Improving winter wheat grain yield and nitrogen use efficiency using nitrogen application time and rate

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Abstract
Preplant nitrogen (N) application, which involves placing nutrients in the soil before seeding, has been an integral part of crop production systems for decades. Some producers are known to apply N at least 21 d before planting. This may increase N loss and lower grain yield. This study evaluated the effect of timing and rate of N application on winter wheat (Triticum aestivum L.) grain yield and N use efficiency (NUE). An experiment with a factorial arrangement of treatments was set up in a randomized complete block design with three replications. Treatments included four N rates (0, 45, 90, and 135 kg ha\(^{-1}\)) with each applied 7 or 30 d before planting, and at Feekes 5 (FK\(_5\)). Grain N was analyzed using LECO CN dry combustion analyzer. The difference method [(Grain N from (fertilized plot – check plot))/N applied was used to compute NUE. Nitrogen application rate significantly affected grain yield (\(P \leq 0.01\)). Although the rate may be temporally and spatially variable, approximately 90 kg N ha\(^{-1}\) was required to obtain yields that differ markedly from the check. Midseason applied N (FK\(_5\)) had similar yields to preplant applied N in two of four site-years and significantly increased yield at one site in 2020. Out of two sites, the timing of N application had a substantial effect on NUE in both years (\(P \leq 0.11\)). In this case, NUE was increased by as much as 9.5% for midseason applied N compared to 30 d before planting N application time.

1 INTRODUCTION

The use of fertilizers, particularly nitrogen (N), in crop production has increased and will continue to rise as human population increases (Galloway et al., 2008; Omara et al., 2019; Vitousek et al., 1997) and projected to reach between 10.9 and 11.2 billion people by the year 2100 (Gerland et al., 2014; United Nations, 2015). Depending on the soil N status and environmental conditions in a given year, fertilizer N can increase crop yield and grain protein content (Teal et al., 2007; Thomason et al., 2000). This depends on the rate, source, method, and timing of N application for winter wheat (Triticum aestivum L.) and that is well-documented in literature (Melaj et al., 2003; Raun & Johnson, 1999; Sowers et al., 1994; Weisz et al., 2001). A study in the United States investigating the timing of N fertilization found that time at which N was applied had little effect on wheat grain yield and soil residual ammonium N (NH\(_4\)–N) (Boman et al., 1995). However, Melaj et al. (2003) showed that N application at tillering resulted in a high wheat grain yield compared with...
N applied at seeding. Although application of N in the fall (September–December) or spring (March–May) may not produce a substantial yield difference, the grain protein content of winter wheat in which N is applied in spring tends to be higher than that of fall (Boman et al., 1995; Brown & Petrie, 2006; Fowler & Brydon, 1989). This is because N applied mid-season or late-season (just before or immediately after flowering) is assimilated by the crops to increase grain N and protein content (Brown & Petrie, 2006; Woolfolk et al., 2002).

Meanwhile, application of N, especially at a high rate, in the fall has been observed to result in lower N use efficiency (NUE) when compared with spring-applied N (Sowers et al., 1994). Nitrogen use efficiency may be further improved by the split application in fall and spring. Mahler et al. (1994) revealed the value of split application where they observed a better response to N when it was split-applied in the fall and spring with NUE reaching as high as 60% compared with a single fall (55%) or spring (53%) application.

A common time for N application among producers is preplant where all the recommended N, determined by laboratory soil testing, is applied before planting. This N is usually applied based on a preplant N rate common in each region (Thomason et al., 2000) without recognizing the annual variability in yield potential and crop response to N (Raun et al., 2011). In Oklahoma, 90 kg N ha$^{-1}$ is reported to be a common rate applied to winter wheat and this is likely to be applied at the same rate every year (Thomason et al., 2000). This is based on the traditional understanding that crop needs for N remain the same every year (Yadav et al., 1997). Dai et al. (2015) found that continuous application of N at the same rate every year led to an annual increase in soil nitrate-N by as much as 18 kg ha$^{-1}$, an indication of the need to make adjustments on the amount of N to apply in each cropping season. Split application of N where one portion is applied preplant and the other mid-season or at a later stage has also been reported in several research studies (Mahler et al., 1994; Randall & Sawyer, 2008; Sowers, Pan, et al., 1994). Preplant N application has not only been attributed by some scholars to a period when producers have adequate time to undertake farm operations but also because field conditions are best for N application with reduced likelihood of soil compaction associated with more rainfall received in spring (Randall & Sawyer, 2008). Most of the research studies that indicated the time for preplant N application either did so at planting (0 d) or within 15 d before planting of wheat seeds (Barbieri et al., 2008; López-Bellido et al., 2005; Melaj et al., 2003; Wuest & Cassman, 1992). It is not uncommon to find research reporting preplant N timing as before planting (Brown & Petrie, 2006; Bushong et al., 2016). This may obscure the ability to pinpoint exactly when N was applied before planting and make an accurate interpretation of yield or any other variables evaluated with N applied preplant at different timings. In some instances, producers apply N at least 21 d ahead of planting seeds (Riley et al., 2001). This may explain why some producers use nitrification inhibitors to slow down the rate of conversion of ammonium (NH$_4^+$) to nitrate (NO$_3^-$) (Boswell et al., 1976; Slangen & Kerkhoff, 1984). Although it is known that preplant N application may result in lower NUE and grain yield in comparison to mid-season sensor-based fertilization (Raun et al., 2002), the role of early preplant N on grain yield, and NUE of winter wheat has not been adequately addressed and documented. It may be possible that N applied preplant at different times may interact with the quantity of N applied to produce different yield and NUE responses. By evaluating different preplant N timings against in-season timing at various N rates, the decision about timing and rate of N application could be improved for producers who use low-tech N management approaches (Arnall & Mullen, 2011). This is particularly important because the 33% global NUE for cereal grains is low (Raun & Johnson, 1999). This study hypothesizes that timing N application 7 or 30 d before planting will lead to lower grain yield and NUE when compared to FK$_5$ application timing and that rate and timing of N application will interact to influence grain yield and NUE.

This work, therefore, aims to evaluate winter wheat grain yield and NUE responses to N applied 30 or 7 d prior to planting, and at Feekes 5 growth stage (FK$_5$).

## 2 MATERIALS AND METHODS

### 2.1 Experimental site and design

Two experiments were established, one at Efav, Stillwater, OK (36°08′12.46″ N, 97°06′26.55″ W), and the second one at Lahoma, OK (36°23′21.76″ N, 98°06′43.31″ W), in 2018 and 2019. The soil at Efav is a Norge loam (fine-silty, mixed, thermic, Udic Paleustoll) whereas at Lahoma is a Grant silt loam (fine-silty, mixed, superactive, thermic, Udic Argualtoll). No-till and conventional tillage systems were used at Efav and Lahoma, respectively. The experimental design was a randomized complete block design with 12 treatment combinations and 3 replications. A factorial arrangement of
treatments that included four N rates (0, 45, 90, and 135 kg ha$^{-1}$) and three N application timings (7 or 30 d before planting, and at Feekes 5 growth stage, FK$_5$) was used in this study. Feekes 5 is a winter wheat growth stage that occurs between 97 and 110 growing degree days (Dhillon et al., 2020). Each experimental unit within the blocks measured 3.0 by 6.1 m and the blocks were separated from each other by an alley of 3 m.

### 2.2 Experimental management and data analysis

Prior to preplant N application in each year, soil samples were collected at 0-to-15-cm soil depth and analyzed for NO$_3^-$, NH$_4^+$, P, K, and soil pH (Table 1). Soil pH was analyzed using a soil/water ratio of 1:1. Nitrate and NH$_4^+$ were analyzed using a 1 M KCl extract and Lachat 8500 Series 2 Flow Injection Analyzer (Hach Company). Mehlich-3 was used to extract P and K followed by quantification using inductively coupled plasma mass spectrometry (ICP-MS).

Urea (46–0–0) was applied (broadcast) to the soil surface 7 or 30 d prior to planting and at FK$_5$. The N applied in conventional tillage at Lahoma was incorporated immediately after application. However, urea N applied at FK$_5$ was not incorporated to avoid interfering with the root growth for wheat plants. The dates for planting of wheat and application of N are presented in Table 2. It is worth noting that the N application time of 7 or 30 d before planting (Table 2) were not very exact because of precipitation events that occurred prior to the intended application date, making it not suitable to apply fertilizer. In other cases, N was placed before the intended application date because of the anticipated precipitation on the target application date. In all cases, we made sure that N was applied within 3 d (±3 d) of the intended application time. Phosphorus was also applied at 19.6 kg P ha$^{-1}$ in all the experimental units to avert P deficiency. Winter wheat was planted and managed under dryland conditions without supplemental irrigation. Weeds were managed using preemergence herbicide (glyphosate, N-(phosphonomethyl) glycine) and postemergence herbicides (Olympus with Propanoxycarbazone-sodium—methyl 2-[[4,5-dihydro-4-methyl-5-oxo-3-propyoxy-l]/1,2,4-triazol-1-yl]carbonyl]amino)sulfonylebenzoate—and Mesosulfuron-Methyl—Methyl 2-[[4,6-dimethoxy-2-pyrimidinyl]amino]carbonyl]amino)sulfonyle-4-[[methylsulfonylamino]methyl]benzoate—as active ingredients; Axiom with Fluafenacet—N-(4-fluorophenyl)-N-(1-methylethyl)2-[[5-( trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]acetamide—and Metribuzin—4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5,(4H)-one—as active ingredients).

Winter wheat grain was harvested using a self-propelled combine and yield recorded from the onboard computer yield monitor (Teal et al., 2007). Grain weight was adjusted to 12.5% moisture content. Wheat grain samples were dried in a forced-air oven at 66°C for 48 h and ground using a Wiley mill (Arthur H. Thomas Co.) to pass through a 140-mesh sieve (100 μm).

Subsamples of ground samples were placed in glass bottles equipped with four stainless steel metallic rods and assembled in polyvinyl chloride pipes that were then placed on an automatic roller for 24 h to deliver a homogenous sample and sample fineness. A total of 200 mg of the finely ground sample for each treatment was analyzed for grain N concentration (%) using an automated LECO CN 628 and LECO CN 828 dry combustion analyzer (LECO Corporation) for 2019 and 2020 grain harvest, respectively. Grain N concentration was then multiplied by grain yield to obtain grain N uptake (kg ha$^{-1}$).

Nitrogen use efficiency was computed using the difference method as defined in the following equation:

$$\text{NUE} \% = \frac{\text{Grain N from (Fertilized plots − Unfertilized plots) \times 100}}{\text{N applied}}$$

The data obtained were analyzed using R statistical package (R Core Team). Analysis of variance was used to evaluate the effect of timing and rate of N application on grain yield, and NUE. Data visualization was achieved using ggplot2 within the tidyverse package (Wickham et al., 2019).

Tables were generated using flextable (Gohel, 2020a) and officer (Gohel, 2020b) packages. Using the agricolae package, treatment means were generated and separated by LSD at the .05 and .12 probability level for grain yield and NUE, respectively (de Mendiburu, 2020). The $p$ values were adjusted using Bonferroni. A .12 probability level was selected for NUE because it appears that detecting a significant NUE difference among treatments is difficult at the commonly used .05 or lower probability level. However, this may need also to be explored by other scholars. Standard errors are shown in all the figures to indicate the precision of mean estimates. Single degree of freedom contrasts were also performed using gmodels package (Warnes et al., 2018) to evaluate yield differences among specific treatment levels.
TABLE 2  Winter wheat planting and N application dates, and methods of N application at Efaw and Lahoma, OK

<table>
<thead>
<tr>
<th>Location</th>
<th>Tillage</th>
<th>Date</th>
<th>N application</th>
<th>Planting</th>
<th>Method</th>
<th>Days&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efaw</td>
<td>No-till</td>
<td>4 Oct.</td>
<td>2 Oct.</td>
<td>–</td>
<td>–</td>
<td>Preplant</td>
</tr>
<tr>
<td>Efaw</td>
<td>No-till</td>
<td>22 Mar.</td>
<td>21 Feb.</td>
<td>–</td>
<td>–</td>
<td>Top dress</td>
</tr>
<tr>
<td>Lahoma CT</td>
<td>CT</td>
<td>14 Sept.</td>
<td>6 Sept.</td>
<td>15 Oct.</td>
<td>4 Oct.</td>
<td>Preplant</td>
</tr>
<tr>
<td>Lahoma CT</td>
<td>CT</td>
<td>5 Oct.</td>
<td>27 Sept.</td>
<td>–</td>
<td>–</td>
<td>Preplant</td>
</tr>
</tbody>
</table>

<sup>a</sup>Indicates the number of days urea was applied before or after planting and should be considered together with the column delineating method.

TABLE 3  Analysis of variance showing the effect of timing and rate of N application on winter wheat grain yield in 2019 and 2020

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>df</th>
<th>Mean square error</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lahma</td>
<td>Efaw</td>
</tr>
<tr>
<td>N rate</td>
<td>3</td>
<td>2.32</td>
<td>3.58</td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>1.45</td>
<td>0.58</td>
</tr>
<tr>
<td>N rate × Time</td>
<td>6</td>
<td>0.08</td>
<td>1.47</td>
</tr>
<tr>
<td>NUE</td>
<td></td>
<td>2</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>123.7</td>
<td>169.4</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>27.3</td>
<td>63.9</td>
</tr>
</tbody>
</table>

3 | RESULTS AND DISCUSSION

3.1 | Grain yield

The interaction between timing and rate of N application did not influence winter wheat grain yield in three of four site-years (P ≥ .08; Table 3). The only site-year where the interaction between the two factors had a substantial effect was at Efaw in 2019 (P = .03; Table 3). Because of the lack of interaction among the two factors in three site-years, the main effects of rate and timing of N application were evaluated. The rate of N application had a significant effect on winter wheat grain yield at Efaw in 2020 and Lahoma in 2019 and 2020 (P < .01; Table 3). Grain yield increased as the rate of N application was increased from 0 to 135 kg ha<sup>-1</sup> (Figure 1). At Efaw (2020), the grain yield in the unfertilized check plot was 3.1 Mg ha<sup>-1</sup> whereas at Lahoma, the control treatments yielded 1.8 and 2.1 Mg ha<sup>-1</sup> in 2019 and 2020, respectively (Figure 1 and 2). Apart from Lahoma (2020), grain yield in these check plots was at least 15% lower (significant) than the grain yield obtained with the application of 90 kg N ha<sup>-1</sup> (Figure 1 and 2). The effect of applying N at Lahoma in 2019 was even detected at a much lower rate of 45 kg ha<sup>-1</sup> where grain yield exceeded that of the unfertilized check plot by 31.5% (Figure 2). This is potentially a case where soil N supplying capacity via mineralization was low, thus, allowing crops to respond to lower N rates. The fact that this phenomenon did not occur at both Efaw and Lahoma in 2020 is an illustration of the need for soil testing prior to planting as crop demand for N is highly variable in both temporal and spatial dimensions. However, N application at 45 and 90 kg ha<sup>-1</sup> resulted in similar grain yields in all the three site-years without interaction between timing and rate of N application (Figure 1 and 2). This was also the case for the contrast between 90 and 135 kg N ha<sup>-1</sup> with the exception of Lahoma in 2019, where the single degree of freedom contrast showed that applying 135 kg N ha<sup>-1</sup> led to 15.6% more grain yield than 2.4 Mg ha<sup>-1</sup> achieved by applying 90 kg N ha<sup>-1</sup> (Table 4; Figure 2). Nitrogen application at 135 kg ha<sup>-1</sup> resulted in grain yield that differed from yield attained with 45 kg ha<sup>-1</sup> application rate at Lahoma in both years. Since there was no yield difference between 45 and 90 kg ha<sup>-1</sup>, it is likely that 45 kg N ha<sup>-1</sup> will produce a substantially lower yield relative to a rate that exceeds 90 kg ha<sup>-1</sup>. It is, however, important to remember that 90 kg N ha<sup>-1</sup> is likely to give a
TABLE 4: Single degree of freedom contrasts evaluating grain yield differences at specific treatment levels

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>N rate&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 vs 90</td>
<td>-1.20</td>
<td>-2.01</td>
<td>-5.64</td>
<td>-0.46</td>
<td>.23</td>
<td>.05</td>
<td>&lt;.01</td>
<td>.64</td>
</tr>
<tr>
<td>45 vs 135</td>
<td>-3.30</td>
<td>-3.05</td>
<td>-7.79</td>
<td>-1.83</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>.08</td>
</tr>
<tr>
<td>90 vs 135</td>
<td>-2.09</td>
<td>-1.04</td>
<td>-2.14</td>
<td>-1.37</td>
<td>.04</td>
<td>.30</td>
<td>.04</td>
<td>.18</td>
</tr>
<tr>
<td>Time&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 vs 7</td>
<td>0.09</td>
<td>-0.64</td>
<td>-0.11</td>
<td>0.32</td>
<td>.92</td>
<td>.53</td>
<td>.91</td>
<td>.75</td>
</tr>
<tr>
<td>30 vs FK&lt;sub&gt;5&lt;/sub&gt;</td>
<td>2.79</td>
<td>-1.02</td>
<td>-0.48</td>
<td>-2.65</td>
<td>.01</td>
<td>.31</td>
<td>.64</td>
<td>.01</td>
</tr>
<tr>
<td>7 vs FK&lt;sub&gt;5&lt;/sub&gt;</td>
<td>2.70</td>
<td>-0.38</td>
<td>-0.36</td>
<td>-2.97</td>
<td>.01</td>
<td>.70</td>
<td>.72</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

<sup>a</sup>Nitrogen rates are in kg ha<sup>-1</sup>.

<sup>b</sup>Nitrogen was applied 30 or 7 d before planting, and at FK<sub>5</sub> growth stage.

FIGURE 1: Effect of rate of N application on winter wheat grain yield at the Efaw experimental site in 2020. The error bar indicates the standard error. Similar letters on top of bars indicate no significant grain yield differences among N levels.

FIGURE 2: Effect of rate of N application on winter wheat grain yield at the Lahoma experimental site in 2019 and 2020. The error bar indicates the standard error. Similar letters on top of bars for each year indicate no significant grain yield differences among N levels.

Substantial yield advantage over wheat production without N fertilization in Oklahoma (Figure 1 and 2). Crops responded to inorganic fertilizer N possibly because of low soil mineralization potential that supplied inadequate N to meet crop demand. There is likely to be less demand for fertilizer N in an environment with high mineralization potential and plant-available water (Schulz et al., 2015). Crops growing in such an environment may be able to compensate for any early season N deficiency during the course of the growing season. Nitrogen application at the right rate leads to elevated yields by increasing the number of spikes and the number of kernels per spike (Abedi et al., 2011; Si et al., 2020). The yield improvement may take on a quadratic pattern as N rates are increased (Russenes et al., 2019; Si et al., 2020; Woodard & Bly, 1998; Yang et al., 2017), meaning that grain yield begins to decrease as applied N reaches a certain level.
The significant grain yield difference due to the interaction between timing and rate of N application at Efaw (2019) (Table 3) suggests that the effect of timing of N application on winter wheat grain yield depended on the rate of N applied. Alternatively, the effect of N rate on winter wheat grain yield was also a function of the time at which N was applied. The interaction plot showed that increasing the N rate and applying it at FK$_5$ resulted in the largest grain yield (Figure 3). Furthermore, application of N earlier than the planting date favored grain yield only when N rates were low. The highest grain yield (4.2 Mg ha$^{-1}$) was obtained with 135 kg N ha$^{-1}$ applied at FK$_5$ (Figure 3). Compared with the production of winter wheat without N fertilization, this timing and rate of N application resulted in an 83.6% higher grain yield.

In addition, application of 135 kg N ha$^{-1}$ at FK$_5$ had a significantly larger grain yield when compared with the grain yield obtained with 90 kg N ha$^{-1}$ applied 30 d before planting. This timing and rate of N application produced a 20.7% grain yield difference (Figure 3). Although the N application rate of 135 kg ha$^{-1}$ was lower than the optimum rate when evaluated using a second-degree polynomial, it was apparently close to a rate required to achieve the peak yield. When this N was applied at FK$_5$, grain yield was likely high because it is the stage at which crop N uptake is high due to accumulation, partitioning, and remobilization of dry matter and N to leaves, stems, grain, and other organs (Zheng et al., 2020). However, when N rate is low, N application at FK$_5$ is unlikely to result in the highest yield as crops will prioritize the accumulation of grain N over starch (Gaju et al., 2014; Walsh & Walsh, 2020). The interaction between timing and rate of N application has also been found to have a significant effect on winter wheat grain yield (Woodard & Bly, 1998; Zebarth & Sheard, 1992). Abedi et al. (2011) reported that the interaction between timing and rate of N application led to increased yields at higher N rates. However, their work also showed that in some cases grain yield began to decline as the rate of application exceeded 240 kg N ha$^{-1}$. This is because N influences wheat grain yield in a quadratic pattern as excess N may become toxic to crops (Si et al., 2020). This result at Efaw suggests that determining an appropriate N rate and time for N application could improve grain yield for winter wheat.

The timing of N application had a significant effect on winter wheat grain yield at Lahoma and Efaw in 2019 and 2020, respectively ($P < .01$; Table 3). Meanwhile, there was no effect of timing of N application on grain yield at Lahoma in 2020 ($P = .42$). Results showed that applying N 30 and 7 d prior to planting resulted in similar grain yields with an average of 2.7 and 3.3 Mg ha$^{-1}$ at Lahoma and Efaw, respectively (Figure 4 and 5; Table 4). This suggests that a producer is likely to attain similar grain yield levels regardless of the preplant timing selected for N application (30 or 7 d before planting).

However, in both cases, grain yield for N applied 30 or 7 d prior to planting was markedly different from N applied at FK$_5$ ($P = 0.01$; Table 4). At Lahoma (2019), N application
prior to planting had on average 2.7 Mg ha\(^{-1}\) grain yield and that was 29.2\% more than yield realized with fertilization at FK\(_3\) (Figure 4). This result is possibly because of volatilization that might have occurred at this site because fertilization was soon followed by heavy rain and a week without precipitation. By applying all the N at FK\(_3\), it also potentially affected the proper establishment of yield components. Head density and number of tillers may be reduced by early season N deficiency particularly when mineralization is low in a given year. In other words, crops that receive early season N are better able to establish more tillers and high leaf area index that allow them to transpire and assimilate more organic materials (Johnston & Fowler, 1992). This likely reduces competition among tillers, thus minimizing the death of some tillers associated with inadequate soil N supply early in the season (Efretuei et al., 2016). This could be the reason why Raun et al. (2002) suggested that winter wheat grain yield potential is maximized with the application of some N preplant. It should, however, be noted that wheat crops that did not receive preplant N may be able to compensate for early season N deficiency by increasing the number of seeds per head and tillers upon N application in-season (Brown et al., 2005).

Conversely, N application (at Efaw in 2020) at FK\(_3\) led to 17.1\% more grain yield relative to preplant N application timings that had an average grain yield of 3.3 Mg ha\(^{-1}\) (Figure 5). This result could be because of the increased likelihood for the loss of N applied before planting via leaching and or denitrification (Beaudoin et al., 2005; Delgado, 2002; Sogbedji et al., 2001). These coupled with plant N loss that occurs in winter wheat during midseason can lead to a significant loss of a portion of preplant N (Kanampiu et al., 1997). This finding corresponded with results reported by Vaughan et al. (1990) that for fall-applied N to achieve the same yield level as spring-applied N, 20\% more N applied in spring has to be added to the fall-applied N. This indicates that applying N after planting may result in more grain yield than N applied preplant. In comparison to preplant N application, Boyer et al. (2012) showed that an additional 100 kg of wheat grain yield could be generated by applying 90 kg N ha\(^{-1}\) in-season. Besides, N placement in-season coincides with a period when uptake of plant nutrients is at its peak as plants synthesize organic compounds and derive energy for growth and development.

For Lahoma in 2020, grain yields were similar across the different N application timings with an average yield of 2.6 Mg ha\(^{-1}\) (Table 4; Figure 4). This similarity in grain yield could be because the soil was able to supply much of the N needed by crops via mineralization (Efretuei et al., 2016). This possibility makes it unlikely for crops to respond to added fertilizer N. The second possible explanation for this lies with N loss or uptake taking place at a similar rate across the three different timings of N application. Crops subjected to similar amounts of N at the time of peak demand, regardless of the time at which N was applied, may achieve similar grain yield levels. Some past studies have shown that N timing does not affect grain yield. For instance, Schulz et al. (2015) reported that a single N application in-season and split application at various stages did result in any yield differences and that split N applied late in the season may not be adequately utilized by crops due to moisture shortage. This could be because of the uniform soil moisture during the course of the growing season that leads to similar biomass (Cousins et al., 2020). This suggests that N applied preplant or in-season are subjected to similar quantities of soil moisture that may affect N uptake or loss in a uniform manner. At the 2020 site, rainfall received was fairly consistent throughout the growing season and the slope over time was near zero (Figure 6). This could have led to similar losses of N for the different N application timings leading to similar grain yields. Similar to the observation at Lahoma in 2020, Boman et al. (1995) detected no dramatic yield differences between N applied preplant and after planting. They, however, stated that N applied in-season could lead to plant tissue damage and suggested that the appropriate time to apply N is in early January (late FK\(_3\)). Furthermore, early season deficiency may occur if N application is delayed until midseason and this needs to be corrected if yield potential for any growing environment is to be realized (Fowler & Brydon, 1989).

**Figure 5** Effect of timing of N application on winter wheat grain yield at Efaw in 2020. The error bar indicates the standard error. Similar letters on top of bars indicate no significant grain yield differences among treatment levels.
3.2 | Nitrogen use efficiency

Nitrogen use efficiency was not affected by the interaction between timing and rate of N application in all the four site-years ($P \geq 0.36$; Table 3). There was also no effect of N rate on NUE for grain winter wheat ($P \geq 0.39$; Table 3). Nitrogen use efficiency across the different application rates had a mean of 20.2 and 21.1% at Efaw and Lahoma, respectively. This could be because much of the applied N went into improving grain yield by accumulating more starch and related organic compounds, thus diluting the grain N concentration (Lollato et al., 2019). This may also explain why at 45 kg N ha$^{-1}$, grain yield was different from that of control treatments in one of three site-years without interaction between the two factors (Figure 1 and 2). This potentially made NUE for higher N rates to match those associated with lower rates. In general, NUE associated with each N rate >0 was low both spatially and temporally that NUE values across the different application rates or timings were below the 33% global estimate (Raun & Johnson, 1999) or a more recent estimate of 35% (Omara et al., 2019).

The timing of N application had a major effect on grain NUE at Lahoma in both 2019 and 2020 ($P \leq 0.11$; Table 3). The timing of N application at FK$_S$ increased NUE by at least 7.3% compared with an NUE of 15.6% achieved with N applied 30 d before planting (Figure 7). This was similar for N applied at FK$_5$ and 7 d before planting with an average of 24.0 and 22.6%, respectively (Figure 7). At the same site in 2020, there was also an advantage of applying N 7 d prior to planting and resulted in 9.6% greater NUE value than 15.6% for N applied 30 d before planting. Although there was no yield advantage at the two N application timings (7 or 30 d before planting), this NUE benefit suggests that the window for N loss, for instance, via leaching, denitrification, volatilization, and/or plant N loss is increased when N is applied 30 d before planting.
Alternatively, by applying N as close as possible to the planting date or after planting like at FK5, there is increased potential for recovery of more N in the grain. Application of N in-season has a greater chance of increasing biomass accumulation (de Oliveira Silva et al., 2020) than preplant, suggesting that more N may be partitioned to the grain (Wuest & Cassman, 1992). Other research work reported a similar observation that in-season N management improves NUE. For instance, Dhillon et al. (2019) found topdressing to have significantly higher NUE values in three out of five site-years. Similarly, Barbieri et al. (2008) noted that delaying and applying N until at least tillering leads to an improvement in NUE. This is because N applied in-season such as at anthesis minimizes N loss pathways, thus making more N available for uptake via transpiration stream (Wuest & Cassman, 1992).

In both years at Efaw, NUE values were similar across the different N application timings (Figure 7). Nitrogen use efficiency averaged 23.3 and 17.1% in 2019 and 2020, respectively (Figure 7). This is possibly because grain N concentration was similar across the different N application timings. In addition, grain yields (2019) did not vary significantly between the different timings of N application, making the resulting NUE similar since grain N concentrations over N application timings were also related. Even though grain yield at FK5 in 2020 was significantly larger than that of the preplant N timings (Figure 5), that did not translate into higher grain N concentration possibly because of the accumulation of starch that diluted the grain N content (Lollato et al., 2019).

4 | CONCLUSION

The timing of N application had an inconsistent effect on winter wheat grain yield with FK5 producing grain yield that was both significantly lower and higher than the yield realized with N applied preplant (7 or 30 d before planting) and an insignificant yield difference between the different timings. However, FK5 is likely to offer an advantage since there is increased potential for the loss of N applied at this stage when compared with N applied preplant with a wider window for loss of N via pathways such as volatilization, denitrification, leaching, and/or plant N loss. This was evidenced in NUE where its values obtained at FK5 were higher than those achieved by applying N preplant at one of the sites. Nitrogen application rate was invaluable for improving winter wheat grain yield that in Oklahoma; about 90 kg N ha⁻¹ may be needed to improve grain yield depending on the soil mineralization potential. Contrary to our hypothesis, the interaction between timing and rate of N application influenced grain yield in only one of four site-years. Nonetheless, this offered insight into the need to appropriately time and apply the right quantity of N to attain the yield potential of a crop growing environment.

**CONFLICT OF INTEREST**

They authors declare no conflicts of interest.

**AUTHOR CONTRIBUTIONS**

Lawrence Aula: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing-original draft; Peter Omara: Methodology, Writing-review & editing; Fikayo B. Oyebiyi: Writing-review & editing; Elizabeth Eickhoff: Data curation, Writing-review & editing; Jonathan Carpenter: Project administration, Supervision, Writing-review & editing; Eva Nambi: Writing-review & editing; Alimamy Fornah: Writing-review & editing; William R. Raun: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing-review & editing.

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