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Research Note: Effects of on-farm and hatchery hatching on broiler performance, intestinal lesions, and immune response during a subclinical necrotic enteritis challenge

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ABSTRACT The effects of traditional and on-farm hatching systems on broiler performance and health under a subclinical necrotic enteritis (**NE**) challenge were evaluated in this study. A 2×2 factorial study explored the effects of place of hatch (on-farm hatched [**OFH**] vs. hatchery hatched [**HH**]) and NE challenge (nonchallenged vs. challenged) on broilers. Cobb 500 eggs (~E19) were acquired from a commercial hatchery; 840 eggs were placed in pens on clean shavings in pre-warmed floor pens and allowed to hatch out, while 927 eggs were placed in a hatcher set under standard practices. On day (**d**) of hatch, all chicks were weighed and randomly distributed to 4 treatments (8 replicate pens each and 30 birds/pen). The OFH birds were placed immediately after sorting while HH birds were placed back in the hatcher overnight to simulate commercial hatchery procedures. After placing HH birds, feed and litter in the challenge group pens were sprayed with a live oocyst coccidia vaccine as a predisposing factor to NE. The small intestines of 3 male chicks per pen were scored for NE lesions (n = 24) on d 8 (peak NE challenge) and jejunal samples were collected from 1 bird per pen for RNA extraction and qPCR on d 8 and d 14. Data were analyzed using JMP Pro17 and significance between treatments was identified by LSD ($P \leq 0.05$). Regardless of the hatching system, the subclinical NE challenge caused a significant reduction in average daily gain (**ADG**) and average daily feed intake (**ADFI**), and increased feed conversion ratio (**FCR**) until d 28 ($P \leq 0.05$). Moreover, OFH birds exhibited significantly better growth ($P \leq 0.05$) through d 28 but had similar performance to HH birds by d 42. There were no significant differences in NE lesion scores between HH and OFH groups. In conclusion, OFH system resulted in better broiler performance compared to HH system under both no-challenge and challenge conditions during the starter and grower periods. This practice may hold potential for further exploration by the industry as an alternative to traditional hatching, aiming to improve the welfare and productivity of broilers.

Key words: on-farm hatching, necrotic enteritis, broiler, immune response, nutrient transporter

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INTRODUCTION

Hatchery practices are among the most important aspects of intensive poultry production systems, with the industry focusing on improving welfare standards in addition to production efficiencies. In traditional hatchery systems, depending on breeder age, egg storage, and setting time, the hatching windows can last up to 48 h (Panda et al., 2015). Once broiler chicks are pulled from the hatcher, they go through a rigorous processing protocol that includes handling, vaccination, cooling, packaging, transportation, and delivery, all occurring over hours before being given access to their first feed and water (van der Wagt et al., 2020). Therefore, chicks experience extended feed and water deprivation, which might last up to the first 72 h of their life, particularly for early-hatched birds (van der Wagt et al., 2020). Along with the delayed feed and water access, these stressors can compromise early chick development and immune function, which may increase susceptibility to intestinal infections (Uni and Ferket, 2004), potentially leading to diminished subsequent performance.

Early access to feed and water will support earlier development of the chick's intestinal tract, increasing

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the size and number of enterocytes that in turn will support its ability to transport nutrients needed for rapid growth (White et al., 2024). This early access to feed can also help the development of the immune system that may be key for overcoming stress and disease challenges. While there are many ways the industry can address delayed access to feed, recent alternative hatching systems can provide early access to feed and water either in the hatcher or in the house (de Jong et al., 2019; Giersberg et al., 2021). On-farm hatching (**OFH**) involves transporting eggs to the broiler farm around day (**d**) 18 of incubation, allowing chicks to hatch directly on the farm. This alternative hatching method has been developed to mitigate the impact of typical stressors in traditional hatchery systems, aiming to enhance chicks' welfare and performance (van de Ven et al., 2009; de Jong et al., 2019). Research indicates that OFH can lead to improved early growth performance and reduced mortality rates in broilers (de Jong et al., 2019; 2020). However, no direct comparisons have been made between place of hatch (**POH**) and necrotic enteritis (**NE**) challenge in terms of broiler performance and health. By comparing the performance and health of broilers hatched on-farm with those hatched in traditional hatcheries, we aim to provide insights into the potential benefits of this alternative hatching practice. Thus, in the current study, we hypothesized that reducing early-life stressors through OFH will enhance broiler resilience to NE and improve overall performance and health.

MATERIALS AND METHODS

Hatching Procedures, Animals, and Management

This project was approved and conducted under the guidelines of the Institutional Animal Care and Use Committee (# 18-136-APSC). Cobb 500 embryonated eggs (n = 1,767) were acquired from a local commercial hatchery at approximately embryonic d 19 (E19). Eggs were weighed and randomly allocated into 2 groups: Hatchery-hatched (**HH**) and OFH. For HH group, a total of 927 eggs were placed in a commercial hatcher set to 36.5°C and 65% humidity to hatch out. The hatching window was considered 'started' approximately when the first 10% of chicks had hatched and was considered completed 48 h later. Following this period, the HH chickens were held overnight in the hatcher to simulate standard hatchery conditions with feed and water withheld during travel prior to placement. Further, HH chicks were removed from the hatcher, sorted, and divided into 2 groups (nonchallenged and challenged) each with 8 pens with 30 birds/pen with similar average body weight (**BW**). Concurrently, 840 eggs, designated as OFH group, were placed on floor pens covered with 8-10 cm of fresh wood shavings on E19. Eggs were directly placed on the shavings with the air sac positioned upward, sides resting against one another. The average environmental temperature from E19 to d 0 was ~35°C and relative humidity was ~50%. Eggs were allowed the same hatching window as that of the HH chicks. At completion of the hatching window, OFH chicks were collected, weighed, and divided into 2 groups (nonchallenged and challenged) each with 8 pens with 30 birds/pen with similar average BW.

Overall, a total of 960 mixed sex birds were randomly allocated to 32 floor pens in a 2 × 2 factorial arrangements that included POH (HH and OFH) and NE challenge (**NEC**) (nonchallenged and challenged). The birds were raised in a controlled environment for 42 d. The starter, grower, and finisher diets were based on corn-soybean meal formulations and provided to the birds from d 0 to 14, d 14 to 28, and d 28 to 42 of age, respectively. Diets were formulated to meet or exceed the nutrient recommendations of Cobb 500 (Table 1). Each pen was equipped with a plastic bucket feeder and an automatic nipple drinker line. Water and experimental diets (in mash form) were provided ad libitum throughout the study period. An automatic ventilation system was used to control the environment, and temperature was maintained as follows: 35°C for first 3 d, then gradually reduced by approximately 3°C each week until it reached 21°C where it remained constant thereafter. Birds and feeders were weighed by pen on d 8 (peak NE challenge), at the end of starter (d 14), grower (d 28), and finisher (d 42) periods. Feed intake (**FI**) was calculated by pen on d 8, 14, 28, and 42. Birds were checked at least twice daily, and any mortality was recorded (including bird weight). Additionally, average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**) were calculated and adjusted for mortality for each period and the cumulative experimental period (d 0−42).

Necrotic Enteritis Challenge

In order to replicate field conditions, birds were challenged with a unique, naturally occurring NE model (Calik et al., 2019b; Emami et al., 2019; 2020; Blue et al., 2023). This condition was induced in 16 pens by spraying a concentrated (10X) commercial coccidiosis vaccine on the litter and feed at the time of bird placement, which, in conjunction with the presence of *Clostridium perfringens* spores in the environment (samples tested and confirmed positive in the laboratory), leads to the development of a NE outbreak approximately 1-wk post vaccine administration. The Coccivac-B52 vaccine was used for this trial (containing live oocysts of *Eimeria acervulina, E. maxima, E. maxima MFP, E. mivati*, and *E. tenella*; Merck Animal Health). The coccidiosis vaccine dosage was chosen based on successful dosage levels used in previous studies in this facility (Calik et al., 2019b; Emami et al., 2019; 2020; Blue et al., 2023).

Lesion Scores

On d 8, 3 male birds were selected based on the average BW of each pen (24/treatment), euthanized by cervical dislocation, and the small intestines were evaluated for NE lesions and scored based on a 0 to

Table 1. Composition of diets (as fed basis, %).[1]

Ingredients (%)	Starter (d 0−14)	Grower (d 14−28)	Finisher (d 28−42)
Corn	59.53	64.12	65.70
Soybean meal	33.50	28.80	26.86
Soybean Oil	2.18	2.60	3.50
Dicalcium phosphate	2.05	1.92	1.70
Limestone	1.11	1.00	0.90
Sodium chloride	0.37	0.37	0.35
DL-Methionine[2]	0.38	0.34	0.29
L-Lysine HCl[3]	0.37	0.35	0.24
L-Threonine[4]	0.15	0.14	0.10
Vitamin/trace mineral premix[5]	0.36	0.36	0.36
Calculated Analysis (% unless specified)			
ME (kcal/kg)	3007	3087	3168
Crude protein	21.81	19.90	18.94
Total phosphorus	0.76	0.71	0.66
Available phosphorus	0.45	0.42	0.38
Calcium	0.90	0.84	0.76
Methionine	0.67	0.61	0.55
Methionine + Cysteine	0.98	0.89	0.82
Lysine	1.32	1.19	1.05
Threonine	0.86	0.78	0.71
Linoleic acid	1.44	1.52	1.55
Dietary cation-anion balance (mEq)	194	174	170

[1]All treatments were fed each diet on ad libitum basis for duration of the experiment phases.
[2]Rhodimet NP99, ADISSEO, GA, USA.
[3]L-Lysine HCl, Ajinomoto Heartland, Inc. Eddyville, IA, USA.
[4]Fenchem Ingredient Technology, Nanjing, China.
[5]Vitamins supplied per kg diet: retinol 3.33 mg, cholecalciferol 0.1 mg, α-tocopherol acetate 23.4 mg, vitamin K3 1.2 mg, vitamin B1 1.6 mg, vitamin B2 9.5 mg, niacin 40 mg, pantothenic acid 9.5 mg, vitamin B6 2 mg, folic acid 1 mg, vitamin B12 0.016 mg, biotin 0.05 mg, choline 556 mg. Minerals supplied per kg diet: Mn 144 mg, Fe 72 mg, Zn 144 mg, Cu 16.2 mg, I 2.1 mg, Se 0.22 mg.

4 scale. The duodenum, jejunum, and ileum were scored separately using the following lesion scoring criteria: 0 = no gross lesions, 1 = thin-walled or friable, 2 = focal necrosis or ulceration, 3 = multifocal coalescing areas (large patches) of necrosis, 4 = severe extensive necrosis (Prescott et al., 1978).

Total RNA Extraction, Reverse Transcription, and Quantitative Real-Time PCR

On d 8 (the first bird selected for lesion scoring from each pen) and d 14, 1 bird from each pen was euthanized by cervical dislocation, and samples from the jejunum were collected to assess the mRNA abundance of interferon gamma (**IFNγ**), sodium-glucose cotransporter 1 (**SGLT1**), and peptide transporter 1 (**PepT1**). These tissue samples were homogenized by a TissueLyser II and total RNA was extracted using RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Total RNA was quantified by spectrophotometry and integrity was evaluated by gel electrophoresis on 1.5% agarose gel in 0.5X TAE buffer. Two micrograms of total RNA were used to synthesize first-strand cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer's recommendation. The mRNA abundance of IFNγ (F: GCTCCCGATGAACGACTTGA; R: TGTAAGATGCTGAAGAGTTCATTCG) SGLT1 (F: GCCATGGCCAGGGCTTA; R: CAATAACCTGATCTGTGCACCAGTA), and PepT1 (F: CCCCTGAGGAGGATCACTGTT; R: CAAAAGAGCAGCAGCAACGA) was measured by quantitative real-time PCR (7500 Fast Real-Time PCR System, Applied Biosystems) using Fast SYBR^{TM} Green Master Mix (Applied Biosystems). Each reaction was performed in a total volume of 10 μL in duplicate wells. Product specificity was confirmed by analysis of the melting curves produced by the 7500 software (version 2.0.3). mRNA abundance was analyzed using glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**) as an endogenous control. Average mRNA abundance relative to GAPDH for each sample was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The calibrator for each gene was the average ΔCt value from the HH-NC group.

Statistical Analysis

Analysis of data was performed using 2-way ANOVA (JMP Pro17) between POH and NE challenge using Fisher's least significant difference (**LSD**) method. The results report the interaction between POH and NE challenge, and the main effects of POH and NE challenge. Results are reported as least square means (**LS Means**) with standard error means (**SEM**). The probability $P \leq 0.05$ was considered significant unless otherwise noted.

RESULTS AND DISCUSSION

The effects of hatching location on broiler growth performance and mortality rate under nonchallenge and challenge conditions are shown in Table 2. There was no significant interaction effect on the growth performance

Table 2. Effect of traditional and on-farm hatching systems on growth performance parameters of broilers during a subclinical necrotic enteritis challenge.[1]

	Treatments[2]									Statistics		
	Hatchery (HH)		On-Farm (OFH)		POH[3]		NEC[4]			*P*-value		
Parameter	NC	NE	NC	NE	HH	OFH	NC	NE	SEM	*POH*	*NEC*	*POH* × *NEC*
0−8 d												
ADG (g)	20.74	17.32	23.27	18.93	19.03[b]	21.10[a]	22.01[a]	18.13[b]	0.28	<0.001	<0.001	0.156
ADFI (g)	24.67	23.55	27.28	25.29	24.11[b]	26.29[a]	25.98[a]	24.42[b]	0.54	0.002	0.021	0.495
FCR	1.18	1.35	1.17	1.33	1.27	1.25	1.18[b]	1.34[a]	0.03	0.541	<0.001	0.940
Mortality, %	0	0.95	0.95	1.24	0.47	1.10	0.47	1.10	0.62	*0.324*	*0.323*	*0.601*
8−14 d												
ADG (g)	48.23	43.87	50.06	45.24	46.05[b]	47.65[a]	49.14[a]	44.55[b]	0.72	0.043	<0.001	0.761
ADFI (g)	63.85	58.17	65.93	61.56	61.01[b]	63.74[a]	64.89[a]	59.86[b]	1.26	0.050	0.001	0.624
FCR	1.32	1.32	1.31	1.36	1.32	1.34	1.32	1.34	0.02	0.590	0.402	0.452
Mortality, %	0	0.52	0	0.92	0.26	0.46	0	0.72	0.40	*0.630*	*0.086*	*0.630*
14−28 d												
ADG (g)	71.51	67.18	73.16	68.64	69.35	70.9	72.33[a]	67.91[b]	0.96	0.123	0.001	0.920
ADFI (g)	126.6	121.2	129.5	125.3	123.9[b]	127.4[a]	128.1[a]	123.2[b]	1.48	0.034	0.005	0.721
FCR	1.77	1.8	1.77	1.82	1.78	1.79	1.77[b]	1.81[a]	0.02	0.629	0.042	0.619
Mortality, %	0	0	0	0.52	0	0.26	0	0.26	0.27	0.360	0.360	0.360
0−28 d												
ADG (g)	50.52	47.08	52.59	47.78	48.8[b]	50.18[a]	51.55[a]	47.43[b]	0.58	0.024	<0.001	0.248
ADFI (g)	82.01	78.73	84.83	80.64	80.37[b]	82.73[a]	83.42[a]	79.62[b]	0.80	0.006	<0.001	0.567
FCR	1.62	1.67	1.61	1.68	1.64	1.64	1.61[b]	1.68[a]	0.01	0.863	<0.001	0.390
Mortality, %	0	1.42	0.95	2.49	0.71	1.72	0.47[b]	1.96[a]	0.69	0.162	0.042	0.619
28−42 d												
ADG (g)	87.20	91.65	85.28	91.36	89.43	88.32	86.24	91.50	0.96	*0.571*	*0.011*	*0.677*
ADFI (g)	156.3	157.6	154.5	160.0	157.0	157.3	155.4	158.8	1.35	*0.921*	*0.221*	*0.451*
FCR	1.79	1.81	1.72	1.75	1.76	1.78	1.80[a]	1.74[b]	0.01	*0.184*	*0.002*	*0.759*
Mortality, %	0.42	0.42	0	0	0.42	0	0.21	0.21	0.15	*0.200*	*0.999*	*0.999*
0−42 d												
ADG (g)	60.67	60.05	61.29	60.80	60.37	61.04	60.98	60.43	0.35	*0.349*	*0.448*	*0.932*
ADFI (g)	103.0	101.4	104.0	104.2	102.2	104.1	103.5	102.8	0.62	*0.141*	*0.586*	*0.485*
FCR	1.70	1.69	1.70	1.71	1.69	1.70	1.70	1.70	0.01	*0.227*	*0.686*	*0.215*
Mortality, %	0.42	1.92	0.95	2.49	1.17	1.72	0.69[b]	2.21[a]	0.37	0.579	0.047	0.874

[a,b]In each column, indicates with different letters are significantly different ($P < 0.05$).
[1]Data represent mean values of 8 replicate pens per treatment.
[2]HH: chicks hatched in typical industry hatcher; OFH: on-farm hatched birds; NC: nonchallenge; NE: necrotic enteritis.
[3]POH: place of hatch.
[4]NEC: necrotic enteritis challenge.

of the birds in terms of ADG, ADFI, FCR, and mortality rate between POH and NE challenge treatments during different experimental periods or the entire course of the study. The NE challenge caused a significant ($P \leq 0.05$) reduction in ADG and ADFI in the 0 to 8, 8 to 14, 14 to 28, 0 to 28 d intervals, and increased FCR in the 0 to 8, 14 to 28, 0 to 28 d intervals. Moreover, the mortality rate in the 0 to 28 ($P = 0.042$) and 0 to 42 ($P = 0.047$) d intervals were significantly higher in NE challenged birds than in nonchallenged birds. Regardless of the NE challenge, OFH birds exhibited significantly ($P \leq 0.05$) greater ADFI values during the 0 to 8, 8 to 14, and 14 to 28 d intervals, and greater ADG values during the 0 to 8, 8 to 14, and 0 to 28 d intervals. The benefits of OFH system, where chicks have immediate access to feed and water post-hatch, have been well-documented under nonchallenge conditions. Accordingly, previous studies observed a significantly greater BW in OFH birds up to d 7 (de Jong et al., 2019) and d 21 (de Jong et al., 2020) compared to HH birds. Such improvements in early growth performance are likely due to the early access to feed and water that accelerates the development of the gastrointestinal tract and reduction in early post-hatch stressors. However, by d 42, the NE challenged birds experienced compensatory growth that reversed a reduction during the starter and grower periods, and no differences in growth performance were observed between POH and NEC treatments over the entire experimental period (d 0−42). Trials conducted under field conditions without a challenge have demonstrated that broilers can compensate for growth reductions, regardless of the hatching system (de Jong et al., 2019; de Jong et al., 2020). One possible explanation for the comparable performance among the treatment groups could be due to the subclinical challenge conditions, as evidenced by lower lesion scores and low mortality rates. It is known that modern broilers have a great genetic potential to support their growth and can compensate for growth performance reduction when the stressor is temporary for a short duration, or mild in nature (Keerqin et al., 2017; Ozlu et al., 2020). Another possible explanation for the compensatory growth could be related to the overnight withholding time in the hatcher in the current study. Ozlu et al. (2020) reported that broilers can compensate for BW reduction with up to 24-26 h of delayed access to feed and water. However, there are also reports suggesting that OFH birds could maintain a growth performance advantage until market age, particularly birds from younger parent flocks (Souza da Silva et al., 2021). Hence, the impact of different hatching systems on broiler performance remains to be elucidated under different and more severe challenge

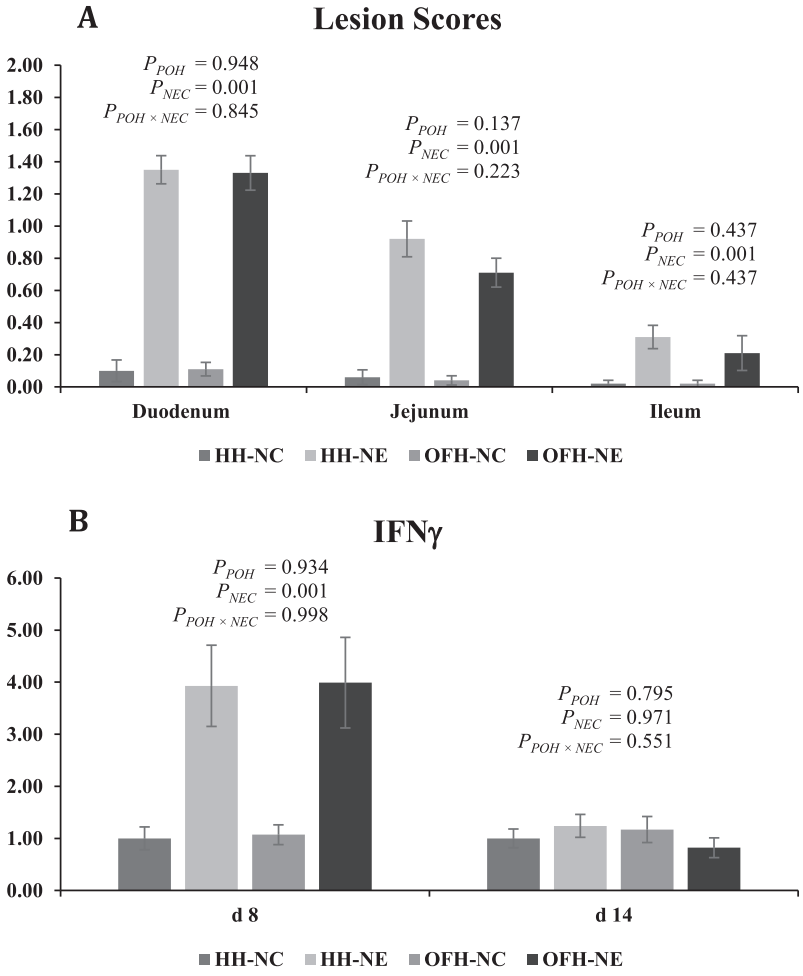

Figure 1. Effect of traditional and on-farm hatching systems on intestinal lesion scores (A) and mRNA abundance of IFNγ (B) during a subclinical necrotic enteritis challenge. HH: chicks hatched in typical industry hatcher; OFH: on-farm hatched birds; NC: nonchallenged; NE: necrotic enteritis; POH: place of hatch; NEC: necrotic enteritis challenge.

conditions to determine their potential benefits and limitations.

IFNγ is a potent pro-inflammatory cytokine, and its mRNA level significantly increases under intestinal challenge conditions such as coccidiosis (Calik et al., 2019a) or NE (Emami et al., 2019) challenges. Concurrent with the lesion scores (Figure 1), IFNγ mRNA abundance was significantly greater in challenged groups regardless of the hatching system on d 8 (Figure 1). However, no significant interaction or treatment main effects were observed for mRNA abundance of IFNγ on d 14 indicating that the birds in the challenged groups started to recover from subclinical NE. Additionally, no significant interactions or main effects were observed among treatment groups in terms of PepT1 and SGLT1 mRNA abundance in the jejunum on d 8 and d 14 (data not shown). To the best of our knowledge, this is the first study exploring the impact of HH and OFH systems on broiler performance and health. An important finding of the current study is that the hatching system had no impact on broiler immune response and nutrient transporters. However, further research is warranted to investigate the broader effects of different hatching systems on broiler immune response under more severe challenge conditions.

In conclusion, OFH birds exhibited in significantly better growth performance in both nonchallenged and under subclinical NE challenge conditions through d 28, but no long-term effects (market age) on performance were observed. This study provides new insights into the relationship between OFH practices and NE challenges, building on previous studies that compared different hatching systems under no-challenge conditions. Overall, even though there were no significant differences in final performance variables in the current study, OFH may significantly contribute to enhanced broiler performance beyond d 28 under more severe disease conditions, extended withholding times, or other field conditions. Additional research could explore the combination of OFH with nondrug feed additives as a strategy to combat various challenges and improve market age performance.

DISCLOSURES

The authors declare no conflicts of interest.

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