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Ensiling Typha (*Typha latifolia*) Forage with Different Additives for Ruminant Feeding: In Vitro Studies

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Abstract: Typha is a wild plant with invasive growth that causes environmental problems in different areas worldwide. This study investigated the ensiling potential of typha (*Typha latifolia*) to be preserved and used for ruminant feeding. Four silage treatments were evaluated: typha with no additives (CON); with formic acid (FA; 4 mL/kg); with urea and molasses (UM; 19 and 24 g/kg, respectively); and with formic acid, urea, and molasses (FAUM; all at previous concentrations). Three bags of each silage were prepared and stored for 4 months before being analysed and fermented in vitro with ruminal fluid from sheep. All of the silages were well preserved, and their pH was greater ($p < 0.05$) for UM and FAUM (8.50 and 7.70, respectively) compared with CON and FA (5.39 and 4.75). The high concentrations of volatile fatty acid and ammonia−nitrogen in the UM and FAUM silages indicated undesirable fermentations and precluded their use in practice. However, neither CON nor FA silages improved the in vitro ruminal degradability of pre-ensiling typha (61.7, 58.2, and 65.2%, respectively), which was lower than that of a medium-quality triticale hay (68.0%). The results indicate that typha silage could replace low-quality forages in ruminant diets, being adequate especially for low-producing animals or for those under maintenance conditions. More studies with different doses of additives are needed to identify the optimal conditions for ensiling typha and increasing its nutritive value.

Keywords: silages; urea; formic acid; molasses; rumen fermentation; gas production

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1. Introduction

The genus *Typha* spp. is made up of between 10 to 15 species of aquatic plants, commonly known by the name of cattail, which refers to its inflorescence. These plants are distributed throughout tropical and subtropical ecosystems, and are found in moist soils, swamps, marshes, or brackish seas throughout the world [1]. Its characteristics, growth pattern, great adaptability to different environmental conditions, and release of compounds that prevent the growth of other plants, give *Typha* spp. plants the ability to easily expand and occupy areas where other vegetation previously existed [2]. This invasive behavior can cause serious problems in different areas of the world, where local populations living near wetlands experience threats to their economic activities, as well as their health and well-being [3].

The productivity of livestock is constrained in many tropical and subtropical areas by the limited supply, low quality, and high cost of the available feedstuffs. Limited feed supply is especially marked during the dry season, as grass growth is dramatically reduced, and the performance and productivity of animals is decreased. Therefore, finding new

sources of ingredients that are locally available and economically affordable is a priority. The implementation of farm practices that involve the use of *Typha* spp. plants as animal feed can help to overcome the problems associated with this plant and to improve the resilience and livelihood of livestock farmers. *Typha* spp. plants are fibrous and have a low nutritive value [4], and thus may be more adequate for ruminant feeding than for other animal species. In fact, previous studies have suggested that *Typha* could be used as animal feed for ruminants in periods of feed shortage, such as the dry season, as the plant is continuously available [5–7]. Nevertheless, these practices require a simple and low-cost storage method to increase feed availability. Silage is a low-cost method to store and preserve forage and is extensively used all around the globe. During ensiling, easily fermentable carbohydrates are converted into organic acids by epiphytic bacteria, effectively reducing the pH and allowing forage preservation [8]. Ensiling *Typha* spp. could be challenging because of their low easily fermentable carbohydrates content, but the ensiling process can be enhanced using additives, such as organic acids, urea, or molasses, as reported for other low-quality forages [8]. Formic acid, a widely used organic acid for helping to decrease pH during ensiling, and molasses are added as substrates to promote lactic acid production, whereas urea is utilized to increase the nitrogen (N) content of silages, especially when low-protein feeds are ensiled [8].

There is little information on the suitability of different additives for ensiling *Typha* spp., but WingChing-Jones and Leal [5] reported that ensiling *Typha domingensis* with urea and molasses increased its energy and crude protein (CP) content compared with the pre-ensiling plant. However, to the best of our knowledge, no previous study has assessed the potential influence of ensiling on the nutritive value of typha. Our hypothesis is that using formic acid as an additive could help to decrease the pH and improve the ensiling process of typha. The aim of this work was thus to evaluate the effects of using formic acid, urea, and molasses as additives on the characteristics, chemical composition, and in vitro ruminal fermentation of typha silages.

2. Materials and Methods

2.1. Typha Samples Collection, Silage Preparation, and Silage Processing

Samples of wild typha (*Typha latifolia*) were harvested manually at different points from water ways with a high level of leachate close to agricultural fields. The sampling area was located in the southwest of Spain (Valdeobispo, Cáceres, Spain, (40°4′55″ North; 6°14′51″ West) during the period of May 2019. The climate of this area is subtropical Mediterranean, with average annual temperatures ranging between 15° and 18 °C. Winters are mild (average of 8 °C) and summers are dry and hot, reaching absolute maximums of 39 °C. The plants were collected during the vegetative stage before blooming, at about 2–3 months old, and they were between 1.5 and 2 m high. After collection, the roots were immediately separated and discarded, and the leaves and stems were transported to the laboratory. The plant biomass was pre-wilted outdoors for 36 h to increase their dry matter (DM) content to about 47% before ensiling. Finally, the plants were cut manually into about 2 × 3 cm pieces using scissors.

The experimental design involved 4 different silage types, with three replicates per treatment. Typha was ensiled without any additive (control); with formic acid (4 mL/kg of plant biomass; FA); with urea and molasses (19 and 34 g/kg of plant biomass, respectively; UM); and with formic acid, urea, and molasses (all additives at the previous concentrations; FAUM). The amount of each additive was selected from previous studies reported in the literature [8]. All silages were created in vacuum-*packed* plastic bags (25 × 40 cm). Three bags per silage treatment were prepared, making a total of 12 silage bags. The typha biomass (400 g) was weighed in a plastic bucket and treated with the corresponding additive. The corresponding amount of additive for each treatment was solubilized in 130 mL of water, and the solution was added to the plastic bucked and mixed thoroughly with the typha. For the control silages, 130 mL of water was added to the bucket. The amount of water added was calculated from the DM determination of the typha to achieve

a target DM of 30–34% in the silages. The mixture was then quantitatively transferred into plastic bags that were sealed using an automatic vacuum-packing machine (Model VM 101H, UniVac, Weesp, The Netherlands). In addition, samples of pre-wilted typha were collected during the process of preparing the silages for chemical composition analysis and in vitro incubations. A detailed description of the sampling and biomass preparation was previously given at a conference [9].

The silages were stored in darkness for 4 months at room temperature (20 °C) before being processed, as described by Samarasinghe et al. [10]. Briefly, the bags were opened, their content was weighed, and they were homogenized through hand mixing. Then, 50 g from each silage bag was immediately weighed, mixed with 500 mL of demineralized water, and the mixture was homogenized in a Waring blender (Waring 24CB10; Waring Commercial, New Hartford, CT, USA) for 40 s. The mixing procedure was repeated after 1 min to prevent heating. The mixture was filtered through 4 layers of cheesecloth, and the pH of the filtrate (silage extract) was immediately measured using a pH meter Crison GPL 21 (Crison Instruments, Barcelona, Spain). Then, 2 ml of the extract was mixed with 40 µL of H_2SO_4 (20%; vol/vol) for preservation and immediately frozen at −20 °C until analysis of the volatile fatty acids (VFA), NH_3-N, and lactic acid. In addition, 50 g of content from each silage bag was weighed and dried at 60 °C for 48 h for determining the DM content. The rest of the silage bag contents were frozen (−20 °C), freeze dried, and ground to 1 mm before analysis of the chemical composition and in vitro ruminal incubations.

2.2. In Vitro Fermentations: Donor Animals and Experimental Procedures

Samples of the pre-ensiling typha and typha silages were incubated in vitro with ruminal fluid from sheep to determine the gas production kinetics and the main fermentation parameters. In addition, a sample of triticale hay was included in the in vitro trials for comparative purposes.

Four adult rumen-fistulated Lacuane sheep (64.7 ± 2.10 kg body weight; female) were used as rumen fluid donors for the in vitro incubations. Sheep handling and ruminal content sampling were performed following the European guidelines for experimental animal protection, and all experimental procedures were approved by the Animal Experimentation Ethics Committee of the Autonomous Community of Madrid (approval number PROEX 035/17). Sheep were housed in pens and fed a mixed diet of alfalfa hay and a commercial concentrate (80:20) at a rate of 48 g DM/kg body weight$^{0.75}$. The diet was administered twice daily at 09:00 a.m. and 17:00 p.m., and the animals had free access to water and to a mineral–vitamin mixture.

For determining the gas production kinetics of the samples, ruminal contents were manually collected from each sheep immediately before the morning feeding, using stainless tweezers (40 cm long) with a shovel on the end. The contents were filtered through 4 layers of cheesecloth into thermal flasks, and immediately transported to the laboratory. The fluid of each individual sheep was mixed with the culture medium of Goering and Van Soest [11] (without including trypticase; 39 °C) in a 1:4 proportion. The procedure was conducted under CO_2 flushing to minimize air exposure.

Then, 200 mg of DM of each sample was accurately weighed into 60 mL glass vials, which were filled with 20 mL of the mixture of rumen fluid and culture medium. The mixture was dosed by means of a peristaltic pump (Watson-Marlow 520UIP31; Watson-Marlow Fluid Technology Group, Cornwall, UK). The vials were sealed with solid rubber stoppers and incubated at 39 °C for 120 h. The amount of gas produced was measured at 2, 4, 6, 9, 12, 24, 30, 36, 48, 60, 72, 96, and 120 h of incubation using a pressure transducer (Delta Ohm DTP704-2BGI, Herter Instruments SL, Barcelona, Spain) and a calibrated syringe. The gas was collected inside a syringe connected to the pressure transducer, until the pressure into the vial was 0. The volume of gas collected in the syringe was then measured, and it was released after each measurement. In addition, 2 bottles with no substrate (blanks) were included for each inoculum (the ruminal fluid from each of the 4 sheep used as donors) for the purpose of correcting the gas production values for the gas released from

the endogenous substrates. A single incubation run was performed and replication (n = 4) was achieved using the ruminal fluid of each individual sheep as the inoculum.

In order to analyze the main fermentation parameters, immediately after measuring the gas produced after 24 h of incubation, the vials were hand-shaken, a sample of 1 mL was taken from each vial using an insulin syringe (introducing the needle through the rubber cup of the vial) and mixed with 0.40 µL of 20% H_2SO_4 to stop fermentation. Samples were frozen at −20 °C until the analysis of the VFA and ammonia N (NH_3-N) content.

The potential in vitro DM ruminal degradability (PDMD) was determined by weighing, in duplicate, 300 mg DM of each sample into 30 µm pore size polyester bags (Ankom Corp F57; Ankom Technology Corp., Fairport, NY, USA), which were placed into 2 jars (1 bag for each sample in each jar) of the Ankom Daisy II incubator (Ankom Technology Corp., Fairport, NY, USA). The ruminal fluids of the 4 sheep were mixed in equal proportion and added to the culture medium of Goering and Van Soest [11] in a 1:4 proportion. Then, 2 l of the final mixture was filled into each jar, and the jars were immediately placed into the incubator. After 120 h of incubation, the jars were opened, and the bags were washed with tap water, dried (60 °C, 48 h), and weighed for calculating the PDMD.

2.3. Chemical Analyses

The DM, ash, and ether extract (EE) content of pre-ensiling typha, typha silages, and triticale hay used as the reference were analyzed following the methods described by the Association of Official Analytical Chemists [12]. The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin content were determined as described by Van Soest et al. [13] and by Robertson and Van Soest [14], respectively, and the results were expressed excluding the residual ash. The N content was measured using the Dumas combustion method with a Leco FP258 analyzer (Leco Corporation, St. Joseph, MI, USA) and the CP content was calculated as N content × 6.25. Concentrations of NH_3-N in the silage extract and in the samples taken from the in vitro fermentations were determined as described by Weatherburn [15] using the phenol–hypochlorite method. Concentrations of VFA in the same samples were analyzed by gas chromatography, as described by García-Martínez et al. [16], using a Shimadzu GC 14B gas chromatograph (Shimadzu Europa GmbH, Duisburg, Germany) fitted with a flame ionization detector and a column packed with Carboxen 1000 (Supelco, Madrid, Spain). The lactic acid concentrations were analyzed using the colorimetric method reported by Taylor [17]. All of the analyses were performed in duplicate.

2.4. Calculations and Statistical Analyses

The DM recovery in the silages was calculated independently for each bag silage as the DM weight of the bag content after 3 months of storage divided by the initial DM in each bag, and it was expressed as the percentage of initial DM.

The model of Gas = PGP × $(1 - e^{(-c \times (t - lag))})$ proposed by Krishnamoorthy et al. [18] was used to fit gas production data with time using an iterative least squares procedure, using the NLIN procedure of the statistical package SAS [19]. In this model, PGP is the potential gas production (asymptotic production), c is the fractional rate of gas production, lag is the time at which gas production starts, and t is the time of gas measurement. In addition, the average gas production rate (AGPR) is estimated using the equation proposed by France et al. [20]: AGPR = PGP × $c/[2 \times (\ln 2 + c \times lag)]$.

Data on the chemical composition and PDMD were analyzed through one-way analysis of variance with the silage treatment (control, FA, UM, and FAUM) as the main effect. In vitro data were analyzed through analysis of variance as a mixed model with the fixed effect of the silage type treatment and the random effect of the inoculum (ruminal fluid) used in the in vitro trial. All of the statistical analyses were performed using the Proc Mixed of SAS [19]. Significance was declared at $p < 0.05$, and p values between 0.05 and 0.10 were considered as a trend. When a significant effect for the treatment was detected, differences between means were assessed using Tukey's test.

3. Results and Discussion

3.1. Chemical Composition and Characteristics of Silages

Silage bags were visually monitored once weekly, and no vacuum failure was detected during the whole storage period. No effluent or signs of molding were detected in any bag upon opening. The content of all of the bags had a sweet–vinegar odor and normal greenish color typical for well-fermented forage silages [8], apart from the UM silages, which had a strong, bad odor and were dark-brown colored.

As shown in Table 1, the amount of fresh matter in the bags was greater ($p < 0.05$) in the silages treated with urea and molasses (UM and FAUM) compared with the CON and FA silages, which was attributed to the addition of molasses. The recovery of DM in the silages ranged from 87.7% for CON to 96.8% for FA silage. These values are consistent with the low production of gas observed in all bags and the lack of effluent in any silage. The DM content of all silages was similar to the expected value (30–32%), although it was greater for the silages treated with formic acid (FA and FAUM) than for the CON silage.

Table 1. Characteristics of typha silages treated with no additive (CON); with formic acid (FA); with urea and molasses (UM); and with formic acid, urea, and molasses (FAUM) [1].

Item	Silage Treatment				SEM [2]	p =
	CON	FA	UM	FAUM		
Fresh matter (g/bag)	410 [a]	404 [a]	440 [b]	442 [b]	3.4	<0.001
Recovery of dry matter (DM, %)	87.7	96.8	88.9	94.1	2.43	0.089
DM (%)	30.4 [a]	34.6 [b]	32.6 [ab]	34.6 [b]	0.98	0.046
pH	5.39 [b]	4.75 [a]	8.50 [d]	7.70 [c]	0.114	<0.001
NH_3-N (g/kg DM) [3]	0.221 [a]	0.00 [a]	9.06 [c]	7.22 [b]	0.156	<0.001
VFA [4] (g/kg DM)						
Acetic	16.6 [b]	8.86 [a]	46.1 [c]	13.7 [ab]	1.15	<0.001
Propionic	3.84 [b]	0.22 [a]	0.99 [a]	0.88 [a]	0.603	0.012
Butyric	17.2 [c]	0.76 [a]	11.8 [bc]	4.84 [ab]	2.34	0.005
Isobutyric	1.42 [b]	0.19 [a]	0.62 [ab]	0.00 [a]	0.262	0.0214
Isovaleric	0.32 [b]	0.00 [a]	0.69 [c]	0.00 [a]	0.0207	<0.001
Valeric	2.73 [b]	0.00 [a]	0.00 [a]	0.00 [a]	0.144	<0.001
Total VFA	42.0 [b]	10.0 [a]	60.2 [c]	19.4 [a]	3.78	<0.001
Lactic acid (g/kg DM)	4.89 [a]	14.5 [a]	19.1 [b]	17.0 [ab]	2.15	0.028

[a, b, c, d] Within each parameter, means in the same row with different superscripts are different ($p < 0.05$). [1] FA: 4 mL of formic acid/kg; UM: 19 g of urea and 34 g of molasses/kg; FAUM: formic acid, urea, and molasses at previous concentrations; [2] SEM: standard error of the mean (n = 3); [3] NH_3-N: ammonia nitrogen; [4] VFA: volatile fatty acids.

In agreement with previous reports [21–23], the silages treated with urea had greater ($p < 0.05$) pH values than the CON and FA silages, which was due to the alkalizing effect of the NH_3-N produced by the enzymatic decomposition of the urea. The urease activity in the forage silages has been reported to be optimum at pH close to 8 and to be inhibited by pH values below 5.0 [24]. The high pH values in the UM and FAUM silages possibly contributed to the high urea decomposition, as indicated by the high NH_3-N concentrations observed for these silages. In fact, the NH_3-N concentrations were low for CON silage and undetectable for FA silage, whereas both UM and FAUM had greater ($p < 0.05$) concentrations (Table 1). The NH_3-N concentration is considered to be an indicator of proteolytic activity during silage fermentation, but in our study, the high NH_3-N concentrations in UM and FAUM silages were probably due to the decomposition of the urea added. The lower concentrations of isobutyric, valeric, and isovaleric acids observed for UM and FAUM compared with the CON silages would suggest a lower proteolytic activity in the silages treated with urea, as these VFA are produced in the degradation of branched-chain amino acids [25].

The NH_3-N content for CON and FA silages accounted for 1.21 and 0.0% of the total N, respectively, which was lower than the maximal recommended value of 12% for

grass silages with 25–35% DM [8]. In contrast, the values for UM and FAUM silages (37.3 and 20.3%, respectively) were above this value. In agreement with our results, Hinds et al. [26] reported that the NH_3-N content accounted for 17.4 to 20.8% of the total N for the sorghum silages treated with urea at similar concentrations (18 g of urea/kg), whereas in the untreated silages the NH_3-N content was lower than 5% of the total N. In our study, the addition of formic acid to the silages containing urea and molasses failed to reduce the pH to adequate levels in the FAUM silage, and urea was extensively degraded, as indicated by the high NH_3-N concentrations.

The concentrations of acetic acid in silages usually range from 10 to 30 g per kg DM [8]. In our study, the CON, FA, and FAUM silages were within this range, but the UM silage showed much greater concentrations (Table 1). As pointed out by Kung et al. [8], moderate concentrations of acetic acid (30–40 g/kg DM) can be beneficial because this acid is a potent antifungal agent. Moreover, when silage is consumed by animals, the acetic acid can be absorbed from the rumen and used either as an energy source or for the synthesis of fatty acids [27]. However, the amount of acetic acid in UM silage can be considered excessive (>40 g/kg DM), and it could indicate unwanted fermentations dominated by clostridia or enterobacteria [28]. It has been reported that treating silages with ammonia or urea results in increased acetic concentrations due to prolonged fermentation, caused by its buffering effect [21].

The CON silage presented greater concentrations ($p < 0.05$) of propionic and butyric compared with the three silages treated with additives. The concentrations of these acids in the silage should be minimal, and preferably undetectable, as they are indicative of fermentation by clostridial microorganisms [8]. Clostridial fermentation can be avoided by the rapid production of lactic acid in the silage, as clostridial microorganisms cannot tolerate high osmotic pressure and low pH conditions that are favored by high concentrations of lactic acid. Although the addition of all additives increased ($p < 0.005$) the concentrations of lactic acid compared with the CON silage (Table 1), the lactic acid concentrations were lower than the values ranging from 20 to 40 g/kg DM silage reported by Kung et al. [8] as the mean values for commonly fed silages. Moreover, the lactic acid/acetic acid ratio in good fermented silages is usually 2.5–3.0 [8], and in our study were below this value (0.32, 1.62, 0.41, and 1.37 for the CON, FA, UM, and FAUM silages, respectively).

Altogether, the results indicate that lactic acid production was low in all silages, and that clostridial fermentations could have been produced in UM and FAUM silages, as indicated by the presence of butyric acid, high pH, and high concentrations of acetic and NH_3-N [8].

The chemical composition of the silages, the pre-ensiling typha, and triticale hay used as a reference is shown in Table 2. Compared with the CON silage, the addition of formic acid reduced ($p < 0.05$) the ash content, whereas the addition of urea and molasses (FAUM silage) increased this content ($p < 0.05$). Although some studies [29,30] have reported that formic acid can cause a decrease in NDF concentrations of ensiled plants due to acid hydrolysis, in our study, this effect was only observed when formic acid was combined with urea and molasses in FAUM silage, which showed lower NDF concentrations than the CON silage. There were no differences between the silages in their ADF and EE content ($p = 0.581$ and 0.081, respectively). As expected, the addition of urea (UM and FAUM silages) caused an increase ($p < 0.05$) in CP content compared with CON and FA silages, which was greater in the FAUM silage probably due to the protective effect of formic acid against CP degradation. The lower concentrations of NH_3-N and the undetectable concentrations of isobutyric, isovaleric, and valeric acids observed in FAUM silage seem to support this hypothesis.

Table 2. Chemical composition (g/kg of dry matter) of typha silages treated with no additive (CON); with formic acid (FA); with urea and molasses (UM); or with formic acid, urea, and molasses (FAUM) of typha biomass before ensiling and of a triticale hay used as a reference [1].

Item	Silage Treatment				SEM [2]	*p* =	Thypha Pre-Ensiling	Triticale Hay
	CON	FA	UM	FAUM				
Crude ash	123 [b]	117 [a]	135 [c]	123 [b]	1.2	<0.001	120	86.0
Crude protein	114 [a]	115 [a]	152 [b]	222 [c]	2.9	<0.001	119	109
Ether extract	39.7	36.9	46.4	48.9	4.52	0.081	48.4	23.8
Neutral detergent fibre	694 [bc]	702 [c]	666 [ab]	643 [a]	7.6	0.002	691	587
Acid detergent fibre	411	404	415	395	10.8	0.581	401	315
Acid detergent lignin	69.2 [ab]	63.1 [a]	73.3 [b]	65.8 [a]	1.64	0.012	66.9	37.4

[a, b, c] Within each parameter, means in the same row with different superscripts are different ($p < 0.05$); [1] FA: 4 mL of formic acid/kg; UM: 19 g of urea and 34 g of molasses/kg; FAUM: formic acid, urea, and molasses at previous concentrations; [2] SEM: standard error of the mean (n = 3).

The chemical composition of the typha used in the present study was similar to that reported by de Evan et al. [4] for samples of *Thypha domingensis* collected in Nigeria and by Wingching-Jones and Leal-Rivera [31] for *Thypha domingensis* collected in Costa Rica. Different factors such as plant maturity, soil characteristics, climate, etc., can influence the chemical composition of typha [4]. As expected, the addition of urea increased the CP content in UM and FAUM silages compared with the pre-ensiling typha (Table 2). The chemical composition of CON and FA silages was similar to that of the pre-ensiling typha, with the exception of EE content, which was numerically lower. A decrease in EE content during the ensiling process has been reported previously, and it was attributed to the activity of lipoxygenases found in the plants [32]. The triticale hay used as a reference in this study had a similar CP content to the pre-ensiling typha, but lower EE, NDF, ADF, and lignin contents.

3.2. In Vitro Ruminal Fermentation of Silages: Ruminal Gas Production Kinetics

The technique of gas production was used as an indicator of the kinetics of the ruminal fermentation of silages, as the amount of gas produced was directly related to the amount of organic matter fermented [33]. The gas production kinetics parameters and gas production curves for all of the tested samples are shown in Table 3 and Figure 1, respectively. Compared with the CON silage, the addition of either formic acid or urea and molasses did not affect the potential gas production (PGP) and the fractional rate of gas production (*c*), but the addition of the three additives in FAUM silage decreased ($p < 0.05$) PGP and increased ($p < 0.05$) *c*. The greater *c* value in the FAUM silage was consistent with the rapid degradation of both urea and molasses [34,35]. Although lower gas production is related to lower degradability, it has to be taken into account that ruminal fermentation of CP produces less gas than that of carbohydrates, and thus high-protein feeds can have lower gas production during in vitro fermentation [35,36]. This could help explain the lower PGP of FAUM silage.

The observed reduction ($p < 0.05$) in Lag (time before fermentation starts) in all of the treated silages compared with the CON silage agreed well with the rapid fermentation of molasses and with the hypothesis that formic acid could accelerate the start of fermentation because of the increased water-soluble carbohydrate content observed previously in the silages treated with this acid [37,38]. Our results agreed well with previous studies, showing improvements in gas kinetics when urea and molasses were added to sugarcane bagasse silages [34] or Leucaena silages [35]. The greater ($p < 0.05$) AGPR observed for all of the treated silages than for the CON silage supported these results, as AGPR is the gas production rate in the initial phases of in vitro fermentation.

Table 3. Parameters of gas production kinetics of typha silages treated with no additive (CON), with formic acid (FA), with urea and molasses (UM), and with formic acid, urea and molasses (FAUM) of typha biomass before ensiling and of the triticale hay used as the reference [1].

Item [2]	Silage				SEM [3]	*p* =	Typha Pre-Ensiling	Triticale Hay
	CON	FA	UM	FAUM				
PGP (mL/g DM)	147 [b]	152 [b]	149 [b]	126 [a]	4.9	0.002	164	206
c (%/h)	4.09 [a]	3.72 [a]	4.21 [ab]	4.94 [b]	0.210	0.002	4.13	3.94
Lag (h)	3.77 [c]	1.81 [a]	2.78 [b]	2.37 [ab]	0.326	0.001	1.33	0.51
AGPR (mL/h)	2.25 [a]	2.60 [b]	3.29 [c]	2.62 [b]	0.066	<0.001	2.35	5.16
PDMD (%)	61.7 [a]	58.2 [a]	77.1 [c]	68.5 [b]	0.90	<0.001	65.2	68.0

[a, b, c] Within each parameter, means in the same row with different superscripts are different ($p < 0.05$); [1] FA: 4 mL of formic acid/kg; UM: 19 g of urea and 34 g of molasses/kg; FAUM: formic acid, urea, and molasses at previous concentrations; [2] PGP: potential gas production; *c*: fractional rate of gas production; *Lag*: time before starting gas production; AGPR: average gas production rate; PDMD: potential dry matter degradability; [3] SEM: standard error of the mean (n = 12).

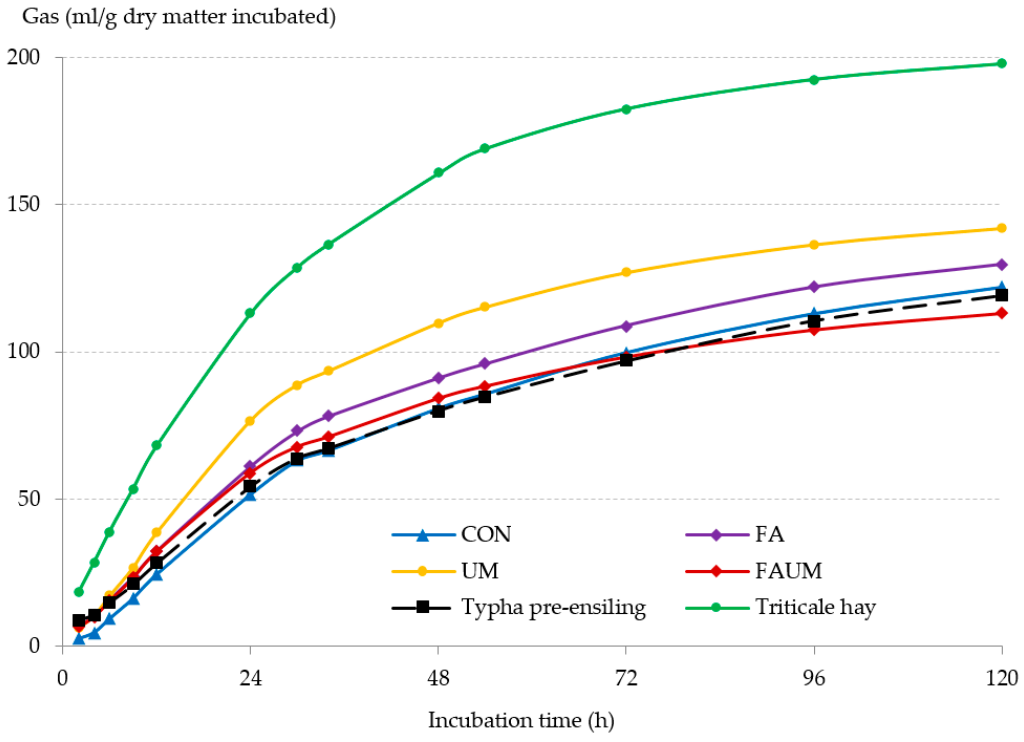

Figure 1. Gas production kinetics of typha silages treated with no additive (CON); with formic acid (FA; 4 mL/kg); with urea and molasses (UM; 19 and 34 g/ kg, respectively); and with formic acid, urea, and molasses (FAUM; 4 mL, 19 g, and 34 g/kg, respectively) of typha biomass before ensiling, and of the triticale hay used as the reference.

The treatment with urea and molasses (UM and FAUM) led to greater PDMD compared with the CON silage, possibly due to an increase in the degradability of the structural carbohydrates, as has been previously reported for silages of other forages [35]. In contrast, the use of formic acid decreased ($p < 0.05$) PDMD, which was difficult to explain, but this effect has also been previously reported by Aksu et al. [39] in maize silage.

During the ensiling period, some fermentable substances were lost, which could explain the PGP reduction observed in the silages compared with the pre-ensiling typha (Table 4). Compared with the triticale hay used as a reference, all typha silages presented lower values of PGP, AGPR, and PDMD, with the exception of UM and FAUM silages, which had a similar PDMD. However, the strong bad odor and dark-brown color of the

UM silage and the high pH of both silages made them an invalid option because a silage with these characteristics should not be used in practical feeding. The lower gas production of the pre-ensiling typha and all typha silages compared with the triticale hay used as the reference for all of the measurement times is shown in Figure 1. The results also indicate that neither CON nor FA silages improved the ruminal degradability of the pre-ensiling typha.

Table 4. Production of volatile fatty acids (VFA) and NH_3-N concentrations after 24 h of in vitro fermentation of typha silages treated with no additive (CON); with formic acid (FA); with urea and molasses (UM); and with formic acid, urea, and molasses (FAUM) and of the typha biomass before ensiling with ruminal fluid from sheep [1].

	Silage Treatment							
Item [2]	**CON**	**FA**	**UM**	**FAUM**	**SEM [2]**	***p* =**	**Typha Pre-Ensiling**	**Triticale Hay**
Total VFA (mmol/g dry matter)	4.33 [a]	4.28 [a]	5.31 [b]	4.65 [a]	0.105	<0.001	4.00	5.74
Molar proportions (mol/100 mol)								
Acetic (Ac)	62.1 [a]	63.9 [b]	65.1 [c]	63.3 [b]	0.28	<0.001	65.9	63.0
Propionic (Pr)	20.0 [a]	22.1 [c]	21.2 [b]	22.5 [c]	0.15	<0.001	18.9	23.2
Butyric	11.7 [c]	8.5 [a]	9.3 [ab]	9.6 [b]	0.23	<0.001	9.1	8.8
Isobutyric	1.84 [b]	1.55 [a]	1.27 [a]	1.40 [a]	0.070	<0.001	1.21	1.25
Isovaleric	2.87 [c]	2.58 [b]	1.87 [a]	2.05 [a]	0.050	<0.001	2.52	1.74
Valeric	1.55 [d]	1.36 [c]	1.09 [a]	1.21 [b]	0.027	<0.001	1.76	2.06
Ac/Pr (mol/mol)	3.12 [a]	2.91 [b]	3.10 [a]	2.83 [b]	0.028	<0.001	3.57	2.74
NH_3-N (mg/L) [3]	257 [a]	251 [a]	314 [b]	359 [c]	2.0	<0.001	246	250

[a, b, c, d] Within each parameter, means in the same row with different superscripts are different ($p < 0.05$); [1] FA: 4 mL of formic acid/kg; UM: 19 g of urea and 34 g of molasses/kg; FAUM: formic acid, urea, and molasses at previous concentrations; [2] SEM: standard error of the mean (n = 12). [3] NH_3-N: ammonia nitrogen.

3.3. In Vitro Ruminal Fermentation of Silages: Fermentative Parameters

After 24 h of in vitro incubation of silages with ruminal fluid from sheep, the total VFA production was greater for UM ($p < 0.05$) than for the other silages (Table 4), which may be due to a greater availability of degradable compounds, such as molasses and urea, for ruminal microorganisms. These results are in good accordance with the greater values of AGPR and PDMD observed for UM silage (Table 2). In agreement with our results, Phesatcha and Wanapat [35] also observed a significant increase in total in vitro VFA production when Leucaena silage was enriched with urea and molasses. Although Zhang et al. [37] observed the same effect when including formic acid in ryegrass silage, no increase in total VFA concentrations was observed by Chamberlain et al. [40] by including formic acid in ryegrass silages, which agreed with our results. The lack of differences in in vitro VFA production between CON and FA silages was consistent with the similar PGP and PDMD values observed for both silages (Table 2).

There were significant differences among silages in all individual VFA. Compared with CON, all of the treated silages had greater ($p < 0.05$) acetic and propionic proportions, but lower ($p < 0.05$) butyric, isobutyric, isovaleric, and valeric proportions. As a consequence of these shifts in VFA profile, the acetate/propionate ratio was lower ($p < 0.05$) for the silages treated with formic acid (FA and FAUM) than for the CON and UM silages. The effects of silage additives on VFA profile in their in vitro fermentation reported in the literature are controversial. Chamberlain et al. [40] and Zhang et al. [37] did not observe significant differences in the ruminal VFA profile when using formic acid as an additive for grass and drooping wild ryegrass silages, respectively. Phesatcha and Wanapat [35] reported no shifts in VFA profile after including molasses and urea in Leucaena silages, except for an increase in propionate proportion, which is in agreement with our results.

The lower proportions of isobutyric, valeric, and isovaleric acids observed for the treated silages compared with the CON silage might be indicative of reduced CP degradation, as these VFA were produced in the degradation of branched-chain amino acids [25], but the NH_3-N concentrations were similar to CON for FA silage and greater for the UM and FAUM silages (Table 4). The greater NH_3-N concentrations observed for UM and FAUM silages agreed well with previous studies using urea as a silage additive. In fact, Yunus et al. [22] and Phesatcha and Wanapat [35] observed increased concentrations of NH_3-N when napiergrass and Leucaena silages, respectively, were treated with urea.

Compared with the pre-ensiling typha, all of the silages had numerically greater VFA production, but it was lower than that of triticale hay. These results are consistent with the lower gas production of the silages and indicate that the silages were fermented in vitro to a lesser extent than the triticale hay. However, the VFA profile of the silages was similar to that of triticale hay, especially for those treated with formic acid (FA and FAUM) that showed comparable acetate/propionate ratios (2.91, 2.83, and 2.74 for FA, FAUM, and triticale hay, respectively).

4. Conclusions

Treating typha with formic acid (4 mL/kg) before ensiling resulted in a well-preserved silage with an adequate pH and low concentrations of propionate and butyrate, but low concentrations of lactic acid. In contrast, the enrichment of typha silages with urea and molasses at the levels used in this study resulted in undesirable fermentations and thus they were not suitable for being used in practice. However, ensiling typha with formic acid did not increase the ruminal degradation of the pre-ensiled plant, which was lower than that of a medium-quality triticale hay. The results indicate that typha silage could replace low-quality forages in ruminant diets, being adequate especially for low-producing animals or for those under maintenance conditions. More studies on increasing the molasses level and decreasing the amount of urea are needed to identify the optimal conditions for ensiling typha and increasing its nutritive value.

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