IMPACT OF NITROGEN FERTILIZER APPLICATION TIMING ON WINTER WHEAT

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IMPACT OF NITROGEN FERTILIZER

APPLICATION TIMING ON WINTER WHEAT

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Abstract: Trials were conducted over eight site-years during the 2016/17 and 2017/18 growing seasons in no-till dryland conditions in Oklahoma. A pre-plant treatment of 100 kg ha^{-1} of N was broadcast applied as ammonium nitrate (AN). When visual symptom differentiation (VSD) was documented between the pre-plant and the non-fertilized check, top-dress applications were performed every seven growing days (GDD>0) until 63 growing days after VSD at all sites. The objective of this study was to investigate the effect of delaying N applications based on in season VSD on winter wheat grain yield and protein. The results of the delayed applications were compared to the "standards" of pre-plant application and application made at first VSD. Considering all the data collected, the application of N and its timing had a significant effect on the yield and protein concentration of winter wheat. A negative effect on grain yield was observed when application was made later than late March. In addition, the optimum top dress timings considering all trials were between the agronomic range of 80 to 100 GDD>0 after planting. At this period, the data suggest no negative impact on yield but quite likely positive event as 100% yield recovery was achieved in relation to the pre-plant and 0 days after VSD between the pre-plant and 0DAVD. A best fit model indicated 100% yield recovery with N applications made to the point of 140 GDD>0 after planting. A significant finding of this work was that reaching maximum achievable yield levels was not related to the point in time at which the crop goes under N stress. Rather, optimum grain yield recovery was mostly associated with the N applications made when the crop was most vigorously growing, during the Feekes 6 and 7 growth stages. Evaluation of N timing on protein concentration showed a trend for increasing protein values with an increasing delay of application. Further studies should consider other environments, early planting dates, multiple N rates, and potential N sources to provide a better understanding of the influence of the N application timing on grain yield and protein.

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CHAPTER I

INTRODUCTION

In 2018, the world demand for nitrogen (N) fertilizers will be 115 million Mt (FAO, 2017). Cereal crops represent 60% of all N fertilizers used in agriculture. The average nitrogen use efficiency (NUE) in agriculture suggested by Raun and Johnson (1999) was 33% in cereal crops. This low NUE represents a growing challenge for producers and researchers due to the rapid increase in world population, which has been accompanied by an increase in the demand for food production (Walsh et al., 2012). In addition, fertilization, especially of N, in excess of crop demand can lead to environmental problems (Stone et al., 1996). According to Raun (2005), a 1% increase in worldwide NUE, considering fertilizer prices in 2005, would be worth $1.1 billion. Raun and Johnson (1999) suggested one of the best ways to improve the efficiency and the use of agricultural resources is by increasing the knowledge about the environment and/or conditions that directly influences the N management in order to reduce losses and inputs in the production systems.

Hard red winter wheat (WW) is grown on more acres of crop land in Oklahoma than any other crop. Producers planted 1.8 million hectares in 2017, making Oklahoma the seventh largest wheat producing state in the nation with approximately 7% of the national production. In both 2015 and 2016 Oklahoma WW harvest brought in $470 million to the state economy (USDA, 2017).

Often in southern Great Plains, higher WW yields are accompanied by low grain protein concentrations (Lollato and Edwards, 2015). Until recently, producers did not expect to receive price premiums for increased protein content; therefore, there was limited interest in managing for it. In the region, producers often target the practices with the highest possible efficiency in an attempt to reduce costs. Thus, producers will often opt for only making one fertilizer application for the entire crop cycle, typically pre-plant. This type of mechanism is forcing a N fertilizer application to be made prior to the time when an accurate yield potential of the crop can be determined. However, with multiple years of low protein wheat production, low crop prices, and the continued development of new cultivars allowing for higher yield productivity, southern Great Plains wheat producers are taking notice to managing for yield, quality, and input efficiency.

The primary objective of this study was to determine the impact of delaying a top-dress N application until after a nitrogen deficiencies were identified on winter wheat grain yield and protein concentration. The secondary objective of the project was to identify the optimum timings of N fertilization in winter wheat.

CHAPTER II

REVIEW OF LITERATURE

Nitrogen Uptake by Winter Wheat

Previous works have shown that both genotype and the environment have a great influence on the nitrogen uptake by the crop. According to Austin (1977), in a study analyzing 47 wheat genotypes, plants generally contained 83% of the total N taken up at physiological maturity. On average, 68% of total N in the plant was present in the grain. Moreover, the author suggested a strong correlation between dry matter accumulation and N content at Feekes 10.5.3 and grain filling. This correlation between biomass and N accumulation was also reported by others (Justes et al., 1994; Girma et al., 2011). Devienne-Barret et al. (2000) presented that when there is not a N deficiency, the total N accumulation is determined by the plant growth rate and the soil N-nitrate concentration. Also, the authors noticed that in situations without stress, wheat can accumulate far in excess more N than the plants need to the maximum growth rate. Girma et al. (2010) documented that at growth stage Feekes 5, 61% of the total N accumulated by the plant was found in winter wheat in a study performed in Oklahoma. Amanda de Oliveira, (2016) in a non-publish data found that the N uptake by hard winter wheat in Kansas at Feekes 10.5.3 would be equivalent to 82%. This stage was reached by the crop when the accumulative growing degrees was around 1500 (°C).

Head Formation and Grain Filling in Winter Wheat

By Feekes 5, the plants become strongly erect. The leaves are formed and all meaningful tiller considering yield has ceased their growth. The growing point that generates new cells for the plant begins to develop an embryo head. At this important stage, the number of spikelets is determined. A deficiency of N at this stage can be detrimental, since the availability of N directly influences the number of seeds per head as well as the size of the seed. An increase in N availability after Feekes 5 will not affect the seed number in each head anymore (Miller, 1992).

Generally, the composition of WW grain is 60-70% starch and 8-15% protein. After Feekes 10.5.3, the yield will be dependent on the starch accumulation. The differences in the amount of starch in the grain will affect the final protein concentration, while N availability affects final protein content. In the process of grain elongation, which lasts until about 10-15 days after Feekes 10.5.3, the starch and protein accumulation begins. The grain will increase in the number of cells, but at this point the accumulation of dry matter is not expressive. The A-type amyloplasts begin to differentiate after 4-5 days after flowering and reach their final number in about 7 days. A-type starches develop along kernel growth to maturation, where A-type granules make up only 3% of the total number of cells, but contribute 50-75% of the total endosperm weight (Parker, 1985). Protein accumulation begins approximately 10 days after Feekes 10.5.3. After the elongation, the process of grain filling start (13 to 40 days approximately after the Feekes 10.5.3). The proteins are deposited on the grain at a faster rate than the starch. At this stage, protein and B-type starch are accumulated concurrently, but via different processes.

More than 60% of the fixed starch comes from the photosynthetic process that occurred during the filling of the grains. During the grain filling stage, the starch supplement may be influenced by various factors arising from the environment conditions such as drought and diseases (e.g., which may interfere with the soluble carbohydrates in the internodes mobilization to the grains). Conversely, within 20 days after Feekes 10.5.3, about 50% of the final protein is already accumulated in the grain. More than 70% of the grain's protein is absorbed before grain filling. The N is translocated from other plant parts but mostly from leaves and stems.

Proteins are source limited differently from starch that is sink-limited. Thus, the amount of protein in the grain is not sensitive to the environment but due to the sensitivity of starch accumulation to environment, the concentration of proteins in the grain can be. Lollato and Edwards (2015), documented that in low yielding environments, the starch accumulation in the grains are reduced, resulting in a high protein concertation in the grains. However, in environments whose productive potential is substantially higher, a starch accumulation was observed, thus significantly reducing the protein content in grains.

Dupont et al. (2003), performing controlled environmental conditions, found that different temperature regimes, moderate (24 °C day and 17 °C night) and high (37 °C day and 28 °C night), have an effect on the accumulation and composition of wheat grains when mineral nutrition (NPK) was implemented post-anthesis. At the moderate temperature regime, post-anthesis plant nutrition (20-20-20, NPK) doubled the flour protein and only slightly increased the kernel weight. However, when the plants were submitted to the regime of high temperatures, the mineral nutrition had no effect on the duration of the accumulation or percentage of protein. The high temperature regime reduced the grain filling stage as well as reduced the dry matter accumulation period and the weight of a single kernel by 50%. Similar results were found by Altenbach et al. (2003) being that when the wheat was submitted to high temperature regimes (37/17 °C or 37/28 °C day / night) during the graining. The periods to reach maximum kernel water content, maximum dry weight and harvest maturity were lower when the culture was submitted to 24/17 °C day / night regimen. However, when plants were restricted from post-anthesis fertilizers (20-20-20, NPK), both at temperature regimes 24/17 °C and 37/17 °C, the concentration of protein in grains was reduced, unlike the accumulation of starch in grains had similar patterns.

Nitrogen Application Timing

The timing of N fertilization has been studied broadly among the majority of cereal crops, well documenting that many factors can influence both grain yield and protein concentration (Fowler and Brydon, 1989; Melaj et al. 2003; Blackshaw, 2004). As the soil cannot supply all the N that WW needs to achieve the full genetic yield potential of the crop, therefore N fertilization is almost a compulsory practice in farms around the world. According to Raun and Johnson (1999), an increase in the nitrogen use efficiency of 20% in cereal crops would represent $4.7 billion in savings per year.

Bushong et al. (2014) stated that if soil moisture levels are adequate enough for initial plant development, application of N fertilizer in the pre-plant would be most beneficial for grain yield and water use efficiency in central Rolling Red Plains because of the better root development at the beginning of the plant life cycle. According to the study, when the crop is rainfed and the moisture levels of the soil are below or average, there is no effect on the of N timing application. However, while the study did evaluate a range of N rates, this work was limited in that it only evaluated all N applied pre-plant or all N applied in-season in one application prior to Feekes 6.

Nitrogen recommendations can be affected by many variables and normally change every year, even in the same location (Morris et al., 2006; Melaj et al. 2003). The N uptake by the plants in the production systems will be influenced by rainfall amount and timing, soil type, crop and cultivar, temperature, among others environment peculiarities. According to the authors, researchers cannot determine a standard calendar application time due to the climate variation year by year.

According to Flowler and Brydon (1989), the timing of N fertilizer application has an effect on the grain yield, grain protein yield and concentration in WW. Grain yield and protein are highly influenced by environmental factors that can change the responses by the timings. Furthermore, late applications can increase the protein concentration, but conversely, will decrease the nitrogen use efficiency (NUE). However, late spring applications can only increase yield and protein yield if there is a very strong limitation in the early spring as water stress that will limit yield more than N at that point. The N rates also can interfere in the residual nitrate in the soil, which can change the N fertilizer response.

Melaj et al. (2003) stated that N remobilization and N uptake from the soil are most greatly affected by the meteorological conditions. Nonetheless, N fertilization at tillering increases grain yield and N recovery when the soil supplies the N for the plants in the early stages. McConkey et al. (2002) noticed that no-till areas often have different rates of N mineralization and N immobilization in the soil, likely due to the C:N ratio and changes in moisture content and thermodynamics of the system.

A quadric response was suggested by Booman et al. (1995) in which the optimum time of N fertilization for maximum forage and grain yield would be mid November and early January, respectively. However, the study used calendar days to best estimate the higher yields top dress timings on the model. Calendar days could be very different among years considering the growth stages of the plants.

Woolfolk et al. (2002) in a study performed in Oklahoma evaluated late foliar N applications in grain yield and total grain N. Two different timings of applications were tested (pre and post Feekes 10.5.3) with 2 different fertilizers (urea ammonium nitrate and ammonium sulfate (21-0-0). The authors noticed that N content in the grains can be increased by performing foliar N applications before or immediately after Feekes 10.5.3 with UAN. No effect of the foliar fertilization was detected in grain yield, straw yield, or straw N.

Morris et al. (2006) performed a study in Oklahoma using three different locations in which one of those was also used in this present study (Lake Carl Blackwell). The first objective of this study was to better understand the relation between harvest response index (Johnson et al., 2000) and NDVI response index ($RI_{Harvest}$) (Mullen et al., 2003), which allows in-season yield predictions based on the N fertilization optimization algorithm (NFOA) (Raun et al., 2002). NFOA estimates the N rate needed based on the yield potential without added fertilizer (YP_0) and a specific response index (RI) for each field. The secondary objective to this study, and most important considering the current objectives, was to determinate if potential yield reductions from early stress can be corrected by utilizing in-season applications. For this study, 15 treatments were implemented as a three by five factorial, consisting in three pre-plant N rates (0, 45 and 90 kg ha^{-1}) with five in-season rates applied at Feekes 5. The top-dress rates were based on the yield potential of the crop (sensor based algorithms) with four different RI for each of the pre-plant rates. The results convey that even when pre-plant N application was removed (0 kg ha^{-1} pre-plant applications), Feekes 5 top-dress applications resulted in maximum yields or near maximum when compared with the others treatments with pre-plant applications. Also, when compared with the standard 90 kg ha^{-1} of N application on pre-plant, top-dress applications (0 kg ha^{-1} of N pre-plant) resulted in maximum yields in all of the six locations. However, a limitation of this work was the lack of documenting when, during the growing season, did the crop experience N deficiency.

In a study coducted in Oklahoma from 2002 to 2006 and 2010, also at Lake Carl Blackwell location, Mohamed et al. (2013) tested an orthogonal treatment structure with five N rates (0, 50, 100, 150 and 200 kg ha^{-1}) and three applications timings (pre-plant, February and March) utilizing urea (46-0-0) as pre-plant and UAN (28-0-0) as top-dress N fertilizer sources. Considering the timings of the applications, the authors found an effect of timing on grain N uptake in at least one treatment for all years, except 2003 and 2010. Acording to the authors, the split applications of 150 kg ha^{-1} resulted in the higherest NUE . The authors also stated that if the demand for N is a function of the yield level to be achieved, since the results shown great variability on the grain N uptake by year, timing and rate, even on the same site, the N recommendations may change every single year.

In a general, most of the publications related with N timing in wheat have found that the timing of application had an effect in the grain yield and grain protein yield and concentration. However, these variables were also highly influenced by environmental factors that can change the crops response to application timings. The resulting conclusion was that the best N application timing is dependent on the weather and enviroment conditions of each year and location.

Pre-plant N applications may provide good resistance to water stress at the beginning of the plant life cycle and also a high NUE in low N rates compared when performed in timings, but at the same time, it suggests higher losses in the system during the plant life cycle and can cause low availability at the end of the plant life cycle, thus reducing yield and protein content. Late spring applications (Feekes 10.5.3) can bring up the protein content of the grain, but cannot increase yield in many scenarios. The highest N requirement normally found in the literature is in the highest plant growth period, which normally occurs after the winter in the green up of the crop. Spring applications, when the weather presents favorable conditions, have been found to possibly recover the N stress in the beginning of the cycle (Flowler and Brydon, 1989; Morris et al., 2006). A good alternative for producers is to split the applications in different timings, if possible and affordable. This strategy can make an in-season evaluation of the crop and weather, which can give a better indication of the best timings of the fertilization than an early season decision.

One of the greatest limitations to the studies discussed previously is the lack of a wide breadth of N application timings in a single study. The majority of the work focuses on multiple rates applied at only a few timings. An additional limitation is the lack of documenting when the crop is actually under N stress. A study which the focus is on the plant ability to recover stress previously documented in the same plant cycle would be critical for a better understanding of N management in WW. A wide range of N applications that span a significant period of the crops growing cycle may provide better parameters to identify the best N timing.

Raun et al. (2001) observed that the yield potential of WW could be predicted in season with the use of an optical sensor. As traditional N fertilizer reccomendations are based on the yield potencial of the crop, Raun suggested N rate calculations could be based on the NDVI readings collected in-season. In the southern Great Plains, studies have shown that this approach works well in WW (Butchee et al., 2011; Arnall et al., 2013). The authors shown that producers can save or make more profit by reducing the N applications when they are not necessary for yield increase. A study that covers a larger window in the times of application is necessary since the results could help the understanding in a physiological view of the plant and also contribute to a more robust understanding of the abilty for WW to overcome N stresses without a negative impact on yield or protein concentration. The work may also provide a clearer view of when in the growing cycle is N application the most beneficial considering grain yield and grain protein. Such results would be decisive regarding the WW N fertilization, because the more progressed the crop is in the growth cycle, the more accurate and precise the yield potential predictions values are. This understand could lead to further refinement of sensor based N management and the improvement of NUE.

CHAPTER III

METHODOLOGY

The study was conducted over the 2016-2017 and 2017-2018 growing seasons. The trials were established at five locations in Oklahoma: Lake Carl Blackwell research farm (LCB) near Perry, OK, Cimarron Valley Research Station near Perkins, OK, Cross Country research farm near Stillwater, OK, and the Raymond Sidwell Research Station near Lahoma, OK. Soil classification, soil characteristics, and previous crop at each site are described in Table 1.

Prior to the initiation of the trials, 0-15 cm composite soil samples, composed of 15 soil cores, were collected from the entire trial area, as outlined in Zhang et al. (2013), and submitted for analyses to Soil, Water and Forage Analytical Laboratory (SWFAL) located in Stillwater, OK. The pH analysis was performed using 1:1 soil to deionized water ratio. If the pH was lower than 6.2, Sikora buffer was added to determine the buffer index. For nitrate-N, calcium sulfate was used as the extractor and analyzed on a flow injection analyzer using cadmium reduction chemistry. For P and K, Mehlich 3 was used as extractor and then analyzed on inductively coupled plasma (ICP) according to Zhang and Henderson (2014). The soil analysis results are described in Table 2.

Seeding rate and planting dates are reported in Table 3. At Perkins2017, Perkins2018 and Stillwater2017, 84 kg ha^{-1} of diammonium phosphate (DAP) was applied in-furrow at planting, due to the low pH at Perkins and the low soil test P at Stillwater, which would limit the yield potential.

At all locations, treatments were arranged in a randomized complete block design (RCBD) with three replications. Plot size was 3.1 m by 6.1 m. All the areas utilized in this study were under no-till management. Due to the field at LCB being in a systematic crop rotation, the project was able to establish two studies at that site each growing season. Best management practices recommended by Oklahoma State University were followed for all pest management.

For the two crop years investigated in this study, the wheat variety Doublestop CL Plus was planted in Perkins and Stillwater. Bentley was planted in both seasons at the LCB and Lahoma locations. Variety selection was based upon expected varietal performance in each soil type and environment.

Each trial evaluated eleven different N fertilization timings (one applied at pre-plant and ten top-dress timings) along with an unfertilized check, creating a total of 12 treatments (Table 4). One N rate across all timings was utilized in the study (100 kg ha^{-1}) applied as ammonium nitrate (AN), 34-0-0. The N fertilizer was broadcast applied to the plots. The AN was utilized over the commonly used urea N source (46-0-0) to remove any impact of environment on N loss through volatilization (Ernst and Massey, 1960; Guangming et al., 1998). This was done as the focus of the study was on the ability of the crop to recover from N stress and not the efficiency of the fertilizer.

The top-dress applications timings were based on the visual nitrogen deficiency (VND) between the pre-plant treatment and the non-fertilized check. In other words, N applications began when the pre-plant N treatment had a clear visual (naked eyes) difference considering the intensity of the green color or bigger biomass accumulation when compared with the check treatment. To be accurate, a GreenSeeker™ sensor was used to collect normalized difference vegetation index (NDVI) from the two treatments. When the difference of NDVI readings was greater than 0.02, VND was confirmed, and the site was considered N responsive. Top-dress treatments were applied at 0, 7, 14, 21, 28, 35, 42, 49, 56, and 63 growing degree days > 0 (GDD>0) after VND (DAVD). GDD>0 values were retrieved from the Mesonet Wheat Growth Day Counter (http://www.mesonet.org/index.php/agriculture/category/crop/wheat/number_of_days_gdd_0). GDD>0 is calculated as:

$$1\ GDD > 0 = \frac{Day\ Max\ Temperature + Day\ Min\ Temperature}{2} - 4.4\ °C \qquad (1)$$

Only if

$$\frac{Day\ Max\ Temperature + Day\ Min\ Temperature}{2} - 4.4\ °C > 0 \qquad (2)$$

or

$$\frac{Day\ Max\ Temperature + Day\ Min\ Temperature}{2} - 4.4\ °C < 30\ °C \qquad (3)$$

At grain maturity, the center 1.5 m of the plots were harvested with a Massey Ferguson 8-XP plot combine (Kincaid Equipment Manufacturing; Haven, KS) in June until late June at all experimental sites. Data for grain yield and percent moisture content were recorded by the onboard Harvest Master Yield monitoring computer (Juniper Systems; Logan, UT) and grain samples were collected from each plots at the same time of the harvest. To standardize all grain yields, the moisture content on the grains was adjusted to 12.5 %. Grain protein was determined post-harvest using near infrared spectroscopy Diode Array NIR Analysis Systems model DA 7000, Perten (Kungens Kurva, Sweden) to analyze grain protein content.

Data was analyzed using JMP 13 PRO® (SAS institute) for year-specific crop production factors, such as grain yield and protein. Data was differentiated using ANOVA methods and Dunnett's to separate the means at P = 0.05. Controls utilized on the test were the check (trt2), pre-plant (trt1) and 0DAVD (trt3). For the pre-plant and 0DAVD comparisons the check treatment was removed from the data. A linear mixed model was implemented using Proc Mixed Statements (SAS® 9.4) defining a quadratic response for GDD>0 from planting as fixed effects and blocks nested in each single trial as random effects (trt3). The data was normalized prior to analyses to the pre-plant treatment and 0DAVD treatment. Normalization was performed on a by rep basis for all locations dividing the other treatments results by the pre-plant or the 0DAVD. Check (trt2) was also removed from the data for this analyses .

CHAPTER IV

RESULTS AND DISCUSSION

Timing of Response to Nitrogen

As the design of this study was to evaluate N fertilizer application after visual N deficiencies, treatments were applied at a range of differing dates across all site-years. Table 5 provides the dates of all treatment applications. Even though the planting date and application of the pre-plant were very similar across all sites, the date of 0DAVD ranged from November 11 to February 5. Even in the same season within the same field, LCB, previous crop impacted 0DAVD by 40 and 45 days in first and second crops, respectively. The range in date of differentiation could also be credited to the different varieties used for this study Austin, (1977) documented that wheat cultivars could have a different N uptake in the early season or to the nitrate content in the soil (Raun et al., 1998). The difference in 0DAVD date across site years, along with the impact of location on the accumulation on GDD’s>0 created a range of dates for 63DAVD of February 12 to May 5.

This range application dates presented an opportunity to evaluate N application over a wide range of physiological growth stages and yet creates a challenge in that there is an inconsistency in growth stages evaluated across site years.

Grain Yield and Protein Response to Nitrogen

To provide a general overview of all trials, Figures 1 through 8 graphically represent the grain yield and grain protein content for each treatment at each site-year. In order to verify the effect of N in the crop an ANOVA test was performed by trial. After a significant effect of N was detected (*P*=0.05), a multiple comparison Dunnett's procedure was performed against the no N check (trt 2) to identify treatments resulting in a significant difference in grain yield and protein concentration (Table 6 and 7, respectively).

For the locations Perkins2017, Stillwater2017, LCB2017a, LCB 2017b, Lahoma2018 and LCB2018a, all treatments significantly increased grain yield above the check (Table 6). At LCB2018b, only 0, 7, 14, and 21DAVD treatments showed significantly higher yields that the check. Perkins2018 was the only site-year show no significant response to nitrogen applications based on Dunnett’s analysis. No N treatment at any site year resulted in a negative effect on yield.

With the exception of Perkins2017, all locations had at least one N application timing, which had a significant positive effect on grain protein concentration when compared to the check (Table 6). During the crop season of 2017/18, among the 44 N applications applied, only 5 did not show a significant effect on protein. While in the 2016/17 cropping season, only 13 of the 42 measured treatments significantly increased protein above the non-fertilized check. This effect can be related with the difference on rainfall between the years. The crop season of 2016-2017 had a substantial increase on rainfall when compared with the crop season of 2017-2018 (Table 12). The increase on the rainfall lead an increase on yields, which could be the reason that protein content on the grains are lower as observed by Lollato and Edwards (2015).

Evaluation of the Timing of Nitrogen Application

In order to analyze the impact on in-season application timing on yield and grain protein concentration as related to the pre-plant (trt 1) and 0DAVD (trt 3), the same comparinson test was performed using pre-plant treatment as control for grain yield and protein (tables 8 and 9 respectively). The same analyses were performed utilizing the 0DAVD as a control, also for grain yield and protein (tables 10 and 11 respectively). The first allows the evaluation of in-season N application while the latter addresses the purpose of the study.

As shown in Tables 8 and 10, only three of the 78 comparisons made back to the pre-plant application were significantly less considering yield. All three of these comparisons resulted when N application was delayed until late march or more. When compared to the 0DAVD only two of the 68 comparisons showed a significant decrease on yield. Of these two significant comparisons, one was the pre-plant application for LCB2017a while the other were the 63DAVD application for LCB2017b. It should be noted that while not always significant a numeric decrease in yield when compared to 0DAVD can be noted in the majority of the N applications made during the month of April. In the 2016/17 cropping year 16 of the 38 comparisons for grain yield made back to the pre-plant N application showed a significant increase in yield, while 8 of the 34 comparisons made to 0DAVD grain yield resulted in a significant yield increase. Conversely, there was no timing during the 2017/18 cropping season in which grain yield was significantly greater than the pre-plant or 0DAVD (Table 8 and 10). These results likely highlight to the difference in spring rainfall between the first and second cropping seasons of the project (Table 12). The higher total rainfall on the 2016-2017 season would have increased the probability on N loss via leaching and was a significant component leading to the higher overall yields. The greater potential loss and higher demand for plant uptake likely led to the improved yields for the in-season applications of the first year of the study when compared with pre-plant. While we hypothesize, in drought conditions, and reduced yield potential, of the second cropping season reduced the impact of N timing on wheat grain yield.

Grain protein concentration was decreased only once when compared to both the pre-plant and 0DAVD treatments (Tables 9 and 11). This one timing, LCB2018b 64DAVD, was the only application made in May. During this time the crop was in the early stages of grain-fill. Of the 78 comparisons for protein concentration performed against the pre-plant treatment, in season application of N significantly increased protein 30 times. Eleven significant positive records in the 2016/17 crop and 19 significant positive responses in the 2017/18 crop. It should be noted that Perkins2018, the one location that did not have a significant yield response to N application, had nine positive grain protein responses to N fertilization. Perkins2017, was the only site not to have a positive response to N application in terms of protein concentration. Perkins2017 was the location with the earliest 63DAVD, which may have influenced this result. Others found similar results regarding the increase of grain protein when the timing of the N fertilization was delayed (Flower et al., 1989; Woolfolk et al., 2002)

Just as with yield, the number of significant positive responses to N application was reduced when in-season treatments were compared to 0DAVD. This indicates that the delaying of N application until first visual deficiency will likely produce higher protein values than when all N are applied pre-plant. There were nine positive responses document in the 2016/17 crop and seven in the 2017/18 crop. Unlike in the pre-plant comparison, Perkins2018 had zero significant difference between latter in-seasons application and the 0DAVD.

N Stress Recovery of Yield Based on GDD>0 from Planting

In order to better understand the response of N timing fertilization on yield, an analyses considering the cumulative GDD's>0 from planting date at the timings applications was performed. A linear model was implemented defining a quadratic response for GDD as fixed effects and blocks nested in each single trial as random effects. The unfertilized treatments were removed from the data set for this analyses. The data was normalized for the pre-plant and 0DAVD treatments as described before. Figures 9 and 10 represent the normalized grain yield pre-plant and 0DAVD. The data was plotted by cumulative GDD's>0 from planting (GDDFP) across all locations. Figure 10 provides fixed effects of GDDFP on grain yield normalized for pre-plant N application (T1), while Figure 10 depicts fixed effect of GDDFP on grain yield normalized for 0DAVD application (T3). By using the estimated coefficients of the model, it may be possible estimate the average time of the applications that yield loss can be observed (Table 13 and 14). The estimated yield response equation (T1 normalized and T3 normalized) considering only fixed effects, may be described as:

$$\hat{y} = 0.3517 + 0.01881 * GDDFP - 0.0001 * GDDFP^2 \quad (4)$$

$$\hat{y} = 0.3458 + 0.01794 * GDDFP - 0.0001 * GDDFP^2 \quad (5)$$

Considering the pre-plant application as the normalizing variable (equation 4), by replacing the values of GDD>0, normalized yield was estimated to significantly decline below 1.0 after 143 GDD>0 from planting. When the in-season results were normalized by 0DAVD (equation 5) N applications after 130 GDD>0 had a significant reduction in yield. The difference in the days to loss for pre-plant and 0DAVD, 143 and 130, also the higher yield achieved when N application was delayed until VSD. The quadratic model provides opportunity to identify the point of highest grain yield. This was 94 GDD>0 and 90 GDD>0 for the pre-plant and 0DAVD respectively. A summation of these models is that N could be applied anytime in season up to mid-April without any statistically significant yield loss, while the more risk adverse date would be mid to late March. The application on N in late January and early February maximized yield. Table 14 provides calendar dates for a range of GDD>0 for all locations.

Table 15 shows the fixed effects of GDDFP on % of protein content on the grain. Plots illustrate (figure 11) the quadratic response of N delay application observed for yield as well as the linear response of N delay application observed for grain protein concentration.

CHAPTER V

CONCLUSIONS

The application of nitrogen and its timing had a significant effect on the yield and protein concentration of winter wheat. The standards of pre-plant application and application made at first visual deficiency were used to compare to the remaining application dates. A negative effect on grain yield was observed when application was made later than late March. In addition, the agronomic optimum top-dress timings considering all trials were between the range of 80 to 100 GDD>0 after planting. At this period, the data suggested no negative impact on yield but quite likely positive event as 100% yield recovery was achieved in relation to the pre-plant and 0DAVD up to 140 GDD>0 after planting. A significant finding of this work was that reaching maximum achievable yield levels was not related to the point in time at which the crop goes under N stress. Rather, optimum grain yield recovery was mostly associated with the N applications made when the crop was most vigorously growing, during the Feekes 6 and 7 growth stages. Evaluation of N timing on protein concentration showed a trend for increasing protein values with an increasing delay of application.

These results are significant for the winter wheat growers of the southern Great Plains as this research documented not only the ability but the need to move from pre-plant and early fall N applications. The window for N application is likely much greater than much wheat producers would have ever considered. This work showed that not only could N be delayed and yield not sacrificed, but N could be delayed, yield maintained, and protein increased. As most producers use a urea based N source for in-season N application, which is known to have increased likelihood of losses as observed for Ernst and Massey (1960) and Guangming et al. (1998), this work also suggested that it is more important to time N application with proper weather events than at first sign of N stress.

The conclusion of this study was based on the results of two similar winters in Oklahoma, which were cold and relatively dry. Further studies should take in consideration the timings of the applications in other environments and conditions. Expending the study in other environments, earlier planting dates, multiple N rates, and potential N sources would provide an even better idea of the influence of the N application timing on grain yield and protein, and how producers should manage it.

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TABLES

Table 1. Soil series classification and description for all experimental sites (Lake Carl Blackwell, Perkins, Lahoma, Stillwater) utilized in the study evaluating the impact nitrogen fertilizer timing on winter wheat, conducted in north central Oklahoma over the 2016-2017 and 2017-2018 winter wheat growing seasons.

Location/Year	Soil Series and Description	Previous Crop
Perkins2017	Teller; (fine-loamy, mixed, active, thermic Udic Agriustoll)	Wheat
Stillwater2017	Kirkland; (Fine, mixed, superactive, thermic Udertic Paleustolls)	Wheat
LCB2017a	Port; (Fine-silty, mixed, superactive, thermic Cumulic Haplustolls)	Canola
LCB2017b	Pulaski; (Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents)	Wheat
Perkins2018	Konawa; (fine-loamy, mixed, active, thermic Ultic Haplustalf) Teller; (fine-loamy, mixed, active, thermic Udic Agriustoll)	Wheat
Lahoma2018	Grant; (Fine-silty, mixed, superactive, thermic Udic Argiustolls)	Wheat
LCB2018a	Pulaski; (Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents)	Wheat
LCB2018b	Pulaski; (Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents	Fallow

Table 2. Soil test results pH, buffer index, nitrogen, Mehlich 3 phosphorus, potassium, sulfate, calcium, magnesium, iron, zinc, boron, and copper concentrations 0-15 cm for all experimental sites (Lake Carl Blackwell, Perkins, Lahoma, Stillwater) utilized in the study evaluating the impact nitrogen fertilizer timing on winter wheat, conducted in north central Oklahoma over the 2016-2017 and 2017-2018 growing seasons..

Location/Year	pH	BI	N	P	K	SO_4	Ca	Mg	Fe	Zn	B	Cu	OM
Unit			kg ha^{-1}						ppm				g kg^{-1}
Perkins2017	5.6	6.6	31	36	193	14	667	178	27.6	0.4	0.1	1.1	11
Stillwater2017	6.1	6.9	31	10	108	14	3187	1541	24.4	0.3	0.2	0.3	19
LCB2017a			31	24	124	8	2965	673	24.1	0.5	0.1	0.9	16
LCB2017b	5.7	7.1	21	28	99	5	1688	341	26.7	0.3	0.1	0.8	9
Perkins2018	na	na	na	25	143	na	na	na	na	na	na	na	na
Lahoma2018	5.6	6.9	21	50	278	7	2424	390	na	na	na	na	na
LCB2018a	5.8	7.2	5	21	91	4	1729	337	na	na	na	na	na
LCB2018b	6.2	na	17	33	151	8	3545	612	na	na	na	na	na

na = not available

Table 3. Planting dates and seed rate for all experimental sites (Lake Carl Blackwell, Perkins, Lahoma, Stillwater) utilized in the study evaluating the impact nitrogen fertilizer timing on winter wheat, conducted in north central Oklahoma over the 2016-2017 and 2017-2018 winter wheat growing seasons.

Trial/year	Planting date	Seed rate (kg ha^{-1})
Perkins2017	10/13/2016	84
Stillwater2017	10/13/2016	84
LCB2017a	10/12/2017	84
LCB2017b	10/12/2017	84
Perkins2018	10/12/2017	84
Lahoma2018	10/12/2017	84
LCB2018a	10/11/2017	84
LCB2018b	10/11/2017	84

The letters a and b stand for different previous crop in the same site and year.

Table 4. Treatment structure for all experimental sites (Lake Carl Blackwell, Perkins, Lahoma, Stillwater) utilized in the study evaluating the impact nitrogen fertilizer timing on winter wheat, conducted in north central Oklahoma over the 2016-2017 and 2017-2018 winter wheat growing seasons. trials performed in Stillwater, Lahoma, Perkins and Lake Carl Blackwell, OK in 2016-2017 and 2017-2018 crop seasons.

Treatment	Fertilizer Timing	Nitrogen Rate (kg ha^{-1})
1	Pre-plant	100
2	Check	0
3	0 DAVD	100
4	7 DAVD	100
5	14 DAVD	100
6	21 DAVD	100
7	28 DAVD	100
8	35 DAVD	100
9	42 DAVD	100
10	49 DAVD	100
11	56 DAVD	100
12	63 DAVD	100

DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented.

Table 5. Dates for all pre-plant and in-season nitrogen applications dates for the experimental sites (Lake Carl Blackwell, Perkins, Lahoma, Stillwater) utilized in the study evaluating the impact nitrogen fertilizer timing on winter wheat, conducted in north central Oklahoma over the 2016-2017 and 2017-2018 growing seasons.

	1	2	3	4	5	6	7	8	9	10	11	12
Trial	Pre-plant	Check	0DAVD	7DAVD	14DAVD	21DAVD	28DAVD	35 DAVD	42DAVD	49DAVD	56DAVD	63DAVD
Perkins2017	10/13/2016	na	11/10/2016	11/17/2016	11/24/2016	12/1/2016	12/21/2016	12/29/2016	1/12/2017	1/23/2017	2/2/2017	2/12/2017
Stillwater2017	10/13/2016	na	11/10/2016	11/17/2016	11/24/2016	12/1/2016	12/21/2016	1/2/2017	1/19/2017	1/30/2017	2/12/2017	2/20/2017
LCB2017a	10/12/2016	na	12/21/2016	1/9/2017	1/19/2017	1/30/2017	2/12/2017	2/20/2017	3/1/2017	3/9/2017	3/19/2017	3/27/2017
LCB2017b	10/12/2016	na	1/30/2017	2/12/2017	2/20/2017	3/1/2017	3/9/2017	3/19/2017	3/27/2017	4/4/2017	4/11/2017	4/19/2017
Perkins2018	10/12/2017	na	12/22/2017	1/23/2018	2/5/2018	2/19/2018	3/3/2018	3/11/2018	3/18/2018	3/26/2018	4/3/2018	4/12/2018
Lahoma2018	10/12/2017	na	12/21/2017	1/22/2018	2/1/2018	2/26/2018	3/6/2018	3/15/2018	3/23/2018	3/30/2018	4/6/2018	4/17/2018
LCB2018a	10/11/2017	na	12/22/2017	1/23/2018	2/5/2018	2/26/2018	3/6/2018	3/15/2018	3/22/2018	3/30/2018	4/9/2018	4/18/2018
LCB2018b	10/11/2017	na	2/5/2018	2/26/2018	3/6/2018	3/15/2018	3/22/2018	3/30/2018	4/9/2018	4/18/2018	4/26/2018	5/2/2018

DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was Documented;
na = not applicable;
Different colors in the table display different months.

Table 6. Winter wheat grain yield (kg ha^{-1}) as affected by the timing of application of 100 kg N ha^{-1} at all trials locations in Oklahoma in 2016-2017 and 2017-2018 crop seasons. Multiple comparison utilizing Dunnett's test (Check without N application as a control) is demonstrated by the asterisks evaluating the effect of N application on winter wheat grain yield.

Trials	Treatments											
	1	2	3	4	5	6	7	8	9	10	11	12
	Pre-plant	Check	0 DAVD	7 DAVD	14 DAVD	21 DAVD	28 DAVD	35 DAVD	42 DAVD	49 DAVD	56 DAVD	63 DAVD
Perkins2017	3144***	1514	3126***	3538***	3676***	na	3768***	3858***	3916***	4029***	4011***	3797***
Stillwater2017	2452*	1429	2929***	3032***	3096***	na	3547***	3225***	3389***	43453***	3381***	3878***
LCB2017a	4022***	2582	4935***	4457***	4792***	4661***	4870***	5580***	5858***	5902***	5671***	5615***
LCB2017b	4166***	2192	4536***	4637***	5079***	5702***	5655***	5651***	5552***	4796***	4030***	3156*
Perkins2018	3845	3389	3774	3844	3903	3576	3624	3781	3262	3345	3003	3399
Lahoma2018	3603***	2080	3413***	**3676*** **	3821***	3559***	3432***	3546***	3052**	2811*	2939**	2830*
LCB2018a	3846***	1448	4376***	4225***	3577**	3954***	4113***	4205***	4402***	3791**	3484**	3091*
LCB2018b	3801	2547	4229*	4696**	2865	4755**	4563*	3321	3254	3139	2630	2792

Significance levels are indicated by the asterisks as:
** is significant at the 0.05 probability level;*
*** is significant at the 0.01 probability level;*
**** is significant at 0.001of probability level;*
The colors displayed in the table illustrates the differences for each of the means comparisons as:
yellow = not significantly different;
green = significantly greater;
DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented;
na = not available.

Table 7. Winter wheat grain protein content (%) as affected by the timing of application of 100 kg N ha^{-1} at all trials locations in Oklahoma in 2016-2017 and 2017-2018 crop seasons. Multiple comparison utilizing Dunnett's test (Check without N application as a control) is demonstrated by the asterisks evaluating the effect of N application on winter wheat grain yield.

	Treatments											
	1	2	3	4	5	6	7	8	9	10	11	12
Trials	Pre-plant	Check	0 DAVD	7 DAVD	14 DAVD	21 DAVD	28 DAVD	35 DAVD	42 DAVD	49 DAVD	56 DAVD	63 DAVD
Perkins2017	12.7	11.9	13.0	13.0	13.5	na	13.3	11.8	12.8	13.4	12.5	13.2
Stillwater2017	10.5	10.4	10.7	11.1	10.8	na	11.0	11.0	11.5***	11.3**	11.2*	12.2***
LCB2017a	7.8	8.5	7.7	7.4	7.6	7.9	7.8	9.0	8.8	9.1	10.3*	10.8**
LCB2017b	7.7	7.3	8.8	8.3	9.3*	9.8**	8.7	9.5**	10.7***	11***	12.9***	13.9***
Perkins2018	12**	9.7	14.3***	13.8***	13.9***	13.5***	15***	13.9***	15***	14.9***	15.5***	15.9***
Lahoma2018	11.5**	9.5	12.6***	**12.2*** **	11.9**	12.8***	12**	12.4***	13.9***	14***	13.4***	13.8***
LCB2018a	11.5	9.5	11.7	11.6	12.1*	12.8***	11.6	12.9***	12.8**	13.2***	14.5***	14.2***
LCB2018b	12.3***	9.5	12.3***	12.6***	12**	12.4***	13.1***	14.5***	14.6***	15***	15.4***	10.1

Significance levels are indicated by the asterisks as:
** is significant at the 0.05 probability level;*
*** is significant at the 0.01 probability level;*
**** is significant at 0.001of probability level;*
The colors displayed in the table illustrates the differences for each of the means comparisons as:
yellow = not significantly different;
green = significantly greater;
DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented;
na = not available.

Table 8. Winter wheat grain yield (kg ha^{-1}) as affected by the timing of application of 100 kg N ha^{-1} at all trials locations in Oklahoma in 2016-2017 and 2017-2018 crop seasons. Multiple comparison utilizing Dunnett's test (pre-plant N application treatment as control) is demonstrated by the asterisks evaluating the effect of N application on winter wheat grain yield.

Trials	Tretaments									
	3	4	5	6	7	8	9	10	11	12
	0 DAVD	7 DAVD	14 DAVD	21 DAVD	28 DAVD	35 DAVD	42 DAVD	49 DAVD	56 DAVD	63 DAVD
Perkins2017	3126	3538	3676	na	3768	3858	3916	4029	4011	3797
Stillwater2017	2929	3032	3096	na	3547*	3225	3389	43453*	3381	3878**
LCB2017a	4935**	4457	4792*	4661	4870*	5580***	5858***	5902***	5671***	5615***
LCB2017b	4536	4637	5079*	5702***	5655***	5651***	5552***	4796	4030	3156**
Perkins2018	3774	3844	3903	3576	3624	3781	3389	3345	3003	3399
Lahoma2018	3413	3676	3821	3559	3432	3546	3052	2811*	2939	2830*
LCB2018a	4376	4225	3577	3954	4113	4205	4402	3791	3484	3091
LCB2018b	4229	4696	2865	4755	4563	3321	3254	3139	2630	2792

Significance levels are indicated by the asterisks as:
** is significant at the 0.05 probability level;*
*** is significant at the 0.01 probability level;*
**** is significant at 0.001of probability level;*
The colors displayed in the table illustrates the differences for each of the means comparisons as:
yellow = not significantly different;
green = significantly greater;
red = significantly lower;
DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented;
na = not available.

Table 9. Winter wheat grain protein content (%) as affected by the timing of application of 100 kg N ha^{-1} at all trials locations in Oklahoma in 2016-2017 and 2017-2018 crop seasons. Multiple comparison utilizing Dunnett's test (pre-plant N application treatment as control) is demonstrated by the asterisks evaluating the effect of N application on winter wheat grain yield.

Trials	Treatments									
	3	4	5	6	7	8	9	10	11	12
	0 DAVD	7 DAVD	14 DAVD	21 DAVD	28 DAVD	35 DAVD	42 DAVD	49 DAVD	56 DAVD	63 DAVD
Perkins2017	13.0	13.0	13.5	na	13.3	11.8	12.8	13.4	12.5	13.2
Stillwater2017	10.7	11.1	10.8	na	11.0	11.0	11.5**	11.3**	11.2*	12.2***
LCB2017a	7.7	7.4	7.6	7.9	7.8	9.0	8.8	9.1	10.3***	10.8***
LCB2017b	8.8	8.3	9.3	9.8*	8.7	9.5	10.7***	11***	12.9***	13.9***
Perkins2018	14.3**	13.8*	13.9*	13.5	15***	13.9*	15***	14.9***	15.5***	15.9***
Lahoma2018	12.6	**12.2**	11.9	12.8	12.0	12.4	13.9**	14**	13.4*	13.8**
LCB2018a	11.7	11.6	12.1	12.8	11.6	12.9	12.8	13.2	14.5**	14.2**
LCB2018b	12.3	12.6	12.0	12.4	13.1	14.5**	14.6**	15***	15.4***	10.1**

Significance levels are indicated by the asterisks as:
** is significant at the 0.05 probability level;*
*** is significant at the 0.01 probability level;*
**** is significant at 0.001of probability level;*
The colors displayed in the table illustrates the differences for each of the means comparisons as:
yellow = not significantly different;
green = significantly greater;
red = significantly lower;
DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented;
na = not available.

Table 10. Winter wheat grain yield (kg ha^{-1}) as affected by the timing of application of 100 kg N ha^{-1} at all trials locations in Oklahoma in 2016-2017 and 2017-2018 crop seasons. Multiple comparison utilizing Dunnett's test (N application timing at 0 growing degree days > 0 after visual symptom differentiation treatment as control) is demonstrated by the asterisks evaluating the effect of N application on winter wheat grain yield.

	1	4	5	6	7	8	9	10	11	12
Trials	Pre-plant	7 DAVD	14 DAVD	21 DAVD	28 DAVD	35 DAVD	42 DAVD	49 DAVD	56 DAVD	63 DAVD
Perkins2017	3144	3538	3676	na	3768	3858	3916	4029	4011	3797
Stillwater2017	2452	3032	3096	na	3547	3225	3389	43453	3381	3878*
LCB2017a	4022**	4457	4792	4661	4870	5580	5858**	5902**	5671*	5615
LCB2017b	4166	4637	5079	5702**	5655**	5651**	5552**	4796	4030	3156***
Perkins2018	3845	3844	3903	3576	3624	3781	3262	3345	3003	3399
Lahoma2018	3603	3676	3821	3559	3432	3546	3052	2811	2939	2830
LCB2018a	3846	4225	3577	3954	4113	4205	4402	3791	3484	3091
LCB2018b	3801	4696	2865	4755	4563	3321	3254	3139	2630	2792

Significance levels are indicated by the asterisks as:
** is significant at the 0.05 probability level;*
*** is significant at the 0.01 probability level;*
**** is significant at 0.001of probability level;*
The colors displayed in the table illustrates the differences for each of the means comparisons as:
yellow = not significantly different;
green = significantly greater;
red = significantly lower;
DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented;
na = not available.

Table 11. Winter wheat grain protein content (%) as affected by the timing of application of 100 kg N ha^{-1} at all trials locations in Oklahoma in 2016-2017 and 2017-2018 crop seasons. Multiple comparison utilizing Dunnett's test (N application timing at 0 growing degree days > 0 after visual symptom differentiation treatment as control) is demonstrated by the asterisks evaluating the effect of N application on winter wheat grain yield.

	Treatments									
	1	4	5	6	7	8	9	10	11	12
Trials	Pre-plant	7 DAVD	14 DAVD	21 DAVD	28 DAVD	35 DAVD	42 DAVD	49 DAVD	56 DAVD	63 DAVD
Perkins2017	12.7	13.0	13.5	na	13.3	11.8	12.8	13.4	12.5	13.2
Stillwater2017	10.5	11.1	10.8	na	11.0	11.0	11.5**	11.3	11.2	12.2***
LCB2017a	7.8	7.4	7.6	7.9	7.8	9.0	8.8	9.1*	10.3***	10.8***
LCB2017b	7.7	8.3	9.3	9.8	8.7	9.5	10.7*	11*	12.9***	13.9***
Perkins2018	12**	13.8	13.9	13.5	15.0	13.9	15.0	14.9	15.5	15.9*
Lahoma2018	11.5	**12.2**	11.9	12.8	12.0	12.4	13.9	14.0	13.4	13.8
LCB2018a	11.5	11.6	12.1	12.8	11.6	12.9	12.8	13.2	14.5**	14.2*
LCB2018b	12.3	12.6	12.0	12.4	13.1	14.5**	14.6**	15***	15.4***	10.1**

Significance levels are indicated by the asterisks as:
** is significant at the 0.05 probability level;*
*** is significant at the 0.01 probability level;*
**** is significant at 0.001of probability level;*
The colors displayed in the table illustrates the differences for each of the means comparisons as:
yellow = not significantly different;
green = significantly greater;
red = significantly lower;
DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented;
na = not available.

Table 12. Monthly Rainfall (millimeters) data for the winter wheat growing season (months of September, October, November and December, January, February, March, April and May) 2016-2017 and 2017-2018 for the locations of Perkins, Stillwater, Lake Carl Blackwell and Lahoma. Data obtained from the Oklahoma Mesonet website.

	2016-2017									
	September	October	November	December	January	February	March	April	May	Total
Perkins	60	54	55	12	67	50	60	230	101	690
LCB	75	18	10	32	62	68	46	259	87	656
Stillwater	65	98	22	10	65	56	49	252	66	684
	2017-2018									
	September	October	November	December	January	February	March	April	May	Total
Perkins	69	144	7	16	4	83	20	66	100	507
LCB	80	176	11	13	5	59	30	51	76	500
Lahoma	54	58	4	2	0	34	24	0	80	253

LCB = Lake Carl Blackwell, OK

Data obtained from the Oklahoma Mesonet website

Table 13. Linear mixed model demonstrating the effect on winter wheat grain yield as relation of growing degree days > 0 from planting at the N application timing across eight trials evaluating the impact nitrogen fertilizer timing on winter wheat, conducted in north central Oklahoma performed between 2016 and 2018.

	Solution for Fixed Effects				
Variable	**Estimate**	**Standard Erros**	**DF**	**t Value**	**Pr > |t|**
Intercept	0.3517	0.1282	239	2.74	0.0066
GDDFP	0.01881	0.002595	221	7.25	<.0001
GDDFP ^{2}	-0.0001	0.000013	220	-7.93	<.0001
	Fit Statistics				
	-2 Res Log Likelihood		-78.5		
	AIC		-74.5		
	Covariance Parameter Estimates				
	Cov Parm		Estimate		
	Location(Block)		0.04072		
	Residual		0.02796		

GDDFP = Growing degree days > 0 from planting;
Treatments where the N applications were not performed were removed from the data;
Normalization was performed on a by rep basis for all locations dividing the other treatments results by the pre-plant treatment.

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Table 14. Linear mixed model demonstrating the effect on winter wheat grain yield as relation of Growing degree days > 0 from planting at the N application timing across eight trials evaluating the impact nitrogen fertilizer timing on winter wheat, conducted in north central Oklahoma performed between 2016 and 2018.

	Solution for Fixed Effects				
Variable	**Estimate**	**Standard Erros**	**DF**	**t Value**	**Pr > |t|**
Intercept	0.3458	0.1576	215	2.19	0.0293
GDDFP	0.01794	0.00314	201	5.71	<.0001
$GDDFP^2$	-0.0001	0.000015	199	-6.58	<.0001

Fit Statistics	
-2 Res Log Likelihood	-80.5
AIC	-76.5
Covariance Parameter Estimates	
Cov Parm	Estimate
Location(Block)	0.02553
Residual	0.02707

GDDFP = Growing degree days > 0 from planting;
Treatments where the N applications were not performed were removed from the data;
Normalization was performed on a by rep basis for all locations dividing the other treatments results by the treatment applied at the first sign of visual nitrogen deficiency of the crop.

Table 15. Linear mixed model demonstrating the effect on winter wheat grain protein content as relation of Growing degree days > 0 from planting at the N application timing across eight trials evaluating the impact nitrogen fertilizer timing on winter wheat, conducted in north central Oklahoma performed between 2016 and 2018.

	Solution for Fixed Effects				
Variable	**Estimate**	**Standard Erros**	**DF**	**t Value**	**Pr > |t|**
Intercept	-0.06729	0.03044	90	-2.21	0.0296
GDDFP	-0.00487	0.03044	90	-0.16	0.8733
GDDFP ^{2}	-0.02565	0.03044	90	-0.84	0.4016
	Fit Statistics				
	-2 Res Log Likelihood		-80.5		
	AIC		-334.8		
	Covariance Parameter Estimates				
	Cov Parm		Estimate		
	Location(Block)		0.005468		
	Residual		0.008005		

GDDFP = Growing degree days > 0 from planting;
Treatments where the N applications were not performed were removed from the data;
Normalization was performed on a by rep basis for all locations dividing the other treatments results by the treatment applied at the first sign of visual nitrogen deficiency of the crop.

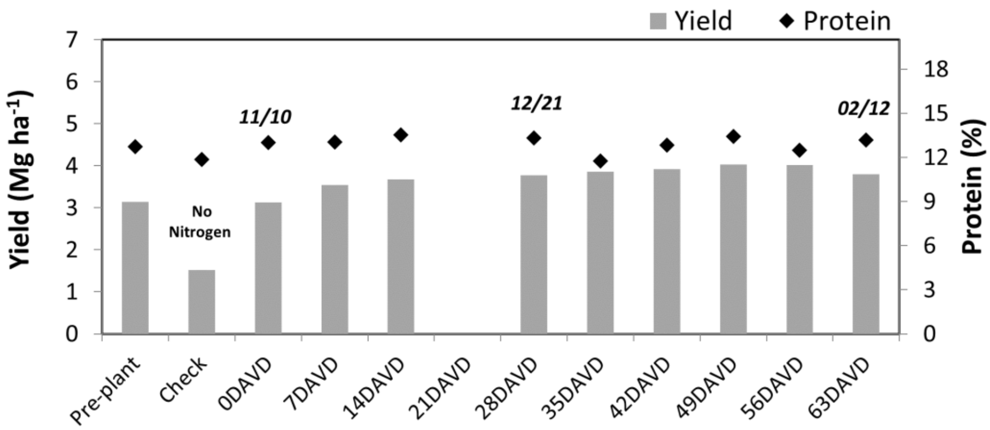
Table 16. Growing degree days > 0 from planting and equivalent calendar days for all experimental sites (Lake Carl Blackwell, Perkins, Lahoma, Stillwater) utilized in the study evaluating the impact nitrogen fertilizer timing on winter wheat, conducted in north central Oklahoma over the 2016-2017 and 2017-2018 winter wheat growing seasons.

	Growing Degree Days>0 from planting						
	0	25	50	75	100	125	150
Perkins2017	10.13.16	11.6.16	12.2.16	1.19.17	2.20.17	3.23.17	4.21.17
Stillwater17	10.13.16	11.6.16	12.2.16	1.23.17	2.27.17	3.27.17	4.21.17
LCB2017	10.12.16	11.5.16	12.1.16	1.24.17	2.28.17	3.29.17	4.23.17
Perk2018	10.12.17	11.6.17	12.2.17	1.27.18	3.8.18	4.3.18	5.1.18
Lahoma2018	10.12.17	11.8.17	12.5.17	1.28.18	3.14.18	4.11.18	5.7.18
LCB2018	10.11.17	11.7.17	12.3.17	1.30.18	3.14.18	4.11.18	5.7.18

GDD>0 obtained from the Oklahoma Mesonet website.

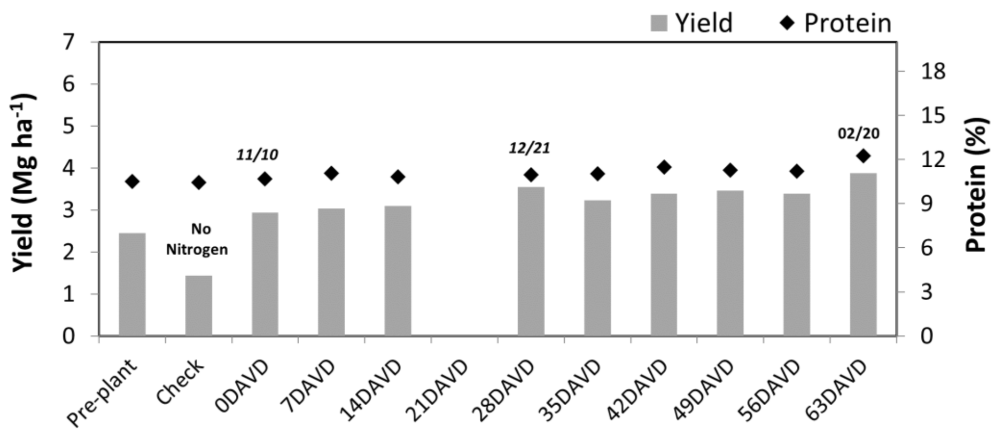
FIGURES

Figure 1. Winter wheat grain yield and protein response to the application of 100 kg N ha^{-1} as affected by the timing of application at Perkins, OK in 2016-2017



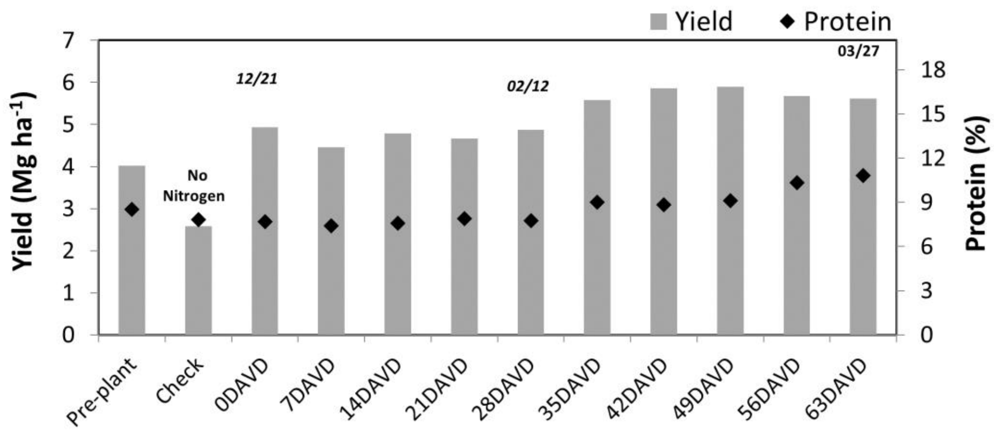
DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented;.
Dates on top of the bars indicates the date of the treatment application;
The treatment 21DAVD is missing due to unavailable data.

Figure 2. Winter wheat grain yield and protein response to the application of 100 kg N ha^{-1} as affected by the timing of application at Stillwater, OK in 2016-2017.



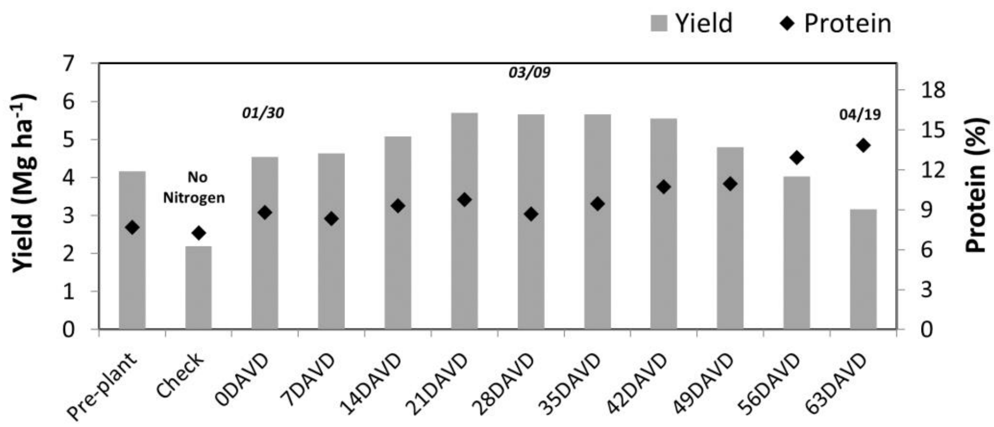
DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented.
Dates on top of the bars indicates the date of the treatment application.
The treatment 21DAVD is missing due to unavailable data.

Figure 3. Winter wheat grain yield and protein response to the application of 100 kg N ha^{-1} as affected by the timing of application at Lake Carl Blackwell, OK (a) in 2016-2017.



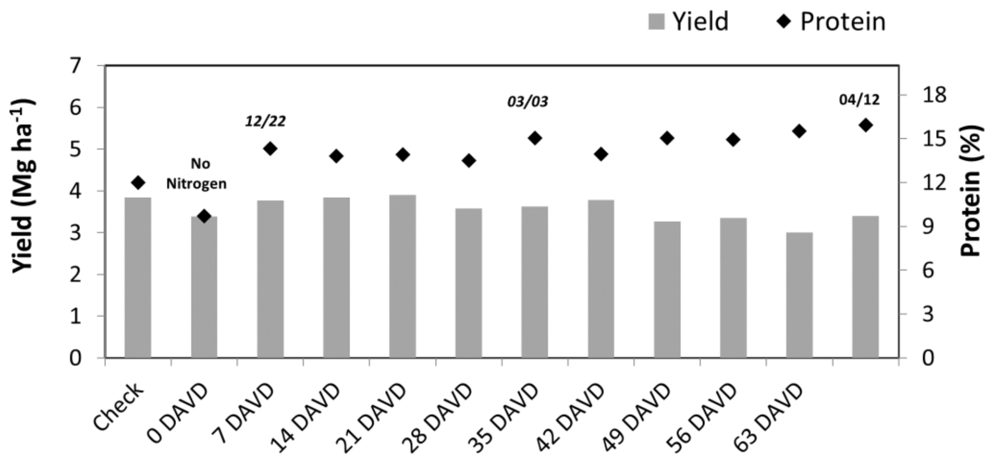
DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented. Dates on top of the bars indicates the date of the treatment application.

*F*igure 4. Winter wheat grain yield and protein response to the application of 100 kg N ha^{-1} as affected by the timing of application at Lake Carl Blackwell, OK (b) in 2016-2017.



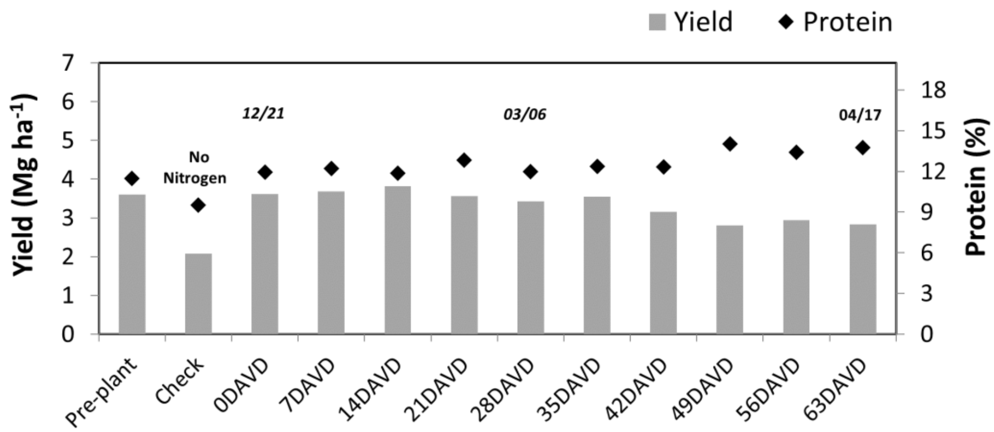
DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented. Dates on top of the bars indicates the date of the treatment application.

Figure 5. Winter wheat grain yield and protein response to the application of 100 kg N ha^{-1} as affected by the timing of application at Perkins, OK in 2017-2018.



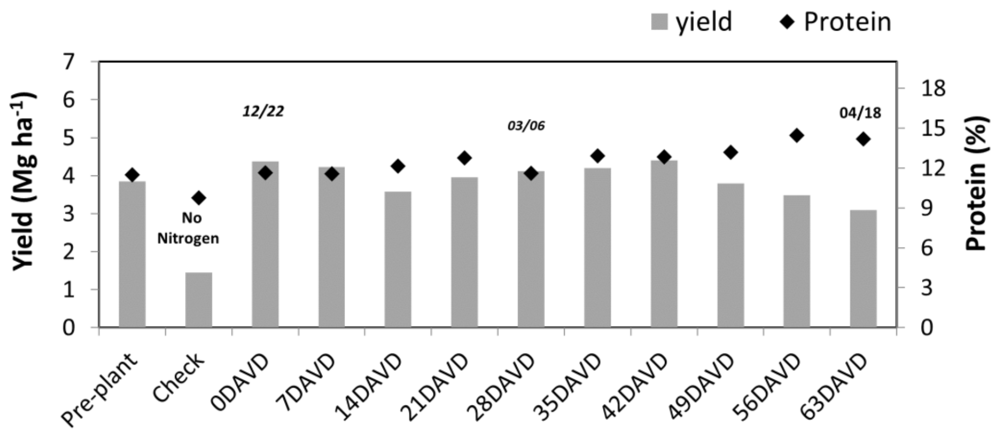
DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented. Dates on top of the bars indicates the date of the treatment application.

Figure 6. Winter wheat grain yield and protein response to the application of 100 kg N ha^{-1} as affected by the timing of application at Stillwater, OK in 2017-2018.



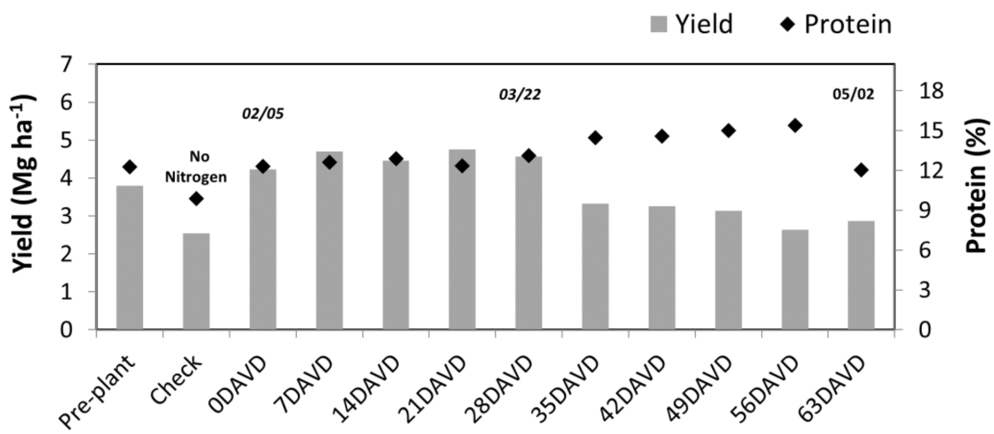
DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented. Dates on top of the bars indicates the date of the treatment application.

Figure 7. Winter wheat grain yield and protein response to the application of 100 kg N ha^{-1} as affected by the timing of application at Lake Carl Blackwell, OK (a) in 2017-2018.



DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented. Dates on top of the bars indicates the date of the treatment application.

Figure 8. Winter wheat grain yield and protein response to the application of 100 kg N ha^{-1} as affected by the timing of application at Lake Carl Blackwell, OK (b) in 2017-2018.



DAVD = Growing degree days > 0 after nitrogen visual symptom differentiation was documented. Dates on top of the bars indicates the date of the treatment application.

VITA

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