

**Supplementary Table S1.** Dates for all pre-plant and in-season nitrogen (N) applications dates for the experimental sites conducted in north central Oklahoma over the 2016-2017, 2017-2018, 2018-2019 and 2019-2020 growing seasons.

	Treatments										
	1	3	4	5	6	7	8	9	10	11	12
	Pre-plant	0DAVD	7DAVD	14DAVD	21DAVD	28DAVD	35 DAVD	42DAVD	49DAVD	56DAVD	63DAVD
Perkins2017	10/13/2016	11/10/2016	11/17/2016	11/24/2016	na	12/21/2016	12/29/2016	1/12/2017	1/23/2017	2/2/2017	2/12/2017
Stillwater2017	10/13/2016	11/10/2016	11/17/2016	11/24/2016	na	12/21/2016	1/2/2017	1/19/2017	1/30/2017	2/12/2017	2/20/2017
LCB2017a	10/12/2016	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	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	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	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	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	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
Newkirk2019	10/24/2018	3/7/2019	3/15/2019	3/22/2019	3/28/2019	4/5/2019	4/12/2019	4/19/2019	4/29/2019	5/7/2019	5/14/2019
LCB2019	11/7/2018	3/8/2019	3/15/2019	3/22/2019	3/28/2019	4/7/2019	4/15/2019	4/22/2019	4/29/2019	5/7/2019	na
Newkirk2020	10/18/2019	2/4/2020	2/25/2020	3/3/2020	3/11/2020	3/23/2020	3/29/2020	4/6/2020	4/14/2020	4/21/2020	4/28/2020
LCB2020	10/15/2019	2/3/2020	2/21/2020	3/5/2020	3/12/2020	3/23/2020	3/29/2020	4/9/2020	4/16/2020	4/21/2020	4/28/2020

na = not applicable; Different colors in the table display different months.

**Supplementary Table S2.** Winter wheat grain yield (kg ha<sup>-1</sup>) and protein (%) as affected by the timing of application of 100 kg N ha<sup>-1</sup> at all trial locations in Oklahoma in 2016-2017, 2017-2018, 2018-2019, and 2019-2020. Multiple comparisons utilizing Dunnett's test (pre-plant treatment as control) are demonstrated by the asterisks (\*) evaluating the effect of N application on winter wheat grain yield and protein. Boxes shaded pink represent grain yield or protein concentration which were statistically less than the pre-plant application, while the green shaded boxes represent a significant increase in grain yield or protein.

		Treatments										
		1	3	4	5	6	7	8	9	10	11	12
		Check	0 DAVD	7 DAVD	14 DAVD	21 DAVD	28 DAVD	35 DAVD	42 DAVD	49 DAVD	56 DAVD	63 DAVD
Perkins2017	Yield	***	ns	ns	ns	na	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	na	ns	ns	ns	ns	ns	ns
Stillwater2017	Yield	*	ns	ns	ns	na	*	ns	ns	*	ns	**
	Protein	ns	ns	ns	ns	na	ns	ns	**	***	*	***
LCB2017a	Yield	***	**	ns	*	ns	*	***	***	***	***	***
	Protein	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	***
LCB2017b	Yield	***	ns	ns	*	***	***	***	***	ns	ns	**
	Protein	ns	ns	ns	ns	*	ns	ns	***	***	***	***
Perkins2018	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	**	**	*	*	ns	***	*	***	***	***	***
Lahoma2018	Yield	***	ns	ns	ns	ns	ns	ns	ns	*	ns	*
	Protein	**	ns	ns	ns	ns	ns	ns	**	**	*	**
LCB2018a	Yield	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	**
LCB2018b	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	***	ns	ns	ns	ns	ns	**	**	***	***	**
Newkirk2019	Yield	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	*	ns	***	**	**	***	***	***	***	***	***
LCB2019	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	na
	Protein	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	na
Newkirk2020	Yield	***	ns	ns	ns	ns	ns	ns	*	*	***	***
	Protein	**	ns	ns	ns	ns	ns	*	***	**	***	***
LCB2020	Yield	***	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	ns	ns	***	***	***	***	***

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 0.001 probability level; ns: not significant at 0.05 probability level; na: not available; For the trial of LCB2020 the actual visual deficiency was noticed at treatment 4.

**Supplementary Table S3.** Winter wheat grain yield (kg ha<sup>-1</sup>) and protein (%) as affected by the timing of application of 100 kg N ha<sup>-1</sup> at all trials locations in Oklahoma in 2016-2017, 2017-2018, 2018-2019 and 2019-2020 crop seasons. Multiple comparison using Dunnett's test (treatment 3 as control) is demonstrated by the asterisks (\*) evaluating the effect of N application on winter wheat grain yield and protein concentration. Boxes shaded pink represent grain yield or protein concentration which were statistically less than the pre-plant application, while the green shaded boxes represents significant increase in grain yield or protein, most dramatic when the timing of N application progressed past Feekes 8.

		Treatments									
		2	4	5	6	7	8	9	10	11	12
		Pre-plant	7 DAVD	14 DAVD	21 DAVD	28 DAVD	35 DAVD	42 DAVD	49 DAVD	56 DAVD	63 DAVD
Perkins2017	Yield	ns	ns	ns	na	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	na	ns	ns	ns	ns	ns	ns
Stillwater2017	Yield	ns	ns	ns	na	ns	ns	ns	ns	ns	*
	Protein	ns	ns	ns	na	ns	ns	**	ns	ns	***
LCB2017a	Yield	**	ns	ns	ns	ns	ns	**	**	*	ns
	Protein	ns	ns	ns	ns	ns	ns	ns	*	***	***
LCB2017b	Yield	ns	ns	ns	**	**	**	**	ns	ns	***
	Protein	ns	ns	ns	ns	ns	ns	*	*	***	***
Perkins2018	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	**	ns	ns	ns	ns	ns	ns	ns	ns	*
Lahoma2018	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
LCB2018a	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	ns	ns	ns	ns	**	*
LCB2018b	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	ns	**	**	***	***	**
Newkirk2019	Yield	ns	ns	ns	ns	ns	ns	*	***	***	***
	Protein	ns	**	ns	ns	ns	***	***	***	***	***
LCB2019	Yield	ns	ns	ns	ns	ns	**	ns	**	ns	na
	Protein	ns	**	ns	ns	ns	***	***	***	***	na
Newkirk2020	Yield	ns	ns	ns	ns	ns	ns	ns	ns	**	***
	Protein	ns	ns	ns	ns	ns	ns	**	*	***	***
LCB2020	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	ns	***	***	***	***	***

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 0.001 probability level; ns: not significant at 0.05 probability level; na: not available; For the trial of LCB2020 the actual visual deficiency was noticed at treatment 4.