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Response of leaf area ratio, specific leaf weight, grain moisture content and quality traits of spring maize (*Zea mays* L.) to nitrogen dose and application timing

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Abstract

A field experiment was conducted at Regional Research Station, Uchani, Karnal of CCS Haryana Agricultural University during two consecutive spring seasons. The study aimed to assess the response of leaf area ratio (LAR), specific leaf weight (SLW), grain moisture content and quality traits of spring maize (*Zea mays* L.) to nitrogen dose and application timing. The experiment comprised four nitrogen doses, N₁ (150 kg N ha⁻¹), N₂ (165 kg N ha⁻¹), N₃ (180 kg N ha⁻¹) and N₄ (195 kg N ha⁻¹) assigned to main plots, and four nitrogen application timings were allocated to subplots, S₁ (50% at sowing + 25% at 8-leaf stage + 25% at tassel initiation), S₂ (25% at sowing + 25% at 4-leaf + 25% at 8-leaf + 25% at silking), S₃ (20% at sowing + 30% at 6-leaf + 40% at flowering + 10% at grain formation) and S₄ (20% at 2-leaf + 30% at 6-leaf + 40% at tassel initiation + 10% at grain formation). The treatments were arranged in a strip plot design with three replications. The results indicated that LAR and SLW were significantly influenced by different nitrogen doses at all growth stages except at 25 DAS during both years. The highest LAR and SLW values were recorded with N₁, which was statistically at par with N₂ but significantly higher than N₃ and N₄. Among the nitrogen application timing, S₃ produced the highest LAR and SLW followed by S₄ and it was higher than S₁ and S₂. Grain moisture content increased with increasing nitrogen doses, with the highest moisture content observed under N₄ (21.34 and 21.17%), which was similar to with N₃ and lowest under N₁ (18.73 and 19.01%) during both the years. In nitrogen application timing, maximum grain moisture content was recorded under S₃ (21.22 and 21.52%) and S₄ (20.43 and 20.80%), while S₁ resulted in the lowest moisture content. Quality traits including lysine, tryptophan and protein content were significantly affected by nitrogen dose and application timing. The highest lysine (2.93 and 2.84%), tryptophan (0.75 and 0.74%) and protein content (10.02 and 9.83%) were obtained with N₄, which was statistically similar to N₃ but significantly superior to N₁ and N₂ during both years. Among the nitrogen application timing, treatment of S₄ recorded the highest lysine (2.89 and 2.83%), tryptophan (0.75 and 0.74%) and protein content (9.81 and 9.64%), which was statistically at par with S₃ but significantly higher than S₁ and S₂.

Keywords: Spring maize, nitrogen dose, application timing, LAR, SLW, moisture, quality traits

Introduction

Maize is an important cereal and multifunctional crop in the Poaceae family, widely used for human food, animal and poultry feed, and industrial products such as maize starch, dextrose, maize syrup and maize flakes (Gul *et al.*, 2021) [8]. However, maize productivity and grain quality are often low in nutrient-limited soils, especially under nitrogen deficiency. Nitrogen is one of the most essential plant nutrients and component of a wide range of biological chemicals that play critical roles in photosynthetic activity and agricultural productivity (Gheith *et al.*, 2022) [6]. Nitrogen application significantly improved dry matter, yield, harvest index of maize (Yadete *et al.*, 2024) [29]. Increasing nitrogen levels increased leaf growth parameters such as specific leaf area (SLA), leaf area index (LAI) and leaf area ratio (LAR), which ultimately contribute to higher grain yield (Amanullah *et al.*, 2007 and Oscar *et al.*, 2006) [2, 20]. The application of nitrogen at 180 kg ha⁻¹ achieved highest grain moisture content than lower dose (Illes *et al.*, 2025) [13]. Grain protein content is particularly responsive to nitrogen fertilization.

Madagoudra *et al.* (2022) ^[18] reported that highest protein content was obtained with 120 kg N ha⁻¹, which was statistically at par with 90 kg N ha⁻¹. Similarly, Aulakh *et al.* (2012) ^[3] observed higher protein content at 175 kg N ha⁻¹, which remained statistically comparable with 150 and 125 kg N ha⁻¹. They also recorded maximum oil content and minimum carbohydrate content at 175 kg N ha⁻¹. Ullah *et al.* (2015) ^[27] found that application of 280 kg N ha⁻¹ resulted in significantly higher crude protein and crude fibre compared to lower nitrogen levels. Likewise, Kaur and Kumar, (2017) ^[15] reported the maximum protein content at 180 kg N ha⁻¹, which was statistically at par with 150 kg N ha⁻¹ along with higher oil content and lower carbohydrate content. Sharma *et al.* (2017) ^[22] also confirmed that increasing nitrogen rates significantly enhanced protein content in maize.

Nitrogen application timing significantly influenced on maize productivity and nitrogen use efficiency (Gheith *et al.*, 2022) ^[6]. The highest specific leaf area (SLA), leaf area index (LAI) and leaf area ratio (LAR) were observed in plots where nitrogen was applied in five split doses, with a larger proportion supplied at later growth stages. While, the lowest SLA, LAI and LAR were recorded in plots that received nitrogen in only three splits, with a greater proportion applied at the time of sowing (Amanullah *et al.*, 2007) ^[2]. Niaz *et al.* (2014) ^[19] reported that maximum leaf area index was obtained with 50% N broadcast-incorporated at seed bed preparation + 50% N top dressed at V₉ + C₂ which was at par with 25% N broadcast-incorporated at seed bed preparation + 75% N top dressed at V₉ + C₂. Hammad *et al.* (2013) ^[10] observed that the highest leaf area index (LAI) and leaf area duration (LAD) were recorded when nitrogen was applied in three equal splits, 1/3 at V₂ stage, 1/3 at V₁₆ and 1/3 N at R₁ stage. Moreover, the crop attained the maximum crop growth rate (CGR) when nitrogen was applied in three equal portions, 1/3 N at seed bed preparation, 1/3 N at V₆ and 1/3 N at VT stage. Therefore, understanding the response of physiological traits such as leaf area ratio and specific leaf weight along with grain moisture and quality traits to different nitrogen doses and application timings is essential for optimizing nitrogen management in spring maize. The present study was conducted to evaluate the response of leaf area ratio, specific leaf weight, grain moisture content and quality traits of spring maize (*Zea mays* L.) to nitrogen dose and application timing with the aim of identifying efficient nitrogen management practices for improved productivity and grain quality.

Materials and Methods

A field experiment was conducted during the spring seasons of 2013 and 2014 at the Regional Research Station, Uchani, Karnal, of CCS Haryana Agricultural University. The experimental site is situated at 29° 43'N latitude and 76° 58'E longitude, at an altitude of 245 m above mean sea level. The soil was sandy loam in texture with an initial pH of 8.10 and electrical conductivity (EC) of 0.36 dsm⁻¹. The experiment was laid out in a strip plot design with three replications. The experiment comprised four nitrogen doses, N₁ (150 kg N ha⁻¹), N₂ (165 kg N ha⁻¹), N₃ (180 kg N ha⁻¹) and N₄ (195 kg N ha⁻¹) assigned to the main plots. Four nitrogen application timing were allocated to subplots, S₁ (50% at sowing + 25% at 8-leaf stage + 25% at tassel initiation), S₂ (25% at sowing + 25% at 4-leaf + 25% at 8-leaf + 25% at silking), S₃ (20% at sowing + 30% at 6-leaf + 40% at flowering + 10% at grain formation) and S₄ (20% at 2-leaf + 30% at 6-leaf + 40% at tassel initiation + 10% at grain formation). The spring maize variety HQPM-1 was used

in the study. Sowing was carried out on 5 March 2013 and 10 March 2014 using a seed rate of 20 kg ha⁻¹. Seeds were dibbled on dry ridges spaced 60 cm apart with 20 cm plant to plant spacing. Irrigation was applied up to half of the ridge immediately after sowing to ensure adequate soil moisture for proper germination. The gross plot size was 15 m², while the net plot size for recording observations was 11.52 m² (3.20 m × 3.60 m). Nitrogen in the form of urea (46% N) was used for the study. Nitrogen was applied at different dose and application timing based on treatments. Recommended dose of nutrients such as phosphorous and potassium at 60:60 kg ha⁻¹ were applied uniformly to all plots at the time of sowing. SSP and MOP were used as a source of phosphorous and potassium in the form of P₂O₅ and K₂O. The Leaf area ratio (LAR) and Specific leaf weight (SLW) were calculated at 25-day intervals using standard formula. The details are given below.

Leaf area ratio (LAR)

It is a ratio of leaf area per plant to weight per plant and is expressed in cm² g⁻¹. This was calculated by using the following formula.

$$\text{LAR} = \frac{\text{Leaf area per plant}}{\text{Weight per plant}} (\text{cm}^2 \text{ g}^{-1})$$

Specific leaf weight (SLW)

It is a ratio of leaf area per plant to leaf weight per plant and is expressed in cm² g⁻¹. This was calculated by using the following formula.

$$\text{SLW} = \frac{\text{Leaf area per plant}}{\text{Leaf weight per plant}} (\text{cm}^2 \text{ g}^{-1})$$

Grain moisture content (%) was calculated at harvest stage by using grain moisture meter and it is expressed in percent. Protein content of grains from each plot was determined by estimating the total nitrogen content of the sample by using the method of modified Kjeldahl's method (Jackson, 1973) ^[14]. The estimated nitrogen was multiplied with conversion factor 6.25 to convert total nitrogen in terms of protein. A representative sample of seeds was taken from each net plot produced for analysis of lysine content (%) and it was determined by using the colorimetric method designed by Tasi *et al.* (1972, Purdue University) ^[26] and tryptophan content (%) of grain was estimated by gloxylic acid. The data recorded for different parameters were analysed with the help of analysis of variance (ANOVA) technique (Gomez and Gomez, 1983) ^[7] for strip plot design using SAS 9.1 software (SAS Institute, Cary, NC). The Tukey procedure was used where ANNOVA was significant and results are presented at 5% level of significance (P = 0.05).

Results and Discussion

Leaf area ratio – LAR (cm² g⁻¹)

Leaf area ratio is the ratio of leaf area to total weight of plant. Results revealed that the doses of nitrogen significantly affected LAR at all the growth stages except at 25 DAS (Table 1). Highest LAR was recorded in plots to which nitrogen was applied in low dose of N₁ (150 kg N ha⁻¹) followed by N₂ (165 kg N ha⁻¹) at 50, 75 DAS and 100 DAS during 2013 and 2014. Lowest LAR was found in plots to which nitrogen was applied in high dose of N₄ (195 kg N ha⁻¹) which was statistically at par with N₃ (180 kg N ha⁻¹) at all the crop growth stages during both the years of study. Similar results were reported by DaMatta *et al.* (2002) ^[4] who reported that LAR decreased with increase of

nitrogen application above optimum level. Similar findings were also reported by Singh *et al.* (2022) ^[25] who observed that nitrogen levels significantly influenced growth indices in wheat. Kumar *et al.* (2024) ^[16] also reported that nitrogen application significantly increased leaf area index and dry matter accumulation, however the response declined beyond the optimum nitrogen level.

Among the nitrogen application timing, the leaf area ratio (LAR) differed significantly at all crop growth stages. The highest LAR was recorded in plots where nitrogen was applied in four unequal splits as S₃ (20% at sowing + 30% at 6-leaf + 40% at flowering + 10% at grain formation). This treatment was statistically at par with S₄ (20% at 2-leaf + 30% at 6-leaf + 40% at tassel initiation + 10% at grain formation), but both were significantly superior to the remaining nitrogen application

timings during both the years of study. The lowest LAR was observed under S₂, where nitrogen was applied in four equal splits as 25% at sowing + 25% at 4-leaf + 25% at 8-leaf + 25% at silking. The LAR increased under split nitrogen application may be attributed to better coordination between crop nitrogen demand and nutrient supply, which enhances leaf development, canopy expansion and nitrogen use efficiency. Similar findings were reported in maize where split nitrogen application improved leaf area traits, growth parameters and nitrogen use efficiency compared with single time application (Shrivastava *et al.*, 2024 and Singh *et al.*, 2025) ^[23, 24]. Furthermore, applying nitrogen in delayed or multiple splits promotes better leaf area development and canopy expansion which ultimately enhances leaf area ratio.

Table 1: Effect of nitrogen dose and application timing on leaf area ratio- LAR (cm² g⁻¹) of spring maize

Treatments	25 DAS		50 DAS		75 DAS		100 DAS	
	2013	2014	2013	2014	2013	2014	2013	2014
Nitrogen dose (kg ha⁻¹)								
N ₁ -150	121.49	117.50	85.26	90.00	81.22	85.50	34.83	37.72
N ₂ -165	120.41	117.86	78.12	81.32	71.99	75.26	30.90	32.95
N ₃ -180	121.34	120.20	72.14	75.19	65.93	68.92	27.50	29.35
N ₄ -195	120.26	119.50	68.76	71.38	62.03	63.71	25.99	27.79
S.E.m. ±	0.55	1.04	1.03	1.16	1.41	1.55	0.98	0.58
C.D.(0.05)	NS	NS	3.55	4.02	4.89	5.36	3.40	2.02
Nitrogen application timing								
S ₁	120.44	118.72	74.55	76.90	68.19	70.41	29.05	29.77
S ₂	115.59	115.59	71.64	74.02	64.41	64.14	26.68	27.39
S ₃	123.77	119.79	79.51	83.93	75.08	81.06	33.05	36.89
S ₄	123.70	120.96	78.58	83.03	73.50	77.78	30.44	33.76
S.E.m. ±	0.52	0.57	0.33	0.74	0.87	1.14	0.85	0.56
C.D.(0.05)	1.78	1.99	1.12	2.57	3.00	3.95	2.94	1.93

S₁ (50% at sowing + 25% at 8-leaf stage + 25% at tassel initiation), S₂ (25% at sowing + 25% at 4-leaf + 25% at 8-leaf + 25% at silking), S₃ (20% at sowing + 30% at 6-leaf + 40% at flowering + 10% at grain formation) and S₄ (20% at 2-leaf + 30% at 6-leaf + 40% at tassel initiation + 10% at grain formation)

Specific leaf weight – SLW (cm² g⁻¹ leaf weight)

Specific leaf weight (SLW) is defined as the ratio of leaf area to leaf weight which indicates the leaf thickness (Table 2). The greater the SLW the lesser will be the photosynthesis in leaf. Analysis of two years of maize data revealed that nitrogen dose significantly influenced SLW at all growth stages except at 25 DAS during both years of investigation. The highest SLW values (197.77 and 200.26 cm² g⁻¹ leaf weight) were recorded in plots receiving the lowest nitrogen dose N₁ (150 kg N ha⁻¹) followed by N₂ (165 kg N ha⁻¹), N₃ and N₄ (180 and 195 kg N ha⁻¹). While lowest SLW was observed under the highest nitrogen dose N₄ (195 kg N ha⁻¹), which was statistically similar with N₃ (180 kg N ha⁻¹) at 50, 75 DAS and 100 DAS during both the years. These results are in agreement with the findings of Fan *et al.* (2022) ^[5] who reported that increasing nitrogen availability tends to reduce SLA due to enhanced leaf area

expansion relative to biomass accumulation, reflecting the plasticity of leaf traits under varying nutrient conditions. Specific leaf weight (SLW) was significantly influenced by nitrogen application timings at all crop growth stages. The highest SLW was recorded when nitrogen was applied in four unequal splits under S₃ (20% at sowing + 30% at 6-leaf + 40% at flowering + 10% at grain formation), which remained statistically similar to S₄ (20% at 2-leaf + 30% at 6-leaf + 40% at tassel initiation + 10% at grain formation) at all growth stages except at 100 DAS in 2014. While, the lowest SLW was observed under four equal splits in S₂ (25% at sowing + 25% at 4-leaf + 25% at 8-leaf + 25% at silking). Similar findings were reported by Amanullah (2009) ^[1] who reported that application of nitrogen in four to five splits enhanced leaf area and plant height leading to improved maize productivity.

Table 2: Effect of nitrogen dose and application timing on specific leaf weight (SLW) (cm² g⁻¹) of spring maize

Treatments	25 DAS		50 DAS		75 DAS		100 DAS	
	2013	2014	2013	2014	2013	2014	2013	2014
Nitrogen dose (kg ha⁻¹)								
N ₁ -150	219.95	211.49	193.74	210.90	282.66	291.58	197.77	200.26
N ₂ -165	218.40	213.03	176.40	188.32	254.10	264.53	180.58	186.89
N ₃ -180	219.32	217.74	160.93	171.52	229.30	249.18	163.83	172.46
N ₄ -195	217.18	217.08	152.98	162.17	213.95	226.88	158.86	166.93
S.E.m. ±	0.50	1.53	2.67	2.96	5.10	6.44	5.17	3.04
C.D.(0.05)	NS	NS	9.24	10.24	17.65	22.30	16.58	10.54

Nitrogen application timing								
S ₁	218.53	216.08	166.56	175.85	237.22	242.26	170.13	167.91
S ₂	210.68	213.37	158.66	167.29	217.15	218.88	154.08	157.26
S ₃	223.06	214.06	181.10	196.88	265.49	288.90	194.90	208.56
S ₄	223.28	215.83	177.73	192.88	260.16	282.13	181.87	192.82
S.E.m. ±	0.49	0.60	1.16	1.30	1.96	6.76	4.10	1.92
C.D.(0.05)	1.68	1.85	4.01	4.51	6.78	23.39	13.16	6.18

S₁ (50% at sowing + 25% at 8-leaf stage + 25% at tassel initiation), S₂ (25% at sowing + 25% at 4-leaf + 25% at 8-leaf + 25% at silking), S₃ (20% at sowing + 30% at 6-leaf + 40% at flowering + 10% at grain formation) and S₄ (20% at 2-leaf + 30% at 6-leaf + 40% at tassel initiation + 10% at grain formation)

Grain moisture content (%)

Grain moisture content of maize was significantly influenced by nitrogen dose and application timing during both the years (Table 3). Grain moisture content was increased with increasing the nitrogen doses. The maximum moisture content was recorded with N₄-195 kg N ha⁻¹ (21.34 and 21.71%) and it was at par with N₃-180 kg N ha⁻¹ (20.69 and 21.17%) as compared to other dose of nitrogen during 2013 and 2014, respectively. Whereas, minimum grain moisture content (18.73 and 19.01%) was recorded in plots to which nitrogen was applied in lower dose of N₁ (150 kg N ha⁻¹) during both the year of study. These findings are consistent with Zhang *et al.* (2021) ^[30] who observed that maize grain moisture content varied by 1.9–4.0 % among different nitrogen application rates (0–450 kg N ha⁻¹). Similar results were also reported by Guo *et al.* (2020) ^[9]. In nitrogen application timing, grain moisture content of maize

varied significantly among the treatments. The highest grain moisture content was recorded under S₃ (20% at sowing + 30% at 6-leaf + 40% at flowering + 10% at grain formation), which registered 21.22% and 21.52% during 2013 and 2014, respectively. This treatment was statistically at par with S₄ (20% at 2-leaf stage + 30% at 6-leaf stage + 40% at tassel initiation + 10% at grain formation) which recorded 20.43% and 20.80% grain moisture content during the respective years. The higher grain moisture content under these treatments may be attributed to the availability of nitrogen during later growth stages, particularly during grain formation which supports kernel development and prolongs physiological activity. While the lowest grain moisture content was observed under S₁ (50% at sowing + 25% at 8-leaf stage + 25% at tassel initiation), with values of 19.25% and 19.77% during 2013 and 2014, respectively.

Table 3: Effect of nitrogen dose and application timing on grain moisture content and quality traits of spring maize

Treatments	Grain moisture content (%)		Lysine content (%)		Tryptophan content (%)		Protein content (%)	
	2013	2014	2013	2014	2013	2014	2013	2014
Nitrogen dose (kg ha ⁻¹)								
N ₁ -150	18.73	19.01	2.79	2.76	0.70	0.70	8.89	8.77
N ₂ -165	19.91	20.29	2.86	2.79	0.72	0.72	9.70	9.55
N ₃ -180	20.69	21.17	2.89	2.83	0.74	0.73	9.95	9.77
N ₄ -195	21.34	21.71	2.93	2.84	0.75	0.74	10.02	9.83
S.E.m. ±	0.41	0.25	0.01	0.01	0.00	0.00	0.02	0.02
C.D.(0.05)	1.33	0.76	0.04	0.02	0.01	0.01	0.07	0.08
Nitrogen application timing								
S ₁	19.25	19.77	2.84	2.77	0.72	0.71	9.38	9.30
S ₂	19.78	20.10	2.85	2.79	0.73	0.72	9.63	9.39
S ₃	21.22	21.52	2.89	2.82	0.75	0.72	9.75	9.58
S ₄	20.43	20.80	2.89	2.83	0.75	0.74	9.81	9.64
S.E.m. ±	0.35	0.28	0.01	0.01	0.00	0.00	0.02	0.02
C.D.(0.05)	1.08	0.83	0.03	0.02	0.01	0.01	0.08	0.07

S₁ (50% at sowing + 25% at 8-leaf stage + 25% at tassel initiation), S₂ (25% at sowing + 25% at 4-leaf + 25% at 8-leaf + 25% at silking), S₃ (20% at sowing + 30% at 6-leaf + 40% at flowering + 10% at grain formation) and S₄ (20% at 2-leaf + 30% at 6-leaf + 40% at tassel initiation + 10% at grain formation).

Quality traits

Lysine, tryptophan and protein content

The quality parameters such as lysine, tryptophan and protein content were significantly influenced by different nitrogen doses (Table 3). The maximum lysine, tryptophan and protein content were recorded with N₄ (195 kg N ha⁻¹), which was statistically similar to N₃ (180 kg N ha⁻¹) but significantly higher than N₁ (150 kg N ha⁻¹) and N₂ (165 kg N ha⁻¹) during both years of investigation. Adequate nitrogen availability enhances amino acid synthesis and protein accumulation in maize kernels, thereby improving grain quality. Similar findings were reported by Madagoudra *et al.*, 2022 ^[18]; Sharma *et al.*, 2017 ^[22] who reported that protein content increased with increasing nitrogen dose. Quality characteristics in maize were reported to be increased with nitrogen supply (Hammad *et al.*, 2011c; Amanullah *et al.*, 2009) ^[11, 1].

In case of nitrogen application timing, maximum lysine, tryptophan and protein contents were observed when nitrogen was applied in four splits as S₄ (20% at 2-leaf stage + 30% at 6-leaf stage + 40% at tassel initiation + 10% at grain formation), which was statistically similar with S₃ (20% at sowing + 30% at 6-leaf + 40% at flowering + 10% at grain formation) but significantly higher than S₁ (50% at sowing + 25% at 8-leaf stage + 25% at tassel initiation) and S₂ (25% at sowing + 25% at 4-leaf + 25% at 8-leaf + 25% at silking). These results corroborated with that of Lindiwe and Erick, (2024) ^[17], Madagoudra *et al.* (2022) ^[18], Hitesh and Renu, (2009) ^[12] reported that application of nitrogen in split doses increased grain protein content. Rizwan *et al.* (2003) ^[21] and Weiser and Seilmeier (1998) ^[28] found that the nitrogen fertilizer application timing influenced grain protein content.

Conclusion

The present study indicated that nitrogen dose and application timing significantly affected leaf area ratio (LAR), specific leaf weight (SLW), grain moisture, lysine, tryptophan and protein content. Among nitrogen doses, N₁ (150 kg N ha⁻¹) recorded higher LAR and SLW, while the lowest values were observed under N₄ (195 kg N ha⁻¹). However, grain moisture content and quality traits such as lysine, tryptophan and protein content were higher with N₄ (195 kg N ha⁻¹) and N₃ (180 kg N ha⁻¹). In case of nitrogen application timing, S₃ (20% at sowing + 30% at 6-leaf + 40% at flowering + 10% at grain formation) and S₄ (20% at 2-leaf + 30% at 6-leaf + 40% at tassel initiation + 10% at grain formation) treatments produced higher LAR, SLW and improved grain quality traits. Lower values of these parameters were recorded under S₂ (25% at sowing + 25% at 4-leaf + 25% at 8-leaf + 25% at silking) and S₁ (50% at sowing + 25% at 8-leaf stage + 25% at tassel initiation). Based on the results, application of 180 kg N ha⁻¹ along with split application as S₃ (20% at sowing + 30% at 6-leaf + 40% at flowering + 10% at grain formation) or S₄ (20% at 2-leaf + 30% at 6-leaf + 40% at tassel initiation + 10% at grain formation) is recommended for improving grain quality traits.

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Conflict of Interest

The authors do not have any conflicts of interest.

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