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Effect of Quebracho Tannin (*Schinopsis quebracho-colorado* (Schltdl.) F.A. Barkley and T. Meyer) on Silage Nutritive Value, Ergovaline Concentration, and Fermentation Parameters of Tall Fescue (*Schedonorus arundinaceus* (Shreb.) Dumort) with Two Dry-Matter Levels

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Abstract: Tall fescue (*Schedonorus arundinaceus* (Shreb.) Dumort) is a cool-season forage grown in the mid-south United States of America that has the potential for spring silage. Ergovaline produced by the fungal endophyte *Neotyphodium coenophialum* is preserved in tall fescue silage and can induce tall fescue toxicosis in livestock. Condensed tannins, such as quebracho (*Schinopsis quebracho-colorado* (Schltdl.) F.A. Barkley and T. Meyer) extract, can bind to the nitrogenous components of ergovaline, rendering it ineffective in the ruminant. Quebracho tannin (QT) can also bind to crude protein, reducing its conversion to ammonia. Quebracho tannin was either not added (0QT) or added at 10 (10QT) or 20 (20QT) g/kg of dry matter (DM) of silage to chopped endophyte-infected tall fescue of 670 g/kg of moisture (high moisture, HM) and 440 g/kg of moisture (low moisture, LM). A moisture × QT interaction affected the final pH (p = 0.02), with the lowest pH observed in HM silages with 0QT and 10QT. The ergovaline concentrations were not significantly different (p = 0.19) among the post-ensiled forages. Ammonia concentrations (g/kg DM) were affected by a moisture × QT interaction (p = 0.05), with greater concentrations observed in HM 0QT compared with HM 10QT, HM 20QT, and LM 20QT. Lactic acid concentrations (g/kg DM) decreased (p = 0.05) with the addition of QT. The moisture × QT interaction (p = 0.02) resulted in higher concentrations of acetic acid in the HM silages with 0QT and 20QT compared to the LM silage with 20QT. The total acid concentrations (g/kg DM) were higher (p < 0.01) at HM but did not differ (p = 0.54) across the QT concentrations. Ensiling tall fescue with quebracho tannin did not reduce the ergovaline concentrations, although proteolysis was reduced at the inclusion of 10 g/kg of QT in the HM silages and 20 g/kg of QT in the LM silages. The results indicate that QT as a tall fescue silage additive showed promise for modifying silage characteristics but it did not reduce ergovaline concentrations at the low QT levels used in this study.

Keywords: tall fescue; quebracho tannin; condensed tannin; tall fescue toxicosis; ammonia; laboratory silo; silage; endophyte

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1. Introduction

Tall fescue (*Schedonorus arundinaceus* (Shreb.)) is a prominent forage in the United States because of its wide range of establishment and adaption, the length of its grazing season, and its resistance to pests and harsh environmental conditions [1]. Tall fescue can be harvested as hay, but frequent spring rains may prevent harvest before excessive maturity.

Harvesting tall fescue as baleage may provide an advantage, as the forage can be baled at moistures higher than hay (<20% moisture), ideally ranging from 45 to 70% moisture [2].

Tall fescue has a symbiotic relationship with the fungal endophyte, *Neotyphodium coenophialum*, which produces ergot alkaloids [3]. Ergot alkaloids are heavily concentrated in the seed with some presence in the leaf and stem tissue [4]. Livestock that consume endophyte-infected fescue show reduced performance and reproductive efficiency [1]. Fresh tall fescue generally contains the highest ergovaline concentrations, whereas tall fescue hay has lower relative concentrations [5]. Tall fescue silage also contains reduced ergovaline compared to fresh or grazed tall fescue [5]; however, Grote et al. [6] observed ergovaline concentrations in silage that were high enough to induce tall fescue toxicosis in sheep (193 ppb).

Some authors have suggested that ergovaline can be bound by condensed tannins (CT), rendering them ineffective in ruminant livestock [7]. Condensed tannins, such as those found in QT (*Schinopsis quebracho-colorado* (Schltdl.) F.A. Barkley and T. Meyer), are polyphenolic compounds synthesized by plants [8]. Condensed tannins are astringent compounds that bind to proteins [9–11] and can also bind to the nitrogenous components of ergot alkaloids [7,12]. Condensed tannins also reduce plant protein degradation into non-protein nitrogen during the ensiling process without affecting the overall fermentation of silages, as measured by pH and lactic acid concentrations averaged across several studies [13].

The objective of this study was to determine the effect of added QT on tall fescue silage's nutritive value, ergovaline concentrations, and fermentation parameters. We hypothesized that the ergovaline concentration and ammonia would be reduced with CT inclusion, whereas high moisture treatments would have higher concentrations of fermentation products.

2. Materials and Methods

2.1. Forage Harvest, Silage Making, and Storage

Tall fescue was harvested at USDA-ARS Dale Bumpers Small Farms Research Center near Booneville, AR (35.1401° N, 93.9216° W), in 2021. In preparation for the study, on 6 April, a 0.4 ha plot of infected tall fescue was mowed, and fresh and dead tall fescue forage material was removed to create a fresh tall fescue stand for the study. The plot was fertilized with 36 kg N from urea on April 21. Vegetative tall fescue was harvested (10 to 15 cm cut length) using a Carter 3-chute forage harvester (Carter Mfg. Co. Inc., Brookston, IN, USA) on 16 June at 1000 h. Forage was spread on concrete and allowed to wilt for either 1 h to 670 g/kg moisture (high moisture (HM)) or 2 h to 440 g/kg moisture (low moisture (LM)). Wilted forage was hand-chopped (<5 cm particle size) with scissors prior to mixing with QT and subsequent packing into polyvinyl chloride (PVC) laboratory silos.

At the time of ensiling, distilled water or the quebracho tannin solution was mixed with the chopped tall fescue (HM and LM) to provide either 0, 10, or 20 g QT/kg forage dry matter (DM). Each combination of moisture and QT treatment had 4 replications, resulting in a total of 24 laboratory silos. Chopped forage was weighed out and QT was added and mixed for each laboratory silo separately prior to packing. Quebracho tannin (QT, Tannin Corporation, Peabody, MA, USA) solutions were made 7 d prior to incorporating with the fescue to allow the QT to completely solubilize. Solutions contained 0.17 and 0.34 g QT/mL of distilled water to achieve QT of 10 and 20 g/kg of silage DM. Chopped forage was weighed to achieve a packing density of 192 kg/m^3 DM, which was spread out in a 61 × 61 × 18 cm plastic container. The QT solution was poured evenly over the chopped forage and then mixed thoroughly by hand. Prior to adding forage to the laboratory silos, a 50 g subsample was taken from each laboratory silo and stored (−20 °C) for pre-ensiled analyses. Forage was then packed into the laboratory silos (10.2 × 29.2 cm). Both ends were capped with a cap PVC fitting (10.2 × 12.7 cm; Fernco—US, Davison, MI, USA) and the top end was fitted with a one-way valve. Laboratory silos were stored at room temperature (23.7 °C) for 60 d.

After 60 d, the laboratory silos were opened and the contents were split into two subsamples. The two post-ensiled subsamples were stored at −20 °C. The pre-ensiled sample and one post-ensiled subsample were lyophilized and then ground through a Wiley mill (Arthur H. Thomas, West Washington Square, PA, USA) to pass through a 1 mm screen for analysis of the ergovaline and nutritive characteristics. A second post-ensiled subsample was analyzed for fermentation parameters at a commercial laboratory.

2.2. Chemical Analysis

Lyophilized pre- and post-ensiled subsamples were used to determine the nutrient content as follows. Ash concentrations were determined by combusting forage samples at 500 °C in a muffle furnace, and the organic matter (OM) was calculated as OM = DM weight − ash weight. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) content in the pre- and post-ensiled forage were analyzed sequentially using a 200 Ankom Fiber analyzer (ANKOM Technology Corporation, Macedon, NY, USA [14]); amylase was included in the NDF procedure and the values were expressed inclusive of the residual ash. The forage lignin content was analyzed using the sulfuric acid method (method 973.18 [15]). Nitrogen was analyzed on the forage samples using the Dumas total-combustion method (Fison's NA2000 Nitrogen/Protein Instrument; method 990.03 [15]). Water-soluble carbohydrates (WSC) were determined from 0.25 g ground subsamples from each pre- and post-ensiled sample with a 2 h extraction in 150 mL of deionized water, followed by gravity filtering through Whatman #1 filter paper. The phenol–sulfuric acid reaction was used to quantify the WSC with a spectrophotometric procedure using dextrose to formulate standards [16]. All laboratory analyses were corrected to a DM basis (method 934.01 [15]).

Lyophilized pre- and post-ensiled subsamples were also used for CT and ergovaline analysis. For CT analyses, purified self-standards were prepared from dried herbage [17]. Biologically active CT was analyzed as protein precipitable phenolics, as reported by Hagerman and Butler [18] and modified by Naumann et al. [19]. Total CT was assayed as described by Terrill et al. [20]. Quantities of total ergovaline (ergovaline + ergovalinine) were determined using high-pressure liquid chromatography (HPLC) with fluorescence detection, as described in Carter et al. [21] with modifications described by Klotz et al. [22]. Briefly, the ground sample was extracted with 80% methanol by mechanical shaking for 2 h. The extract solution was filtered with a solid phase extraction (SPE) C18 column and a 0.2 µm polytetrafluoroethylene filter (PTFE filter) before being injected into HPLC. Separation was achieved using a Kinetex C18 column (Phenomenex Inc., Torrance, CA, USA) 100 mm × 4.6 mm with 2.6 µm particle size at a flow rate of 1.2 mL/min. Elution solutions were (A) 0.1 M ammonium acetate:acetonitrile, 97:3 v/v, and (B) 100% acetonitrile. The linear binary gradient was 22% mobile phase B at the initial time for 0.5 min with a linear change to 35% B during the next 20 min, which was increased to 58% B during the following 8 min, stepped up to 100% B in 0.5 min and maintained for 5 min to wash the column, and decreased to 22% B in 0.5 min and maintained for 5 min prior to the subsequent injection. Ergovaline was detected with excitation at 310 nm and emission at 420 nm.

The fermentation parameters were determined by the Dairy One Forage Testing Laboratory (Ithaca, NY, USA) for the silage fermentation profiles. The forage pH was determined by blending 50 g samples at 2000× g for 2 min in 750 mL of deionized water and then filtering the mixture through cheesecloth. The pH was measured using a Thermo Orion Combination Sure-Flow pH Electrode and a Thermo Orion 410 A meter (Thermo Fisher Scientific, Waltham, MA, USA) that was calibrated with buffers referenced to NIST SRMs: the pH 4 buffer contained potassium hydrogen phthalate and the pH 7 buffer contained sodium phosphate dibasic and potassium phosphate monobasic.

Ammonia-N was analyzed using a peristaltic pump (Timberline Instruments, 1880 S. Flatiron Ct. Suite I, Boulder, CO, USA), which directed the sample, caustic, and absorbing solutions into a diffusion cell [23]. Overall acids were determined by taking an aliquot of extract mixed at a 1:1 ratio with 0.06 M oxalic acid containing 100 ppm trimethylacetic acid (internal standard). Samples were injected into a gas chromatograph (Perkin Elmer Clarus

680) containing a packed column (Supelco) with the following specifications: 2 m × 2 mm Tightspec ID, 4% Carbowax 20M phase on 80/120 Carbopack B-DA. To analyze lactic acid, an aliquot of the extract was used with a biochemistry analyzer (YSI 2950D-1 or 2700 SELECT) equipped with an L-lactate membrane (YSI Incorporated Life Sciences, Yellow Springs, OH, USA).

2.3. Statistical Analysis

The silage chemical composition and fermentation parameters were analyzed using SAS 9.4 PROC GLIMMIX (SAS Inst. Inc., Cary, NC, USA) to test the effects of moisture, QT, and moisture × QT in ANOVA for a 2 × 3 factorial arrangement. The LSMEANS option was used to generate the individual treatment means. The changes in chemical composition that occurred between pre- and post-ensiling were analyzed as stated for the individual components and whether the changes differed from zero using the LSMEANS statement in SAS. Significance was detected at $p \leq 0.05$ and tendencies at $p > 0.05$ and ≤ 0.10. To help explain the significant treatment effects, the main effects and interactions were further separated using pairwise F-protected *t*-tests.

3. Results

3.1. Pre- and Post-Ensiled Forage

The pre- and post-ensiled forage chemical components are reported in Table 1. The amount of total CT in the pure QT was 30%. The resultant condensed tannin concentrations were below the detectable limits in both the pre- and post-ensiled silages. Pre-ensiled NDF concentrations (g/kg DM) tended ($p = 0.07$) to be higher in 10QT compared to 20QT, but there were no significant differences in NDF concentrations between the pre-ensiled fescue without QT and either 10QT ($p = 0.55$) or 20QT ($p = 0.10$). A moisture × QT interaction ($p < 0.01$) was observed for pre-ensiled ADF (g/kg DM) concentrations, which were higher ($p < 0.05$) in LM 10QT than in the other treatments. Hemicellulose (g/kg DM) pre-ensiled concentrations tended ($p = 0.08$) to be higher in the HM treatments than in the LM treatments. A moisture × QT interaction ($p = 0.01$) was observed for pre-ensiled OM (g/kg DM) concentrations, which were found to be greater ($p < 0.05$) in HM 0QT than in HM 20QT and all the LM QT combinations, but were not different ($p = 0.31$) from those found in HM 10QT. Water-soluble carbohydrates (g/kg DM) in the pre-ensiled samples tended ($p = 0.09$) to be higher in the HM than in the LM combinations. Pre-ensiled ergovaline concentrations tended to be higher ($p = 0.06$) in 20T than in 0T.

Table 1. Pre- and post-ensiled forage chemical components of tall fescue ensiled in laboratory silos at different moistures without quebracho tannin (0QT) or with tannin at 10 (10QT) or 20 (20QT) g/kg total silage DM.

	Forage Treatment Combinations [1]									
Item	**High**			**Low**				**Effect *p*-Values**		
Pre-Ensiled [2]	**0QT**	**10QT**	**20QT**	**0QT**	**10QT**	**20QT**	**SEM** [3]	**Moisture**	**Tannin**	**Moisture × Tannin**
NDF, g/kg DM	612	616	608	612	614	598	5.2	0.38	0.07 [4]	0.64
ADF, g/kg DM	339 [b]	337 [b]	339 [b]	339 [b]	355 [a]	334 [b]	3.4	0.17	0.03 [5]	0.01
Hemicellulose, g/kg DM	272	279	268	272	259	265	5.2	0.08	0.58	0.17
ADL, g/kg DM	33	36	35	34	38	35	1.4	0.52	0.13	0.80
OM, g/kg DM	929 [a]	923 [ab]	898 [c]	916 [b]	911 [b]	914 [b]	4.2	0.45	<0.01 [6]	0.01
N, g/kg DM	17	17	16	17	17	16	0.4	0.96	0.13	0.57
WSC, g/kg DM	149	149	121	128	127	119	10.5	0.09	0.16	0.56
Ergovaline, ppb	203	191	229	193	235	233	12.6	0.23	0.06 [7]	0.11
Post-ensiled										

Table 1. *Cont.*

	Forage Treatment Combinations [1]									
Item	**High**			**Low**				**Effect *p*-Values**		
Pre-Ensiled [2]	**0QT**	**10QT**	**20QT**	**0QT**	**10QT**	**20QT**	**SEM [3]**	**Moisture**	**Tannin**	**Moisture × Tannin**
NDF, g/kg DM	616 ^{x}	626 ^{xy}	630 ^{y}	628 ^{xy}	627 ^{xy}	617 ^{x}	5	0.93	0.66	0.07
ADF, g/kg DM	364	364	368	365	367	355	4.3	0.38	0.62	0.15
Hemicellulose, g/kg DM	253 ^{y}	262 ^{x}	262^{x}	263 ^{x}	260 ^{xy}	262 ^{x}	2.8	0.24	0.27	0.09
ADL, g/kg DM	38	39	37	37	42	38	2.5	0.61	0.33	0.69
OM, g/kg DM	918 ^{a}	914 ^{ab}	889^{d}	914 ^{ab}	905 ^{bc}	899 ^{cd}	3.8	0.70	<0.01 [6]	0.05
N, g/kg DM	18 ^{a}	17 ^{b}	17^{b}	17 ^{b}	17 ^{b}	17 ^{b}	0.2	0.23	0.027	0.04
WSC, g/kg DM	67	57	44	88	87	85	6.5	<0.01 [8]	0.14	0.35
Ergovaline, ppb	212	230	236	224	242	201	14.3	0.75	0.38	0.19

[1] Forage treatment combinations included tall fescue ensiled at either high moisture (670 g/kg; HM) or low moisture (440 g/kg; LM) with quebracho tannin at the concentrations specified above. [2] OM = organic matter; NDF = neutral-detergent fiber with inclusive ash; ADF = acid detergent fiber; hemicellulose = NDF − ADF analyzed sequentially; ADL = acid detergent lignin; WSC = water-soluble carbohydrates; DM = dry matter; N = nitrogen. [3] Standard error of the mean. [4] Means for 10QT tended to be higher than 20QT ($p \leq 0.10$). [5] Means for 10QT were higher than 20QT ($p \leq 0.05$). [6] Means for 20QT differed from 0QT and 10QT ($p \leq 0.05$). [7] Means for 0QT were higher than those for 20QT ($p \leq 0.05$). [8] Means for LM were higher than those for HM ($p \leq 0.05$). [9] Means for 10QT were higher than for 0QT ($p \leq 0.05$). ^{a,b,c,d} Within a row, means with different superscripts differ at $p \leq 0.05$. ^{x,y} Within a row, means with different superscripts tended to differ at $p \leq 0.10$.

Tendencies ($p = 0.10$) for moisture × QT interactions were observed for post-ensiled NDF and hemicellulose concentrations. Neutral detergent fiber concentrations tended to differ between HM 0QT and HM 20QT ($p = 0.07$) and HM 20QT and LM 20QT ($p = 0.07$). Hemicellulose concentrations tended to be the lowest ($p = 0.06$) in HM 0QT compared with all other treatments. Moisture × QT interactions ($p \leq 0.05$) were detected for OM and N (g/kg DM) concentrations. Post-ensiled OM (g/kg DM) concentrations were the highest ($p \leq 0.05$) in HM 0QT, HM 10QT, and LM 0QT and the lowest in HM 20QT. Nitrogen concentrations in the post-ensiled forage were the highest ($p = 0.05$) in HM 0QT compared with all other treatment combinations. Post-ensiled WSC concentrations were higher ($p < 0.01$) in LM vs. HM, but were not affected by QT ($p = 0.14$) or the moisture × tannin level interaction ($p = 0.35$). Ergovaline concentrations did not differ among the moisture or tannin treatments ($p \geq 0.19$).

3.2. Changes in Chemical Components

The changes in the forage chemical components between pre-ensiling and post-ensiling are presented in Table 2. The moisture × tannin level interaction did not affect any of the measured changes ($p = 0.26$). The changes in NDF concentrations were significant ($p < 0.05$) in HM 20QT and LM 20QT, but NDF concentrations were not affected ($p = 0.41$) by moisture or QT. ADF Concentrations increased ($p < 0.05$) in all moisture and QT treatments between pre- and post-ensiling, except in LM 10QT, but were not affected ($p \geq 0.15$) by the main effects or their interaction. Hemicellulose concentrations decreased ($p < 0.05$) in HM 0QT and HM 10QT, but not in the other moisture and QT combinations, resulting in a tendency for a greater ($p = 0.06$) decrease in hemicellulose concentrations in HM vs. LM. Changes in ADL concentrations were not affected ($p \geq 0.70$) by moisture, tannin level, or their interaction. Organic matter concentrations decreased ($p < 0.05$) in HM 0QT and LM 20QT, but the changes in the OM concentrations were not affected by moisture, QT, or their interaction ($p \geq 0.26$). Nitrogen concentrations increased ($p < 0.05$) in HM 0QT and HM 20QT, but were not affected by moisture, QT, or their interaction ($p \geq 0.44$). WSC concentrations decreased ($p < 0.05$) in all moisture and tannin treatments during the ensiling process, but to a greater extent ($p < 0.01$) in the HM treatments than the LM treatments, without being impacted by QT or its interaction with WSC ($p = 0.56$). Ergovaline

concentrations increased ($p < 0.03$) in HM 10QT but were not affected by moisture, QT, or their interaction ($p \geq 0.12$).

Table 2. Changes in forage chemical components between pre-ensiling and post-ensiling for tall fescue ensiled in laboratory silos at different moistures without quebracho tannin (QT) or with tannin at 10 (10QT) or 20 (20QT) g/kg total silage DM.

	Forage Treatment Combinations [1]									
	HM			**LM**				**Effect *p*-Values**		
Item [2]	**0QT**	**10QT**	**20QT**	**0QT**	**10QT**	**20QT**	**SEM** [3]	**Moisture**	**Tannin**	**Moisture × Tannin**
NDF, g/kg DM	4.4	10	22.5 *	15.8	13	18.3 *	8.2	0.61	0.41	0.64
ADF, g/kg DM	23.9 *	26.9 *	28.9 *	22.5 *	12	21.2 *	5.7	0.15	0.57	0.39
Hemicellulose, g/kg DM	−19.5 *	−16.9 *	−6.4	−9.3	1	−2.9	6.4	0.06	0.32	0.55
ADL, g/kg DM	5.1	3.3	1.2	2.6	0	3.1	2.9	0.89	0.76	0.70
OM, g/kg DM	−10.7 *	−8	−8.5	−1.9	−6.4	−14.5 *	4.3	0.69	0.46	0.26
N, g/kg DM	1.5 *	0.4	1.1 *	0.6	0.7	0.9	0.4	0.49	0.54	0.44
WSC, g/kg DM	−82.3 *	−92.4 *	−77.0 *	−39.8 *	−40.2 *	−34.7 *	9.65	<0.01	0.56	0.84
Ergovaline, ppb	8.7	39.1 *	7.4	30.5	6.8	−31.8	17.6	0.26	0.12	0.19

[1] Forage treatment combinations included tall fescue ensiled at either high moisture (670 g/kg; HM) or low moisture (440 g/kg; LM) with quebracho tannin at the concentrations specified above. [2] OM = organic matter; NDF = neutral-detergent fiber with inclusive ash; ADF = acid detergent fiber; hemicellulose = NDF − ADF analyzed sequentially; ADL = acid detergent lignin; WSC = water-soluble carbohydrates; DM = dry matter; N = nitrogen. [3] Standard error of the mean. * Means differed from zero ($p \leq 0.05$), indicating that there was a statistical change in concentrations between pre- and post-ensiling.

3.3. Fermentation Parameters

The fermentation parameters are presented in Table 3. A tendency ($p = 0.08$) for a moisture × QT interaction was detected for silage moisture, with higher ($p < 0.001$) moisture in all HM QT combinations compared to LM combinations, whereas LM 10QT and LM20QT showed higher moisture ($p = 0.04$) than LM0QT. Silage moisture was higher ($p < 0.0001$) in HM compared to LM and moisture affected the QT, with higher moisture found in 20QT ($p = 0.01$) compared to 0QT. Moisture × QT interactions ($p < 0.05$) were observed for the silage pH, ammonia (g/kg DM), acetic acid (g/kg DM), and the lactic-to-acetic-acid ratio. The pH in LM 0QT and LM 10QT was higher ($p < 0.02$) than in all the HM combinations. The silage pH in LM 20QT was higher ($p < 0.04$) than in HM 0QT or HM 10QT. Ammonia concentrations were higher in HM 0QT compared with HM 10QT, HM 20QT, and LM 20QT, whereas LM 0QT and LM 10QT showed intermediate concentration levels. Ammonia-N concentrations expressed in g/kg of total N tended ($p = 0.09$) to be higher in HM vs. LM treatments. Lactic acid concentrations were higher ($p = 0.05$) in silages with 0QT compared with those with 10QT and 20QT. Acetic acid concentrations were higher ($p < 0.02$) in HM 0QT and HM 20QT than in LM 20QT (moisture × QT interaction; ($p = 0.02$)), whereas all other treatments showed intermediate concentration levels. The lactic-to-acetic-acid ratio was higher ($p < 0.02$) in LM 0QT compared with LM 10QT and HM 20QT, whereas all other treatments showed intermediate ratios. Tendencies for moisture × QT interactions ($p \leq 0.10$) were observed for propionate and butyrate concentrations. Only HM 10QT had detectable concentrations of propionate, which were higher than in the other treatment combinations. Butyrate concentrations were higher ($p < 0.01$) in HM vs. LM silages and in 20CT compared with 0QT and 10QT. Total silage acids were higher ($p < 0.01$) in HM vs. LM silages.

Table 3. Post-ensiled fermentation parameters of tall fescue ensiled in laboratory silos at different moistures without quebracho tannin (QT) or with tannin at 10 (10QT) or 20 (20QT) g/kg total silage DM.

	Forage Treatment Combinations [1]									
	HM			LM				Effects *p*-Values		
Item [2]	0QT	10QT	20QT	0QT	10QT	20QT	SEM [3]	Moisture	Tannin	Moisture × Tannin
Moisture, %	71.7 ^{x}	72.8 ^{x}	72.2 ^{x}	55.0 ^{z}	58.7 ^{y}	61.1 ^{y}	1.17	<0.01	0.03 [4]	0.08
pH	4.2 ^{c}	4.2 ^{c}	4.4 ^{b}	4.6 ^{a}	4.6 ^{a}	4.5 ^{ab}	0.05	<0.01	0.88	0.02
Ammonia, g/kg DM	8.3 ^{a}	7.0 ^{b}	7.3 ^{b}	7.6 ^{ab}	7.6 ^{ab}	7.1 ^{b}	0.30	0.52	0.03 [5]	0.05
AmmN, g/kg N	67	60	65	60	60	60	2.80	0.09	0.42	0.41
Lactic Acid, g/kg DM	63.8	58.2	59.4	62.8	58.4	55.0	2.40	0.38	0.05 [6]	0.61
Acetic Acid, g/kg DM	6.4 ^{a}	5.2 ^{ab}	6.5 ^{a}	5.1 ^{ab}	6.1 ^{ab}	4.7 ^{b}	0.50	0.07	0.95	0.02
Lactic/Acetic Ratio	10.4 ^{abc}	11.2 ^{abc}	9.1^{c}	12.3 ^{a}	9.6^{bc}	11.7 ^{ab}	0.78	0.14	0.40	0.03
Propionate, g/kg DM	0.0 ^{y}	0.3 ^{x}	0.0 ^{y}	0.0 ^{y}	0.0 ^{y}	0.0 ^{y}	0.10	0.11	0.09 [7]	0.08
Butyrate, g/kg DM	7.2 ^{y}	10.4 ^{y}	15.2 ^{x}	1.0 ^{z}	2.0 ^{z}	3.4 ^{z}	1.20	<0.01	<0.01 [8]	0.10
Total Acids, g/kg DM	78	74	81	68	66	63	2.80	<0.01	0.54	0.17

[1] Forage treatment combinations included tall fescue silage at either high moisture (670 g/kg; HM) or low moisture (440 g/kg; LM) ensiled with quebracho tannin at the concentrations specified above. [2] DM = dry matter, N = nitrogen. [3] Standard error of the mean. [4] Means for 20QT were higher than means for 0QT ($p \leq 0.05$). [5] Means for 0QT differed from those for 10QT and 20QT ($p \leq 0.05$). [6] Means for 0QT were higher than those for 20QT ($p \leq 0.05$). [7] Means for 10QT tended to be higher than those for 0QT and 20QT ($p \leq 0.10$). [8] Means for 20QT tended to be higher than those for 0QT and 10QT ($p \leq 0.05$). ^{a,b,c} Within a row, means with different superscripts differ at $p \leq 0.05$. ^{x,y,z} Within a row, means with different superscripts differ at $p \leq 0.10$.

4. Discussion

4.1. Chemical Components

Many nutritive values were similar regardless of the moisture or QT levels in the pre- and post-ensiled forages. Few differences were observed among the QT treatments due to the low QT inclusion rates of 10 and 20 g/kg of silage DM compared to up to 50 g/kg of silage DM used in other studies that reported measurable effects [24–26]. The overall goal was to identify the lowest concentration of dietary CT that would result in desirable silage fermentation characteristics while also avoiding potential palatability issues once fed to ruminants. The rates for this study were chosen based on previous research related to QT inclusion rates and their corresponding effects on palatability and intake in ruminants. Reduced feed intake has been observed in sheep when the QT inclusion rate was 30 g/kg DM [6]. In addition, the CT concentration of the QT extract was low at 30%. Other reported CT concentrations for QT include a 78% CT rate reported by Norris et al. [27] and a 70% CT rate reported by Benchaar et al. [28], whereas others have reported concentrations as high as 91% CT [29]. The low CT concentration in QT used in our study may have precluded changes in the nutritive values and fermentation during the ensiling process. However, the effect of CT concentration is unknown in other studies that have examined the addition of QT to silage fermentation [24–26] because they did not report the CT concentration of the QT. However, despite the low CT inclusion, the effects of QT were detected in this study.

4.2. Pre- and Post-Ensiled Forage

Both ADF and OM concentrations reflected the moisture and QT interactions in the pre-ensiled samples, whereas the NDF concentration tended to decrease in QT20 compared to QT10 silage. Furthermore, the ergovaline concentration tended to be higher in 20T compared to 0T. These differences did not show a trend and are not readily explainable as the chopped tall fescue was adequately mixed prior to ensiling. Both hemicellulose g/kg DM and WSC g/kg DM concentrations tended to be higher in the HM treatments compared to the LM treatments. The forage utilized for the LM treatments had a longer wilting time compared to that used in the HM treatments; during this time, WSC could have been lost to respiration. However, the trend for higher hemicellulose concentrations in HM was unexpected, as hemicellulose was not lost during respiration.

In the post-ensiled samples, the interaction between moisture and QT treatments persisted for OM concentrations, but there was also a strong negative effect of QT addition, with lower OM concentrations observed at greater levels of QT addition. Interactions were also shown to be significant for N concentrations, and trends were detected for NDF and hemicellulose concentrations, which are better explained by the changes in these variables (between pre- and post-ensiled) that are discussed in the following section. Higher WSC concentrations were observed in the LM silage compared to the HM silage. Water-soluble carbohydrates are the primary substrate of fermentation [30] and have a negative relationship with silage moisture [31]. Ergovaline concentrations did not decrease with QT inclusion as we had hypothesized, with higher ergovaline concentrations in 20QT compared to 0QT in the pre-ensiled forage and no reductions observed in the post-ensiled forage, indicating that the nitrogenous components of ergovaline were not bound by QT.

4.3. Changes in Forage Chemical Components

Neutral-detergent fiber concentrations increased in the post-ensiled samples, particularly for HM 20QT and LM 20QT, where the concentrations increased by 22.5 and 18.3 g/kg DM in HM 20QT and LM 20QT, respectively. Acid detergent fiber concentrations increased in all treatment combinations, except for 10QT. Hemicellulose concentrations declined with QT additions across nearly all treatments, with a tendency for greater loss at HM, indicating increased fermentation at HM [32]. Previous research reported decreases in NDF concentrations and increases in ADF concentrations in silages, as hemicellulose was fermented during the ensiling process [31,33]. Acid detergent lignin concentrations did not change regardless of silage moisture. Nieman et al. [31] observed a greater reduction in ADL concentrations in baleage of 598 g/kg moisture, but not in baleage of 520 g/kg moisture, whereas Coblentz et al. [33] did not observe higher ADL concentrations in baleage of 361 g/kg of moisture compared to baleage of 457 g/kg of moisture. Few changes were observed in OM concentrations, with reductions observed only in HM 0QT and LM 20QT, but a general tendency for reduced concentrations was observed across all treatment combinations. Nitrogen concentrations also showed few changes and were higher in HM 0QT and HM 20QT compared with the other treatments. Higher reductions in WSC were observed in the HM treatments, which were consistent with the moisture effects observed in the post-ensiled forage.

No reductions in ergovaline concentrations were observed in any of the treatments compared to the control (0QT), and the only difference was an increase in ergovaline in HM 10QT. If the nitrogenous components of ergovaline were bound to CT, the bound ergovaline would not be detectable, resulting in reduced ergovaline concentrations. However, cautious interpretation is necessary, as it is unknown if the CT in QT would be bound to ergovaline at the pH levels observed in these silages. For example, in vitro studies showed that sainfoin (*Onobrychis viciifolia* Scop.) CT and protein complexes were stable between pH 3.5 and 7.0, whereas in vivo studies showed that these complexes have low binding affinity at pH values below 3.0, as observed in the anterior duodenum of sheep [34]. The binding affinity of CT in QT was found to be stable between a pH of 5.0 and 7.0 in gelatin [35]. The pH of post-ensiled forage in this study ranged from 4.2 to 4.6. If CT in QT was binding to ergovaline, it should have been observed in the pre-ensiled samples. Although the forage pH was not measured in the pre-ensiled samples, the pH has been observed to be around 6.32–6.41 in fresh alfalfa (*Medicago sativa* L.)-orchardgrass (*Dactylis glomerata* L.) mixtures [36] and 5.54, 5.60, and 5.51 in fresh-meadow fescue (*Schedonorus pratensis* (Huds.) P. Beauv), orchardgrass, and tall fescue [37], respectively.

Low CT and moderate ergovaline concentrations in the silages may have been too low to detect consistent differences in ergovaline concentrations. Tall fescue (vegetative) ergovaline concentrations averaged 213 ppb. Vegetative or boot-stage ergovaline levels were lower than in tall fescue at the mature seed-head stage (200–308 ppb reported by Turner et al. [38]). Post-ensiled ergovaline concentrations did not decrease across all treatments. Roberts et al. [5] reported a 60% reduction in ergovaline concentrations in spring

silages of 530 g/kg of moisture, whereas Turner et al. [38] reported no differences in ergovaline concentrations in endophyte-infected tall fescue silage that ranged from 701–837 g/kg of moisture. There was no difference between moistures despite the possibility of ergovaline reduction due to less light, heat, and oxygen exposure with shorter wilting time in HM [39,40].

4.4. Fermentation Parameters

The silage moisture ranged from 722 to 550 g/kg of moisture, which is an acceptable range for silage fermentation for which Shinners [2] recommends ranges of 450–700 g/kg of moisture. The moisture concentration affects both the rate and extent of silage fermentation, as microbial populations require moisture to carry out fermentation [41]. Higher concentrations of moisture result in higher organic acid production and lower pH [41]. The moisture was higher in the HM treatments, as designed, and ranged from 717 to 728 g/kg of moisture. However, in the LM treatments, it was lower and more variable, ranging from 550 to 611 g/kg of moisture. This variation was caused by inconsistent drying during wilting, although the forage was consistently raked during wilting to maintain similar forage moisture concentrations. Despite this variation, the differences due to forage moisture concentrations were still detected. The silage pH ranged from 4.2 to 4.6, indicating adequate fermentation across all treatments. Kung et al. [42] suggested that for grass silages with moisture between 75 and 65%, normal pH values should be 4.3–4.7. Quebracho tannin did not affect pH, which is consistent with the results of a meta-analysis on the effects of CT on silage fermentation [13].

The inclusion of CT reduced ammonia concentrations. High moisture silage ammonia was reduced with 10QT while LM silages were reduced with 20QT compared to control silages with no QT. The lower requirement for QT at HM was likely due to the higher production of ammonia in the HM treatments. High-moisture silages showed higher levels of microbial activity, resulting in higher ammonia concentrations from protein degradation [42,43]. However, the inclusion of CT can reduce proteolysis by binding to N and creating a compound resistant to degradation [44]. This interaction prevents the loss of forage crude protein through ammonia volatilization and provides livestock with crude protein rather than non-protein N.

Of the volatile fatty acids measured, acetic acid concentrations were generally higher at HM, but the effects of QT were inconclusive. Both lactic acid and butyrate concentrations were affected by QT, where lactic acid decreased with QT inclusions and butyrate increased. Salawu et al. [24] observed reduced lactic acid concentrations in perennial ryegrass silages with QT addition at both 5 and 50 g/kg DM (27.5 g/kg of lactic acid for the control, and 1.5 and 5.9 g/kg of lactic acid for additions of 5 and 50 g/kg of QT, respectively). Salawu et al. [24] also observed increased acetic acid (11.2 g/kg of acetic acid for the control, and 46.9 and 30.6 g/kg of acetic acid for additions of 5 g/kg and 50 g/kg of QT, respectively) and reduced butyrate concentrations (34.9 g/kg butyrate for the control, 17.2 and 4.2 g/kg of butyrate for additions of 5, and 50 g/kg of QT, respectively) with QT inclusion. In a meta-analysis, Jayanegara et al. [13] did not observe differences in lactic or acetic acid concentrations with the addition of CT but did observe a reduction in butyrate concentrations. Propionate levels were low across all treatments and propionate is generally undetectable or less than 0.1 g/kg in silages [42]. As expected, the total acid concentrations were higher in the HM treatments (77.9 g/kg D) compared to the LM treatments (66.2 g/kg DM), as acid production generally increases with silage moisture [41], although no effect of QT was observed for the total acid concentrations. Lactic acid concentrations decreased with the addition of QT, but these decreases were compensated for by the increased butyrate concentrations.

5. Conclusions

Tall fescue ensiled with QT at concentrations of 10 and 20 g/kg of silage DM showed few effects on the nutritive and fermentation parameters. Ergovaline concentrations were slightly increased or remained the same after fermentation regardless of moisture levels

or quebracho tannin concentrations. Moisture drove several fermentation parameters, including higher acid production in the high-moisture silage. At low levels of QT in the silages, the effects of QT on ensiling were limited or inconsistent. Tall fescue ensiled with 20 g QT/kg of silage DM reduced proteolysis regardless of moisture concentrations, without affecting the other fermentation parameters. In general, the addition of QT at 10 and 20 g/kg of DM did not reduce ergovaline concentrations. The issue of condensed tannins binding to ergovaline during ensiling requires further examination, with a particular focus on the silage pH, as well as the condensed tannin and ergovaline concentrations, which can enhance the neutralization of ergovaline without causing palatability issues as a result of the addition of condensed tannins.

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