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# Application of poultry litter and moisture effects on rye–ryegrass–fescue baleage

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**Abstract**

Subsurface poultry litter application improves nutrient utilization compared with surface application through reduced nutrient runoff, and increased forage nutritive value. This study compared the effects of subsurface (SUB) or surface (SURF) poultry litter application against controls (CON; no litter) for baled silages made at high moisture (HM) or low moisture at (LM) Booneville, AR, in 2018. Mixtures of cereal rye (*Secale cereale* L.), annual ryegrass (*Lolium multiflorum* Lam.), and tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] baled silages were compared for nutritive value and silage fermentation characteristics. Data were analyzed as a split-plot design with three fertilizer treatments as whole plots, and initial bale moisture as subplots. Pre-ensiled P and K were greater ($P < .05$) for the mean of SURF and SUB than for CON. Neutral detergent fiber was greater ($P = .02$) in CON versus the mean of SURF and SUB. In post-ensiled bales, propionic ($P < .05$) and succinic acid ($P < .05$) concentrations were greater in the mean of SURF and SUB than in CON, and greater in SUB than in SURF. Moisture had positive ($P < 0.05$) relationships with fermentation acids, and negative relationships with pH, water-soluble carbohydrates, and starch. Overall, very few differences between SUB and SURF were detected, baleage nutritive value was not improved with SUB poultry litter application, and fermentation characteristics were most affected by the initial bale moisture.

## 1 | INTRODUCTION

Poultry litter is a common fertilizer for pasture and forage production systems in the southeastern United States. However, the practice of surface-spreading or broadcasting poultry litter allows nutrient loss during and after application. For example, N is lost through ammonia volatilization (Moore et al., 1996; Pote & Meisinger, 2014), and other nutrients are lost after heavy rainfall via surface runoff (Pote et al., 2003; Sharpley, 1997). To minimize these environmental effects, poultry litter can be incorporated into the soil or applied below the soil surface. Subsurface application of poultry litter is an effective technique for reducing volatilization (Pote & Meisinger, 2014; Pote et al., 2011) and losses from runoff (Pote et al., 2003, 2011; Watts et al., 2011) while enhancing plant growth and minimizing disturbance of plants and soil structure (Pote et al., 2011). Because of the greater retention of nutrients, subsurface application has also been observed to increase the yield of pasture forages (Pote et al., 2003, 2011, 2012)

**Abbreviations:** ADF, acid detergent fiber; ADICP, acid detergent-insoluble crude protein; ADL, acid detergent lignin; CON, no litter control; CP, crude protein; DM, dry matter; HM, high moisture; LM, low moisture; NDF, neutral detergent fiber; NDICP, neutral detergent-insoluble crude protein; SUB, subsurface application treatment; SURF, surface application treatment; WSC, water-soluble carbohydrates.

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**TABLE A** Useful conversions

| To convert Column 1 to Column 2, multiply by | Column 1 suggested unit | Column 2 SI unit |
|---|---|---|
| **0.304** | **ft** | **m** |
| 2.54 | inch | cm |
| 25.4 | inch | mm |
| 0.405 | acre | ha |
| 28.4 | oz | g |
| 454 | lb | g |
| 0.454 | lb | kg |
| 1.12 | lb acre$^{-1}$ | kg ha$^{-1}$ |
| 16.02 | lb ft$^{-1}$ | kg m$^{-3}$ |
| 2.24 | ton acre$^{-1}$ | Mg ha$^{-1}$ |
| 5/9 (˚F – 32) | ˚F | ˚C |

and increase N concentrations in bermudagrass [*Cynodon dactylon* (L.) Pers.] (Pote et al., 2012) and orchardgrass (*Dactylis glomerata* L.) (Kulesza et al., 2016).

Poultry litter application, particularly subsurface-applied poultry litter, has been observed to increase the N concentrations of crops; however, N application has also been observed to reduce plant water-soluble carbohydrates (WSC) concentrations, which are important for organic acid production (Coblentz et al., 2014; Lechtenburg et al., 1972; Muck et al., 2003) in silages. Coblentz et al. (2014) observed a linear decline in WSC for oat (*Avena sativa* L.) with an increased urea fertilization rate. Additionally, both the rate and extent of silage fermentation are affected by dry matter (DM) concentration (Muck & Kung, 2007). Microbial populations require moisture to carry out fermentation; greater DM concentrations result in a less acidic pH and decreased organic acids, thereby requiring more time to reach anaerobic stability (Muck & Kung, 2007). These relationships are well described by regression analyses that demonstrate increased lactic acid and total acids with increasing initial bale moisture (Coblentz & Akins, 2019).

This project aimed to integrate the two technologies, linking subsurface poultry litter application with baled silages at high and low moisture levels. We aimed to determine if subsurface application of poultry litter will result in greater nutritive value than forage amended with SURF applied poultry litter, and, if so, what moisture was best for preserving these nutrients in baled silages. Our hypothesis was that rye (*Secale cereale* L.)–ryegrass (*Lolium multiflorum* Lam.)–tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] plots receiving subsurface application of poultry litter would produce forage with a greater nutritive value than plots receiving surface applications of the poultry litter or no litter. Furthermore, all plots amended with poultry litter should exhibit acceptable fermentation in baled silages.

**Core Ideas**

- Application of poultry litter increased P and K compared with forages receiving no litter.
- Subsurface poultry litter application did not increase the forage nutritive value.
- Fermentation characteristics were most influenced by initial bale moisture.

## 2 | METHODS

### 2.1 | Description of field site and experimental layout

A 15.5-acre field site on the USDA-ARS Dale Bumpers Small Farms Research Center, located near Booneville, AR (35.1401˚N, 93.9216˚W) was selected for the experiment. The base forage of this location is a mixed tall fescue–bermudagrass pasture that naturally transitions from tall fescue in the spring to bermudagrass in the summer and back to tall fescue in the fall. The soil type is a Leadvale silt loam (fine-silty, siliceous, semiactive, thermic Typic Fragiudults). Pretest soil fertility analyses of the nutrient availability concentrations indicated P, K, Ca, Mg, and $SO_4$–S at 22, 58, 1503, 95, and 8 parts per million, respectively; the soil pH was 6.3 (University of Arkansas Diagnostic Laboratory, Fayetteville, AR). The site was divided into three experimental blocks based on the slope location. All blocks were the same size (6.0 acre), and blocks were separated by a 50-ft buffer to prevent any potential leaching of nutrients down the slope, thereby contaminating the next field block. Within each block, three adjacent rectangular 2.0-acre plots (433 by 200 ft) were arranged in a northeast to southwest orientation. Plots were allocated randomly to one of three treatments within each block.

### 2.2 | Application of poultry litter and planting

On 26 Oct 2017, 2.0 tons acre$^{-1}$ of poultry litter was applied with a litter spreader (30.0-ft broadcast width; Salford) to one plot in each of the three blocks as the surface-applied poultry litter treatment (SURF); this represents the conventional application method for poultry litter. The spreader kept 30.0 ft from the plot borders to ensure that litter was contained within the experimental unit. Some minimal application overlap occurred. Likewise, 2.0 tons acre$^{-1}$ of poultry litter was applied on one plot in each block via a mechanized subsurface poultry litter application method (internal auger system)

(SUB). The equipment used was a tractor-drawn prototype and was described further by Pote et al. (2011). The application bands were 2.0 inches wide and 3 inches deep, with eight bands per spreader pass, configured with 15-inch band spacings. The litter nutrient analysis was 3.99, 3.07, and 3.34% N, $P_2O_5$, and $K_2O$, respectively (University of Arkansas Diagnostic Laboratory, Fayetteville, AR USA). Briefly, the subsurface applicator was designed with four trench openers with a fluted coulter to slice the soil. Each coulter was followed by a double-disk opener, which formed the trench. Following the deposition of litter in the trench, the injected litter was covered with soil. This no-till band technique minimizes soil disturbance and has the added benefit of pulverizing the poultry litter, thus precluding the need for pre-grinding the litter (Pote et al., 2011). The final three plots (one per block) were control (CON) plots that did not receive poultry litter or commercial fertilizer.

On 30 Oct. 2017, 'Elbon' cereal rye at 100 lb acre$^{-1}$ and 'Marshall' annual ryegrass at 20 lb acre$^{-1}$ were drilled into a tall fescue sod with a John Deere Model 1590 no-till drill (Deere and Co.) configured with 7.5-inch row spacings. Tall fescue remaining after grazing in September was clipped prior to planting; tall fescue was not chemically suppressed. In SUB plots, planting rows were offset from the litter rows to ensure that annuals were not planted directly into poultry litter bands to prevent the possibility of hindered germination and/or early growth of the seeded forages (Lin et al., 2017).

## 2.3 | Harvest

At 1400 h on 9 Apr. 2018, each of the nine plots was mown and rolled-conditioned in swaths with a pull-type New Holland 7230 mower–conditioner (CNH America) to a 6-inch stubble height. The maximum cutting width of the mower–conditioner was 10 ft, and the swath width after conditioning was 5.5 ft. Prior to mowing, four 0.82-ft$^2$ quadrats were placed randomly in each plot and cut to a 2-inch stubble height for later separation of each quadrat into percentages of cereal rye, annual ryegrass, and tall fescue. Clipped forages were separated by hand and dried for 48 h under forced air at 131 ˚F. The percentages of cereal rye, annual ryegrass plus tall fescue, and other (spring annuals and broadleaf weeds) were calculated on a DM basis. Annual ryegrass and tall fescue were combined into one category, as annual ryegrass and tall fescue plants were difficult to distinguish after cutting.

At 1000 h on 10 Apr. 2018, two adjacent swaths were merged, and baled at 1130 h into 4.0- by 4.0-ft round bales (1,455 ± 154 lb, wet basis) with a John Deere 469 Silage Special baler. In total, 26 bales were produced. Fourteen bales were baled and wrapped within 1 h to create a high-moisture (HM) grouping (overall mean = 59.5% moisture). The remainder of the bales (12 bales) were baled 3 h later (1430 h), wrapped within 1 h, and designated as the low-moisture (LM) bale grouping (overall mean = 52.3% moisture); therefore, the LM grouping was close to the ideal range (45–55% moisture) for baled silages suggested by Shinners (2003). The SUB treatment produced seven HM bales and six LM bales. The SURF treatment produced five HM bales and four LM bales. The CON treatment produced only two HM bales and two LM bales. All forage from the plots was baled; however, bales that did not meet a threshold diameter of 3.2 ft were ejected from the baler and weighed to determine plot yield but were not sampled for forage analysis. Dry matter concentrations from bales harvested from the same moisture treatment and from the same plot were used to calculate the DM yield from incomplete bales. All bales meeting the 3.2-ft diameter threshold were tagged, measured, weighed, and core sampled prior to wrapping. Six core samples (1.0 inches in diameter and 2.0 ft deep) were obtained with a Uni-Forage Sampler (Star Quality Samplers) at evenly spaced areas from the round side of the bale, such that all of the round side of the bale were sampled. Air was displaced from the sample holes with painters' drop cloth packed with a wooden dowel. All core samples were composited by bale and sealed in plastic freezer bags, and placed on ice in insulated coolers, then taken to the research laboratory for processing.

Each bale was wrapped with 29.5-inch by 4,921-ft Sunfilm Silage Wrap (AEP Industries Inc.) (thickness = 25 μm) by a Model SBW8500 single-bale wrapper (Vermeer Corporation) programmed to apply eight plastic layers (32 table revolutions) to each bale. After wrapping, the bales were stored on a gravel pad until the final post-ensiled sampling date on 9 Oct. 2018.

## 2.4 | Post-ensiled sampling procedures

After a storage period of 183 d, nine evenly spaced cores were taken from the entire circumference of the rounded side of each bale using a 2.0-ft probe. All samples were collected, composited by bale, placed in freezer bags, and stored on ice in insulated coolers until transport to the laboratory for processing and laboratory analysis.

## 2.5 | Laboratory procedures

For pre-ensiled samples, one 0.88-oz subsample was taken from the freezer bags after thorough mixing. The subsample was dried for 48 h at 221 ˚F to determine the initial moisture concentration of each bale. The remaining sample (10–15 oz) was stored in an ultralow freezer (−176 ˚F) pending subsequent lyophilization and then ground through a Model 4 Wiley Mill equipped with a 1-mm (0.04-inch) screen (Thomas Scientific). Water-soluble carbohydrates, starch, Ca, P, K, Mg, K,

and S were determined in a commercial lab (Rock River Laboratory) by the following methods. Water-soluble carbohydrates were analyzed following Dubois et al. (1956) and starch was determined following Hall (2008). For mineral concentrations, the samples were digested at 248 ˚F in acid to extract minerals, then analyzed via inductively coupled plasma - optical emission spectrometry.

For post-ensiled samples, one 0.88-oz subsample was taken from each bale and dried for 48 h at 221 ˚F to determine the final moisture concentration of each silage. The remainder of the sample was placed in an ultralow freezer at −176 ˚F. The following week, 7.0 oz of the thoroughly mixed post-ensiled sample was shipped to a commercial lab for determination of the pH and fermentation products by the following methods. For pH, forage samples were mixed with deionized water and read with a combination pH electrode in the commercial laboratory. Volatile fatty acids were extracted from the sample in a 1:10 ratio of the sample to deionized water and centrifuged, then the supernatant was combined with calcium hydroxide and copper sulfate and centrifuged again, and the supernatant analyzed by high performance liquid chromatography equipped with a reverse-phase ion exclusion column and a refractive index detector (Waters Corporation). For $NH_3$–N, the same supernatant was produced as in the volatile fatty acids procedure and was analyzed with a Skalar San++ Segmented Flow SA 5000 Analyzer (Skalar) on the basis of the Berthelot reaction (Krom, 1980). Another 3.5 oz of the sample was lyophilized, ground as described for pre-ensiled samples, and sent to the lab for analysis following the same methods listed above for WSC and starch determination.

The fiber composition of the dried ground forage samples was analyzed sequentially for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) via the batch procedures outlined by the manufacturer for an Ankom200 Fiber Analyzer (Ankom Technology). The concentrations of total N within each sample were determined via a rapid combustion procedure (Association of Official Analytical Chemists, 2005), followed by conversion to crude protein (CP) with a factor of 6.25. Neutral and acid detergent-insoluble CP (NDICP and ADICP, respectively) were determined through a nonsequential NDF and ADF procedure that excluded sodium sulfite and heat-stable α-amylase; residual CP was determined by the combustion procedure described previously.

## 2.6 | Statistical analyses

All analyses of the initial and final bale characteristics and the pre- and post-ensiled nutritive value were analyzed by PROC GLIMMIX in SAS Version 9.4 (SAS Institute Inc.); the experimental design was a randomized complete block with three field blocks, based on field slope. Data were analyzed as a split-plot design with three amendment treatments (SURF, SUB, and CON) designated as whole plots and the initial bale moisture (HM or LM) as subplots. Fertilizer treatments and baling moisture were evaluated as fixed effects, whereas block and block × treatment were random. Bales within a plot were averaged prior to analysis. The model was run with the interactions first, and no significant interactions were detected. For ease of interpretation, logical contrast statements generated by LSMestimate in PROC GLIMMIX were used to compare least square means for fertilizer treatment and moisture: (a) CON vs. SURF + SUB, (b) SURF vs. SUB, and (c) HM vs. LM. Logical contrasts were only included in tables if the main effect was significant ($P \leq .05$); if the main effect was not significant, the analysis of that variable was stopped. Throughout the experiment, significance was declared at $P \leq .05$. Silage fermentation products and final pH were regressed on the initial bale moisture across the poultry litter amendment and initial moisture treatments. Responses over the range of initial bale moisture were evaluated for linear, quadratic, and cubic trends with PROC REG in SAS. The most appropriate polynomial model was identified on the basis of a significant ($P \leq .05$) coefficient for each polynomial order, coupled with the greatest possible coefficient of determination ($R^2$).

**TABLE 1** Yield components of forage following no application of poultry litter (CON), or poultry litter applied by surface (SURF) and subsurface (SUB) methods at Booneville, AR, in 2018. Values are the means of three replicates

| Treatment | Yield | Cereal rye | TF + RG | Other |
|---|---|---|---|---|
| | DM lb acre$^{-1}$ | | % | |
| CON | 890 | 35.5 | 45.8 | 18.7 |
| SURF | 1,340 | 55.5 | 42.3 | 2.2 |
| SUB | 1,695 | 74.1 | 18.0 | 7. 9 |
| SD | 390.0 | 34.16 | 37.62 | 16.70 |

*Note*. TF, tall fescue; RG, annual ryegrass; Other, spring weeds; DM, dry matter.

# 3 | RESULTS AND DISCUSSION

## 3.1 | Field data

Means and standard deviations for yields and botanical composition are presented in Table 1. Yields were numerically greatest in SUB, followed by SURF and CON. Interpretation of these differences requires caution, as these data are only from one harvest in one year. However, the yield data are presented here because of their relationship with botanical composition. There was large variation in botanical composition within treatments: The contribution from cereal rye in the mixture was numerically greater in SUB, followed by SURF and CON. The differences in botanical composition were probably caused by differences in cereal rye yield, as other

**TABLE 2** Initial characteristics of wrapped silage bales following no application of poultry litter (CON), or poultry litter applied by surface (SURF) and subsurface (SUB) methods, with all treatments baled at high (HM) or low (LM) moisture at Booneville, AR, in 2018

| Treatment | Bale moisture | Wet weight | Dry weight | Density | Bale height | Volume |
|---|---|---|---|---|---|---|
| | % | lb | | lb of DM[a] $ft^{-3}$ | ft | $ft^3$ |
| Application | | | | | | |
| CON | 47.7 | 1,344 | 684 | 13.2 | 4.10 | 51.91 |
| SURF | 57.9 | 1,470 | 609 | 11.9 | 4.07 | 51.20 |
| SUB | 62.0 | 1,529 | 568 | 11.4 | 4.03 | 50.85 |
| SEM[b] | 3.06 | 1,18.0 | 43.8 | 0.26 | 0.046 | 1.306 |
| Moisture | | | | | | |
| HM | 59.8 | 1,562 | 598 | 11.8 | 4.07 | 51.20 |
| LM | 52.0 | 1,333 | 642 | 12.5 | 4.07 | 51.55 |
| SEM | 2.45 | 108.0 | 25.3 | 0.47 | 0.030 | 0.706 |
| Contrasts[c] | | | | | | |
| | *P*> *t* | | | | | |
| CON vs. SURF + SUB | .013 | – | – | – | – | – |
| SURF vs. SUB | .243 | – | – | – | – | – |
| HM vs. LM | .006 | .016 | <.001 | – | – | – |

[a]DM, dry matter.
[b]SEM, pooled standard error of the mean.
[c]Contrasts: (a) CON vs. SURF + SUB = control vs. the mean of surface and subsurface applications; (b) SURF vs. SUB = surface vs. subsurface; (c) HM vs. LM = high moisture vs. low moisture. *P*-values for logical contrasts are only included in the table if the main effect was significant ($P \leq .05$); if the main effect was not significant, the analysis of that variable was stopped and no *P*-values are shown.

contributors, such as annual ryegrass (Smith & Kallenbach, 2006) and tall fescue (Islam et al., 2011) peak in growth later in spring than cereal rye. Yields are also discussed because of the associated effects on drying time. Initial bale moisture was difficult to control in this experiment because of the forage response to poultry litter amendment, with yields increasing from CON to SURF to SUB (Table 1). These yield differences probably contributed to moisture differences in the pre-ensiled bales because baling of the HL and LM treatments was based on time, rather than by measured moisture.

## 3.2 | Pre-ensiled bale characteristics

Lower-yielding plots dried faster than higher-yielding plots, resulting in the significant treatment differences for moisture; SURF + SUB was wetter at baling than CON forages (60.0 vs. 47.7%), but SURF and SUB did not differ in moisture (Table 2). High-moisture bales had greater wet weights, and LM bales had greater dry weights. These differences are expected for bales over the generally recommended moisture range for this conservation technique (45–55%)(Shinners, 2003); similar trends have been observed in other studies (Coblentz & Akins, 2019). However, differences in dry weights did not directly translate into differences in bale density, which has also been observed previously (Coblentz & Akins, 2019). No application or moisture effects were observed for bale density (Table 2). Coblentz and Akins (2019) observed that bales at ideal moisture (51.1%) were the densest, compared with bales of 67.5, 61.5, and 45.8% moisture. All bales in this study met the minimum recommendation for bale density of 10.1 lb DM $ft^{-3}$ (Jennings, 2011).

## 3.3 | Nutritive value

### 3.3.1 | Pre-ensiled bales

The nutritive value variables for pre-ensiled bales are presented in Table 3. Both P and K concentrations were greater in SURF + SUB than in CON. Phosphorus was also greater in HM than in LM (Table 3). No differences were observed for Ca, Mg, or S concentrations. Neutral detergent fiber was greater in CON than in SURF + SUB; however, there was no difference between SURF and SUB, and NDF was greater in LM than in HM. Concentrations of NDICP were greater in LM than in HM (Table 3). No differences were observed for application method or moisture for ADF, ADICP, ADL, starch, and WSC.

Greater concentrations of P and K in SURF + SUB than in the CON (Table 3), were expected, as CON received no fertilizer and the initial soil P and K concentrations were low. No differences in mineral concentration were observed between SURF and SUB, despite subsurface applications having been documented as reducing nutrient loss (Pote & Meisinger, 2014). Warren et al. (2008) and Pote et al. (2003, 2012) did

**TABLE 3** Pre-ensiled concentrations of selected carbohydrates and minerals, crude protein (CP), and fiber-bound CP fractions of fiber components of wrapped silage bales following no application of poultry litter (CON), or poultry litter applied by surface (SURF) or subsurface (SUB) methods, with all treatments baled at high (HM) or low (LM) moisture at Booneville, AR, in 2018

| Treatment | P | K | Ca | Mg | S | CP | NDF | ADF | ADL | NDICP | ADICP | Starch | WSC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % of DM | | | | | | | | | % of CP | | % of DM | |
| Application | | | | | | | | | | | | | |
| CON | 0.27 | 1.4 | 0.46 | 0.13 | 0.15 | 12.3 | 53.5 | 28.3 | 5.0 | 6.91 | 3.4 | 5.0 | 11.9 |
| SURF | 0.34 | 1.86 | 0.41 | 0.14 | 0.16 | 13.4 | 50.9 | 26.1 | 4.3 | 7.25 | 3.8 | 5.3 | 12.9 |
| SUB | 0.34 | 1.99 | 0.41 | 0.13 | 0.15 | 13.4 | 49.4 | 25.6 | 3.4 | 6.56 | 3.05 | 5.5 | 12.8 |
| SEM | 0.016 | 0.153 | 0.029 | 0.004 | 0.005 | 0.29 | 1.01 | 0.78 | 0.51 | 0.333 | 0.305 | 0.46 | 0.98 |
| Moisture | | | | | | | | | | | | | |
| HM | 0.33 | 1.82 | 0.42 | 0.13 | 0.15 | 12.89 | 50.59 | 26.41 | 3.75 | 6.55 | 3.21 | 5.36 | 11.6 |
| LM | 0.30 | 1.68 | 0.43 | 0.13 | 0.15 | 13.12 | 51.92 | 26.94 | 4.68 | 7.26 | 3.62 | 5.22 | 13.4 |
| SEM | 0.014 | 0.141 | 0.026 | 0.003 | 0.003 | 0.180 | 0.833 | 0.479 | 0.409 | 0.201 | 0.218 | 0.428 | 0.70 |
| Contrasts[a] | | | | | | | | | | | | | |
| | $P > t$ | | | | | | | | | | | | |
| CON vs. SURF + SUB | .003 | .006 | – | – | – | – | .019 | – | – | – | – | – | – |
| SURF vs. SUB | .649 | .252 | – | – | – | – | .184 | – | – | – | – | – | – |
| HM vs. LM | .044 | – | – | – | – | – | .038 | – | – | .003 | – | – | – |

*Note*. ADL, acid detergent lignin; NDICP, neutral detergent insoluble CP; ADICP, acid detergent insoluble CP; WSC, water-soluble carbohydrates; °DM, dry matter; SEM, pooled standard error of the mean.

[a]Contrasts: (a) CON vs. SURF + SUB = control vs. the mean of surface and subsurface applications; (b) SURF vs. SUB = surface vs. subsurface application; (c) HM vs. LM = high moisture vs low moisture. *P*-values for logical contrasts are only included in the table if the main effect was significant ($P \leq .05$); if the main effect was not significant, the analysis of that variable was stopped and no *P*-values are shown.

not observe differences in bermudagrass P or K concentrations in response to surface- and subsurface-applied poultry litter. Ashworth et al. (2020) did not observe differences in P, K, in corn (*Zea mays* L.) grain or Ca, Mg, or S in corn silage amended with subsurface- and surface-applied poultry litter.

No differences in CP were detected in between CON and SURF + SUB or between SURF and SUB (Table 3). Similarly, Ashworth et al. (2020) did not consistently observe differences in corn CP concentration when comparing surface and banded litter treatments with an unfertilized control. Ashworth et al. (2020) also did not observe differences in CP concentration in corn plants among three distances of banded subsurface poultry litter and surface-applied poultry litter. However, Pote et al. (2012) detected differences in bermudagrass CP in three hay cuttings in a year harvested after applying subsurface and surface poultry litter at 3.0 ton acre$^{-1}$ in spring. Further work by Kulesza et al. (2016) compared subsurface applications at high (4.8 ton acre$^{-1}$ in 2012 and 3.3 ton acre$^{-1}$ in 2013) and low (2.4 ton acre$^{-1}$ in 2012 and 1.7 ton acre$^{-1}$ in 2013) rates with surface applications at high and low rates. Although the difference was not always significant, Kulesza et al. (2016) observed that CP concentrations in subsurface treatments were greater than in the respective surface treatments at the same litter application rate, and that low rates of subsurface-applied produced forage with the same CP as forage harvested from high-rate surface treatments. In this study, 2.0 ton acre$^{-1}$ was applied in fall, with rye–ryegrass–fescue harvested in spring; therefore, the application rates may have been too low to create a response in CP concentration (Table 3).

Concentrations of NDF were lower in SURF + SUB than in CON (Table 3). Botanical composition may have contributed to the greater NDF concentrations in CON because it was visually observed on the day of baling that all plots contained dead plant material from clipping that occurred the previous year. The dead plant material was incorporated into the bale after raking; however, the dead material was not collected with quadrat clippings (Table 1) and the proportion of dead material in each bale is unknown. Though all plots were treated similarly, CON plots and, to a lesser extent, the SURF plots, had less cereal rye mass accumulation (Table 1), potentially allowing the CON bales to have a greater proportion of dead material than higher-yielding SURF and SUB plots. Despite the statistical difference in NDF, the values among the fertilizer treatments are numerically close, and all three treatments produced bales of with a similar nutritive value (Table 3).

Moisture effects were detected for NDICP, and trends were also detected for starch and ADL. Concentrations were greater in the LM treatments for NDICP and ADL, and in HM bales for starch. Although wilting time could potentially increase indigestible plant components through the loss of soluble carbohydrates concentrations via respiration (McDonald & Edwards, 1976), the differences in wilting time between HM and LM were minimal (only 3 h). Therefore, these

**TABLE 4** Post-ensiled concentrations of selected carbohydrates, crude protein (CP), and fiber-bound CP components of wrapped silage bales following no application of poultry litter (CON), or poultry litter applied by surface (SURF) or subsurface (SUB) application, with all treatments baled at high (HM) or low (LM) moisture at Booneville, AR, in 2018

| Treatment | Bale moisture | CP | NDF | ADF | ADL | NDICP | ADICP | Starch | WSC |
|---|---|---|---|---|---|---|---|---|---|
| | % | % of DM | | | | % of CP | | % of DM | |
| Application | | | | | | | | | |
| CON | 45.7 | 11.4 | 48.1 | 28.5 | 5.9 | 4.18 | 4.09 | 3.2 | 11.0 |
| SURF | 56.4 | 12.6 | 45.0 | 27.3 | 4.7 | 3.81 | 4.18 | 3.3 | 12.6 |
| SUB | 60.5 | 12.6 | 43.8 | 26.8 | 4.1 | 3.36 | 3.66 | 2.8 | 11.1 |
| SEM | 2.88 | 0.33 | 0.94 | 0.76 | 0.74 | 0.212 | 0.225 | 0.28 | 0.87 |
| Moisture | | | | | | | | | |
| HM | 58.7 | 12.3 | 44.3 | 26.9 | 4.2 | 3.46 | 3.81 | 3.0 | 11.8 |
| LM | 49.7 | 12.0 | 46.9 | 28.2 | 5.5 | 4.11 | 4.14 | 3.1 | 11.3 |
| SEM | 2.40 | 0.26 | 0.71 | 0.46 | 0.66 | 0.190 | 0.184 | 0.18 | 0.58 |
| Contrasts[a] | | | | | | | | | |
| | $P > t$ | | | | | | | | |
| CON vs. SURF + SUB | .006 | – | .018 | – | – | .018 | – | – | – |
| SURF vs. SUB | .197 | – | .287 | – | – | .041 | – | – | – |
| HM vs. LM | <.001 | – | .002 | .013 | .030 | .003 | – | – | – |

*Note*. ADL, acid detergent lignin; NDICP, neutral detergent insoluble CP; ADICP, acid detergent insoluble CP; WSC, water-soluble carbohydrates; DM, dry matter; SEM, pooled standard error of the mean.

[a]Contrasts: (a) CON vs. SURF + SUB = control vs. the mean of surface and subsurface applications; (b) SURF vs. SUB = surface vs. subsurface applications; (c) HM vs. LM = high moisture vs. low moisture. *P*-values for logical contrasts are only included in the table if the main effect was significant ($P \leq .05$); if the main effect was not significant the analysis of that variable was stopped and no *P*-values are shown.

differences (Table 3) are unlikely to have been caused by wilting effects.

### 3.3.2 | Post-ensiled bales

Bale moisture in post-ensiled bales was lower in CON than in SURF + SUB, which did not differ from each other (Table 4). Neutral detergent fiber followed the same trend as in the pre-ensiled bales, with greater NDF in CON than in SURF + SUB. Greater concentrations of NDICP were observed in CON than in SURF + SUB and greater concentrations in SURF than SUB (Table 4). Low moisture bales had more NDICP, ADF, and ADL than HM bales. No differences for application method or moisture were observed for ADICP, starch, or WSC.

Although no differences were observed for NDICP in pre-ensiled bales, NDICP was greater in CON than in SURF + SUB, and SURF had more NDICP than SUB in post-ensiled bales. Numerically lower values for NDF and NDICP concentrations were observed in post-ensiled bales than in pre-ensiled bales (Table 3 and Table 4), with pre-ensiled NDF averaging 51.3% but in post-ensiled bales averaging only 45.6%. Similarly, NDICP averaged 6.90% of CP in pre-ensiled bales but was numerically reduced to 3.98% of CP in post-ensiled bales. Reductions in NDICP are expected as part of the proteolytic processes associated with silage fermentation (Dewar et al., 1963). Coblentz and Akins (2019) also observed reductions in NDICP in post-ensiled versus pre-ensiled bales. Hemicellulose is one of the three fiber components that make up NDF (cellulose and lignin are the others) and hemicellulose is the most digestible of the fibers (Van Soest, 1994). Breakdown of hemicellulose in silage has previously been examined (Dewar et al., 1963), and Coblentz and Akins (2019) and Coblentz et al. (2017) also observed reductions in NDF during the ensiling process. Muck (1988) stated that reductions in hemicellulose also decrease the concentration of NDF, although the amount of indigestible fiber remains the same. Therefore, it is likely that hemicellulose and hemicellulose-associated CP were metabolized during the ensiling process, reducing concentrations in the post-ensiled bales, whereas cellulose and lignin fibers were minimally increased or unchanged in post-ensiled bales (Table 3 and Table 4).

Final bale moisture was greater in HM silage bales, averaging 58.7% compared with 49.7% for LM bales (Table 4). Greater concentrations of NDF, NDICP, ADF, and ADL were observed for LM versus HM. Coblentz et al. (2017) also observed greater NDICP and NDF, but not ADF or ADL in dry bales (36.1% moisture) than in ideal bales (47.5% moisture). In general, the fiber-related variables were greater in the LM bales (Table 4).

**TABLE 5** Post-ensiled moisture and fermentation products of wrapped silage bales following no application of poultry litter (CON), or poultry litter applied by surface (SURF) or subsurface (SUB) application methods, with all treatments baled at high (HM) or low (LM) moisture at Booneville, AR, in 2018

| Treatment | pH | $NH_3$–N | Propionic acid | Succinic acid | Lactic acid | Acetic acid | Butyric acid | Ethanol | 2,3-Butanediol | Total acids | Total alcohols | Propionic acid | Lactic acid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % of N | % of DM | | | | | | | | | %of total acids | |
| Application | | | | | | | | | | | | | |
| CON | 4.76 | 0.48 | 0.12 | 0.10 | 0.83 | 0.14 | 0.13 | 0.57 | 0.01 | 1.31 | 0.58 | 0.09 | 0.61 |
| SURF | 4.70 | 0.59 | 0.28 | 0.16 | 1.15 | 0.18 | 0.20 | 0.52 | 0.03 | 1.95 | 0.55 | 0.14 | 0.59 |
| SUB | 4.76 | 0.61 | 0.50 | 0.21 | 1.11 | 0.15 | 0.24 | 0.60 | 0.05 | 2.23 | 0.66 | 0.21 | 0.51 |
| SEM | 0.120 | 0.037 | 0.064 | 0.017 | 0.112 | 0.034 | 0.086 | 0.15 | 0.010 | 0.220 | 0.14 | 0.026 | 0.036 |
| Moisture | | | | | | | | | | | | | |
| HM | 4.67 | 0.60 | 0.37 | 0.18 | 1.18 | 0.17 | 0.21 | 0.56 | 0.04 | 2.10 | 0.60 | 0.17 | 0.57 |
| LM | 4.81 | 0.53 | 0.23 | 0.14 | 0.88 | 0.14 | 0.17 | 0.57 | 0.03 | 1.56 | 0.59 | 0.13 | 0.57 |
| SEM | 0.081 | 0.027 | 0.049 | 0.013 | 0.080 | 0.028 | 0.053 | 0.091 | 0.01 | 0.148 | 0.088 | 0.02 | 0.024 |
| Contrasts[a] | | | | | | | | | | | | | |
| | $P > t$ | | | | | | | | | | | | |
| CON vs. SURF + SUB | – | – | .018 | .014 | – | – | – | – | – | – | – | 0.035 | – |
| SURF vs. SUB | – | – | .035 | .048 | – | – | – | – | – | – | – | 0.063 | – |
| HM vs. LM | – | – | .054 | .047 | .035 | – | – | – | – | .030 | – | – | – |

*Note*. DM, dry matter; SEM, pooled standard error of the mean.

[a]Contrasts: (a) CON vs. SURF + SUB = control vs. the mean of surface and subsurface applications; (b) SURF vs. SUB = surface vs. subsurface applications; (c) HM vs. LM = high moisture vs. low moisture. *P*-values for logical contrasts are only included in the table if the main effect was significant ($P \leq .05$); if the main effect was not significant the analysis of that variable was stopped and no *P*-values are shown.

## 3.4 | Silage fermentation characteristics

Bale pH did not differ among application treatments or moisture levels (Table 5). Propionic acid and succinic acid were greater in SURF + SUB than in CON, and both propionic acid and succinic acid were greater in SUB than in SURF. No differences for litter application method were detected for lactic acid, acetic acid, butyric acid, ethanol, 2,3-butanediol, total acids, or total alcohols. However, because of the partially confounded relationships between application method and bale moisture, with the lower initial bale moisture for CON than in SUB + SURF, the fermentation results are more difficult to interpret. To better interpret the relationship of moisture and fermentation products, regression analyses of final bale moisture and the various fermentation products were completed across all application methods and bale moisture treatments (Table 6).

The strong relationships between bale moisture and silage fermentation products and amounts have been documented in several studies (Coblentz & Akins, 2019; Müller et al., 2007; Shinners et al., 2009) and were corroborated in this study. Regression equations were evaluated for linear, quadratic, and cubic trends, and the *P*-values provided are from the best fitting regressions with the lowest *P*-value and the greatest coefficients of determination. All fermentation parameters had relationships with moisture, which are detailed in Table 6. These relationships were expected (Table 6), as moisture is known to influence the production of fermentation acids in silages, generally with positive but polynomial relationships between silage moisture and acid production (Muck, 1988). Negative relationships between WSC and initial bale moisture are expected (Table 6), as WSCs are a primary substrate for fermentation (McDonald et al., 1991). However, the coefficients of determination for many of the relationships described in Table 6 are lower than previously reported (Coblentz & Akins, 2019). Previous work with orchardgrass (*Dactylis glomerata* L.)–alfalfa (*Medicago sativa* L.) baleage by Coblentz and Akins (2019) reported greater coefficients of determination for fermentation products, such as lactic acid ($R^2 = 0.935$), pH ($R^2 = 0.869$), total acids ($R^2 = 0.825$), WSC ($R^2 = 0.943$), and $NH_3$–N ($R^2 = 0.919$). The coefficients of determination reported in this study ranged from 0.405 to 0.625 for the same variables. Potentially, the coefficients of determination were not as strong in this study because of the great variation in botanical composition (Table 1) among the bales.

Except for 2,3-butanediol and total acids, which had linear relationships with initial bale moisture, the relationships between the remainder of the fermentation variables and ini-

**TABLE 6** Regression equations, coefficients of determination, and *P*-values for fermentation parameters as a percentage of dry matter, regressed against initial bale moisture across poultry litter application and moisture treatments at Booneville, AR, in 2018

| Parameter | Equation | $R^2$ | *P*-value |
|---|---|---|---|
| pH | $y = (3 \times 10^{-5})x^3 - 0.0071x^2 + 0.4648x - 4.8792$ | 0.4268 | .006 |
| $NH_3$–N | $y = (5 \times 10^{-6})x^3 - 0.0013x^2 + 0.1134x - 2.3421$ | 0.4905 | .002 |
| Lactic acid | $y = (-5E \times 10^{-5}) \times 3 + 0.0083 \times 2 - 0.4502x + 8.6337$ | 0.4045 | .009 |
| Propionic acid | $y = (-3 \times 10^{-5})x^3 + 0.0047x^2 - 0.2198x + 3.2279$ | 0.4179 | .007 |
| Succinic acid | $y = (-5 \times 10^{-6})x^3 + 0.0008x^2 - 0.0364x + 0.5531$ | 0.4879 | .002 |
| Acetic acid | $y = (4 \times 10^{-5})x^3 - 0.0067x^2 + 0.3703x - 6.6032$ | 0.8055 | <.001 |
| Butyric acid | $y = (-1 \times 10^{-6})x^3 + 0.0008x^2 - 0.0702x + 1.8382$ | 0.4522 | .004 |
| Ethanol | $= (6 \times 10^{-5})x^3 - 0.0126x^2 + 0.7977x - 15.866$ | 0.3742 | .015 |
| 2,3-Butanediol | $y = 0.00182x - 0.06486$ | 0.2010 | .022 |
| Total acids | $y = 0.05606x - 1.24163$ | 0.6252 | <.001 |
| Total alcohols | $y = (7 \times 10^{-5})x^3 - 0.0128x^2 + 0.8168x - 16.295$ | 0.3856 | .012 |
| Water-soluble carbohydrates | $y = -0.0006x^3 + 0.0959x^2 - 5.0068x + 96.94$ | 0.4459 | .004 |
| Starch | $y = -0.0002x^3 + 0.0269x^2 - 1.4558x + 29.236$ | 0.4746 | .002 |
| Lactic acid (% of total acids) | $y = -0.5x + 84.229$ | 0.2638 | .007 |
| Propionic acid (% of total acids) | $y = -0.0002x^3 - 0.0031x^2 + 2.5855x - 86.283$ | 0.3741 | .015 |

tial bale moisture were best described by cubic relationships (Table 6). Cubic relationships describe a situation in which fermentation product production was limited with low bale moisture, followed by an increase in production with increased bale moisture. Coblentz and Akins (2019) observed cubic relationships for initial bale moisture and lactic acid, acetic acid, and total acids.

Additional interesting points were the low pH of all post-ensiled bales in relation to the total acids produced and the high levels of propionic acid, regardless of the treatment (Table 5). Coblentz and Akins (2019) observed total acid values in alfalfa–orchardgrass bales as high as 5.7% of DM at >60% moisture, with the pH remaining at >5.0. Kung et al. (2018) suggested that typical lactic acid levels for grass silage at 65 to 75% moisture averaged 4.3 to 4.7% of DM. Lactic acid levels in this study ranged from 0.51 to 1.64% of DM (Figure 1), despite relatively high moisture levels. Coblentz and Akins (2019) described a cubic relationship for lactic acid and initial bale moisture, with very low lactic acid production under <45% moisture and reaching a plateau at around >65% moisture. An explanation for this plateau, may be that as bale moisture increased beyond 65% moisture, less desirable fermentation products were formed, namely propionic acid with lactic acid as the substrate (McDonald et al., 1991).

Kung et al. (2018) reported that the typical amount of propionic acid for grass silage at 65 to 75% moisture should be <0.1% of DM, whereas the propionic acid levels in this study ranged from 0 to 0.82% of DM (Figure 2). It has been suggested that clostridia and propionic acid-producing bacteria, which use lactic acid as a substrate, could be the cause of increased propionic acid in wetter silages (McDonald et al., 1991).

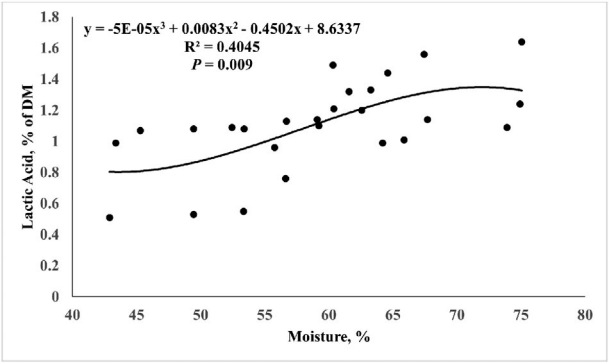



**FIGURE 1** Concentration of lactic acid as a percentage of dry matter (DM) in rye–ryegrass–fescue silage bales with or without poultry litter amendment at Booneville, AR, in 2018, expressed as a function of the initial bale moisture

1991). Lactic acid as a percentage of total acids was shown to have a negative linear relationship with final bale moisture (Figure 3), but the relationship had a low coefficient of determination. However, it was observed that propionic acid as a percentage of total acids had a negative quadratic relationship with initial bale moisture, though it also had a low coefficient of determination (Figure 4). Potentially, lactic acid was converted to propionic acid by clostridia and propionic acid-producing bacteria (McDonald et al., 1991), reducing the proportion of lactic acid within of total acids, whereas propionic acid as a proportion of total acids increased with final bale moisture (Figure 4).

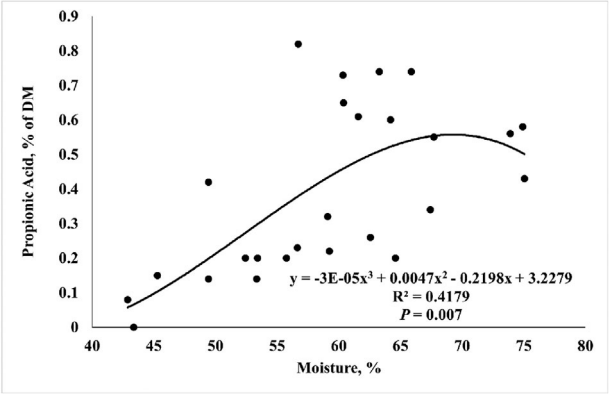



**FIGURE 2** Concentration of propionic acid as a percentage of dry matter (DM) in rye–ryegrass–fescue silage bales with or without poultry litter amendment at Booneville, AR, in 2018, expressed as a function of the initial bale moisture

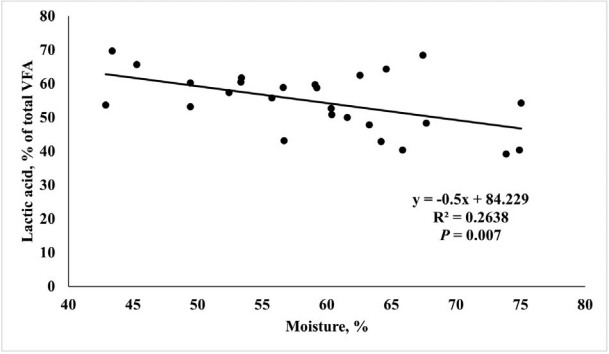



**FIGURE 3** Concentration of lactic acid as a percentage of total volatile fatty acids in rye–ryegrass–fescue silage bales with or without poultry litter amendment at Booneville, AR, in 2018, expressed as a function of the initial bale moisture

## 4 | CONCLUSIONS

Poultry litter application with subsurface technology did not greatly improve the forage's pre-ensiled nutritive value or subsequent post-ensiled fermentation parameters compared with bales from plots with surface-applied poultry litter. However, many differences existed between the control and litter-amended plots, including greater mineral concentrations and less NDF in litter-amended plots. However, some forage nutritive value variables were likely to have been influenced by botanical composition. Bale moisture greatly influenced fermentation parameters, with increased acid production associated with increased moisture.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

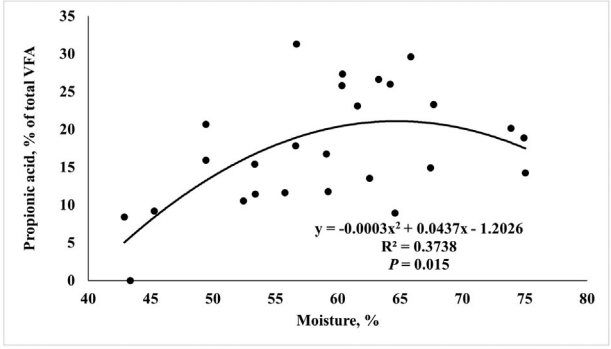



**FIGURE 4** Concentration of propionic acid as a percentage of total volatile fatty acids in rye–ryegrass–fescue silage bales with or without poultry litter amendment at Booneville, AR, in 2018, expressed as a function of the initial bale moisture

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