.06	*

^{a,b} Means in the same line with common superscripts are different at a p value < 0.05; *** p-value < 0.001; * p-value < 0.05; NS: p value > 0.05; EFE: exogenous fibrolytic enzymatic; γ irradiation: gamma irradiation; SEM: standard error of means.

3.4. Ruminal Degradability and Energy Utilizable

Gamma irradiation alone significantly improved DMD, NDFD, ME, and NEL by 12.4%, 8.9%, 5.5%, and 7.5% compared to the control, respectively. EFE supplementation alone had no significant effect. However, adding EFE to gamma-irradiated *Typha latifolia* further enhanced DMD, NDFD, ME, and NEL by 4.9%, 7.1%, 5.7%, and 8.0% compared to gamma irradiation alone, respectively (Table 5).

Table 5. Effects of gamma irradiation pretreatment and exogenous fibrolytic enzyme supplementation on the ruminal degradation and energy utilizable of *Typha latifolia*.

	Control	γ Irradiation	EFE	γ Irradiation + EFE	SEM	*p* Value
Ruminal degradability						
Dry matter (g/kg dry matter)	379 ^{c}	426 ^{b}	386 ^{c}	447 ^{a}	11.1	***
Neutral detergent fiber (g/kg neutral detergent fiber)	247 ^{c}	269 ^{b}	253 ^{c}	288 ^{a}	8.5	*
Energy utilizable (MJ/kg dry matter)						
Metabolizable energy	5.28 ^{c}	5.57 ^{b}	5.45 ^{bc}	5.89 ^{a}	0.241	*
Net energy for lactation.	2.68 ^{c}	2.88 ^{b}	2.81 ^{bc}	3.11 ^{a}	0.153	*

^{a,b,c} Means in the same line with common superscripts are different at a p value < 0.05; *** p value < 0.001; * p-value < 0.05; EFE: exogenous fibrolytic enzymatic; γ irradiation: gamma irradiation; SEM: standard error of means.

3.5. Ruminal Microbiota

Table 6 indicates that gamma irradiation alone significantly increased ruminal bacterial populations by 25.7% while decreasing protozoa by 10.6% compared to the control. EFE supplementation alone had no significant effect. However, the addition of EFE to gamma-irradiated *Typha latifolia* further boosted ruminal bacterial abundance by 21.2% compared to gamma irradiation alone.

Table 6. Effects of gamma irradiation pretreatment and exogenous fibrolytic enzyme supplementation on ruminal microbiota at the end of ruminal fermentation of *Typha latifolia*.

	Control	γ Irradiation	EFE	γ Irradiation + EFE	SEM	*p* Value
Bacteria (10^8 cells/mL)	11.3 ^{c}	14.2 ^{b}	11.8 ^{c}	17.2 ^{a}	0.46	***
Protozoa (10^5 cells/mL)	3.78 ^{a}	3.38 ^{b}	3.77 ^{a}	3.37 ^{b}	0.21	*

^{a,b,c} Means in the same line with common superscripts are different at a p value < 0.05; *** p value < 0.001; * p-value < 0.05; EFE: exogenous fibrolytic enzymatic; γ irradiation: gamma irradiation; SEM: standard error of means.

4. Discussion

Gamma irradiation alone at 150 kGy significantly altered the chemical composition of *Typha latifolia*, reducing NDF by 12.6% while increasing NFC by 49.6% and RS by 44.1%. Similar trends were observed in irradiated agro-industrial by-products, such as rice straw—at 500, 1000, 1500, and 2000 kGy, which significantly reduces NDF compounds— [30] and sugarcane bagasse—at 100, 250, 400, and 1000 kGy, which significantly increases RS [31] due to cleavage of glycosidic bonds by irradiation, which leads to the formation of sugars like glucose and maltose [32]. In addition, the 11.1% increase in ash content of gamma-irradiated *Typha latifolia* may result from the release of hydrogen, carbon dioxide, and carbon monoxide from samples due to the gamma irradiation process, which reduced organic matter in gamma-irradiated rice straw at 500, 1000, 1500, and 2000 kGy [30] and soybean straw at 100 kGy [33]. Gamma irradiation also increased TP by 17.3%, TT increased by 16.1%, and antioxidant activity increased by 5.7%, as seen in the increase in TP and antioxidant activity of irradiated almond skins at 12.7 and 16.3 kGy [34]. This rise in phenolic content could be attributed to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by gamma irradiation [34]. These chemical modifications contributed to a shift in the ruminal microbiota by reducing ruminal protozoa by 10.6%, likely due to increased tannin content [35], while promoting rumen bacterial proliferation 25.7%, likely as a result of reduced protozoal predation on bacteria [36]. The combined effects of these chemical and microbial shifts positively influenced ruminal fermentation dynamics, leading to an increase in the PGP by 14.6%. These changes improved the ruminal DMD by 12.4%, NDFD by 8.9, ME by 5.5%, and ENL by 7.5%. These findings are consistent with previous in situ studies on irradiated soybean

straw at 100 and 150 kGy, which enhanced DM degradation kinetics and effective DM degradability of sheep [33]; previous in vitro studies on irradiated barley straw, sorghum straw, and wheat chaff at 100 and 150 kGy saw improved organic matter digestibility and energy digestibility of sheep [37]. Moreover, gamma irradiation led to a decrease in ruminal NH_3-N and branched-chain VFA, suggesting a reduction in microbial protein degradation. This decrease in ruminal ammonia may be attributed to increased microbial assimilation of ammonia driven by enhanced energy availability or increased tannin content, which protects CP from ruminal degradation [35], or structural modifications of proteins induced by gamma irradiation, such as those observed in gamma irradiated soybean meals and irradiated cotton seed meals at 25, 50, and 75 kGy that reduce CP degradation in the rumen [38,39]. In contrast, treatment of *Typha latifolia* with EFE produced from *Trichoderma longibrachiatum* at 4 μL/g DM did not alter its chemical composition but significantly enhanced the fractional gas production rate by 8.1%, without affecting ruminal microbial populations or fermentation efficiency. This finding is consistent with previous studies in which EFEs from *Trichoderma longibrachiatum* at 4 μL/g DM treatment accelerated the fermentation of already-fermentable compounds of *Posidonia oceanica* by 10.3% [15], and EFEs at 0.51, 1.02, 2.55, 5.10, and 25.5 μL/g DM enhanced the initial fermentation and organic matter degradation of alfalfa stems only during the first 19 h of incubation [40]. The acceleration of rumen fermentation can enhance ruminal turnover, thus improving feed intake [41]; consequently, EFE treatment may contribute to improved intake of *Typha latifolia* in ruminant nutrition.

Unlike EFEs applied to non-irradiated *Typha latifolia*, the application of EFEs to gamma-irradiated *Typha latifolia* resulted in the partial conversion of NDF into RS and NFC. This is likely due to the disruption of lignocellulosic bonds, the reduction in fiber crystallinity, and the modification of cell wall integrity induced by gamma irradiation [31,33], which enhanced the enzymatic hydrolysis of fiber into simpler sugars [16]. These structural changes facilitated a shift in the ruminal microbiome, specifically promoting ruminal bacterial growth by 21.2% compared to gamma irradiation alone while leaving protozoan populations unaffected. This finding is consistent with previous in vitro studies using rumen simulation techniques, where supplementation with EFEs produced from *Trichoderma longibrachiatum* applied to rolled barley grain increased ruminal cellulolytic bacterial populations tenfold without affecting protozoal numbers [42]. In addition, the dual treatments exhibit faster ruminal microbiota colonization and fermentation, as indicated by a 28.6% reduction in time to initiate gas production compared to the control, and reductions of 24.7% and 22.2% compared to gamma irradiation alone and EFE supplementation alone, respectively. This led to improved ruminal degradability, as evidenced by increases in DMD by 4.9% and NDFD by 7.1% compared to gamma irradiation alone. Moreover, energy utilization parameters were enhanced, with ME increased by 5.7% and NEL by 8.0% relative to gamma irradiation alone. These findings align with previous research indicating that physical pretreatment with microwave irradiation enhances the levels of EFE produced from *Trichoderma longibrachiatum* in improving the ruminal degradability and NEL of *Posidonia oceanica* [15]. Furthermore, the combined treatment increased VFA production by 10.3% and the propionic acid proportion by 12.3% compared to gamma irradiation alone, while decreasing acetate proportions by 4.9% relative to gamma irradiation alone. Consequently, the acetate-to-propionate ratio was significantly reduced by 6.8% compared to gamma irradiation alone and by 7.9% compared to the control. These shifts, though numerically limited, are biologically meaningful within the context of rumen fermentation, where even small reductions in the acetate-to-propionate ratio are often associated with improved energy efficiency [43] and potentially reduced methane emissions, as propionate acts as a hydrogen sink, thereby limiting ruminal methanogenesis [44].

5. Conclusions

The integration of gamma irradiation as a physical pretreatment to EFE supplementation significantly enhances the enzymatic hydrolysis of the lignocellulosic compound of *Typha latifolia* into simple sugars in the pre-ruminal phase. This induces a shift in ruminal microbiota by decreasing protozoal populations and stimulating bacterial proliferation, leading to improved ruminal fermentation kinetics, degradability, and energy efficiency. Furthermore, this combined approach enhances VFA production, particularly of propionic acid, while reducing acetate, branched-chain fatty acids, and ruminal NH_3-N concentrations, without perturbing ruminal pH homeostasis. This integrated strategy presents a novel approach for enhancing the efficacy of EFE applications, to improve the ruminal fermentation of lignocellulosic feedstuffs, optimizing nutrient utilization in ruminants. Further investigations are needed to validate its effectiveness across diverse lignocellulosic feed, reduce energy input requirements, and comprehensively assess the cost-effectiveness of this pretreatment strategy at a larger scale to evaluate its practical viability. Additionally, the potential adverse effects of gamma irradiation on plant material—such as the accumulation of harmful oxidative by-products, generation of free radicals, and possible degradation of beneficial constituents—should be carefully examined in future study. In this context, future in vivo studies are strongly recommended to evaluate the safety of this approach in terms of animal health, feed quality, animal performance, and overall food safety.

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Abbreviations

The following abbreviations are used in this manuscript:

ADL	Acid detergent lignin
C	Fractional rate of gas production
CP	Crude protein
DM	Dry matter
DMD	Dry matter degradability
DPPH	2,2-diphenyl-picrylhydrazl-hydrate
EE	Ether extract
EFE	Exogenous fibrolytic enzyme
G_P	Gas pressure
Gv	Gas volume
Lag	Time at which gas production starts
ME	Metabolizable energy
NDF	Neutral detergent fiber
NDFD	Neutral detergent fiber degradability
NEL	Net energy for lactation
NFC	Non-fiber carbohydrate
P_{atm}	Atmospheric pressure
PGP	Potential gas production
RS	Reducing sugars
SEM	Standard error of means

TP	Total polyphenols
TT	Total tannins
V_f	Volume of the bottle
VFA	Volatile fatty acids
Vi	Volume of inoculum added at the start of incubation
γ irradiation	Gamma irradiation

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