Effect of various concentration of butyric acid on growth performance, intestinal lesion scores, and body composition of broilers raised on used litter

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Primary Audience: Nutritionists, poultry producers

SUMMARY

There is still a need for the evaluation of alternative feed additives, including organic acids such as butyric acid, when fed in broiler diets without antibiotic growth promoters. The removal or limitation of growth promoting antibiotics from commercial production changes the opportunities for feed additives in the poultry industry and allows for the evaluation of replacement feed additives that may not have been cost effective when growth promoting antibiotics are available. Therefore, an experiment was conducted to evaluate the effects of various concentrations (200 ppm, 400 ppm, and 800 ppm) of coated butyric acid on growth performance, intestinal lesion score, and body composition of 42-day-old broilers raised on used litter. The addition of the coated butyric acid at 400 ppm and 800 ppm increased BW and 800 ppm improved mortality corrected feed conversion ratio (**FCRm**) over the 42-d period compared with the negative control (**NC**) fed broilers. Although performance was improved, the ratio of lean mass to fatty mass in the broilers was unchanged suggesting a generalize growth response. No significant differences were observed in lesion scores in the duodenum and jejunum and overall lesion scores were low. Both 400 and 800 ppm of butyric acid were able to increase 42-d broiler body weight in comparison to the NC fed birds, but 800 ppm of butyric acid was required to also improve the FCRm in comparison to the NC fed birds.

Key words: butyric acid, antibiotic alternatives, coccidiosis, natural infection model, broiler

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DESCRIPTION OF PROBLEM

Avian coccidial disease, endemic across worldwide broiler production, and can be exacerbated by the reuse of litter should oocysts shed from previous flocks build up and sporulate in the current flock. Coccidial species can infect the gastrointestinal tract from the duodenum to the ceca resulting in localized tissue damage and reduced performance and without treatment, potentially result in morbidity and mortality (Chapman, 2014). The morbidity and mortality associated with poultry coccidia was estimated to be cost producers ~US$14.04 billion in 2016 worldwide (Blake et al., 2020). Historically, the broiler industry has managed coccidia with a combination of ionophores and chemical anticoccidials shuttle programs. Recently, the use of ionophores has been reduced or eliminated in some flocks in response to both governmental regulation and market demand. Therefore, the development

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and validation of alternative additives is critical to maintain the healthful and efficient production of broilers under commercial conditions.

Butyric acid is a volatile fatty acid, and a normal metabolite synthesized in the intestinal flora of poultry (Józefiak et al., 2004). The inclusion of 300 ppm and 400 ppm butyric acid has been shown to improve FCR in unchallenged broilers raised on clean litter (Kaczmarek et al., 2016). In a second experiment, 1,000 ppm Na-butyrate did not alter the performance of unchallenged broilers, however, it was able to partially ameliorate the loss of BW gain (**BWG**) in broilers fed nutritionally reduced diets (Bortoluzzi et al., 2017). Tributyrin is the ester composed of butyric acid and glycerol that reduces the rapid absorption of butyric acid mimicking the effects of coating butyric acid allowing it to reach the small intestine where it can have beneficial effects (Smith et al., 2012). The use of 2,500 ppm of tributyrin improved FCR and increased BWG 7 d post infection when fed to broiler chickens orally gavaged with 10^3 *E. maxima* oocysts (Hansen et al., 2021). The inclusion of 1,000 ppm of either uncoated or microencapsulated butyric acid in the starter phase (1-21 d) followed by 500 ppm in the finisher phase (21−42 d) did not affect the performance in broilers challenged with 2×10^5 sporulated *E. acervulina* oocysts and 2×10^4 *E. maxima* and *E. tenella* oocysts, but FCR was improved in the chickens fed the microencapsulated butyric acid over the 1 to 21 d period (Ventura et al., 2025). Therefore, the current experiment was conducted to investigate the influence of various concentrations of coated butyric acid on broiler chicken growth performance, body composition, oocyst shedding, and intestinal lesion scores when broilers were grown on reused litter.

MATERIALS AND METHODS

Animals and Experimental Diets

This experiment was conducted according to the guidelines of the Virginia Tech Institutional Animal Care and Use Committee. In total, 1,650 male broiler chicks (Heritage Breeders, Salisbury, MD) were randomly assigned to five treatment groups with 12 replicate pens of 25 chicks per treatment. Treatments consisted of a positive control that received the nontreated control diet with clean pine shaving litter (**PC**); a negative control that received the same nontreated control diet, but were housed on used litter (**NC**); and three treatment groups that received either 200, 400, or 800 ppm butyric acid (ButiPEARL - Kemin Industries, Des Moines, IA) added on top of the control diet and housed on used litter (NC + 200, NC + 400, NC + 800, respectively). Pens shared 2 boarders with adjacent pens and when a boarder was shared, a 6-inch solid plastic boarder was used at the bottom of the shared pens to reduce cross contamination among all pens. The natural challenge model was generated by using seeder birds to shed coccidial spores into the litter before experimental birds were placed into the pens. The used litter was generated by placing approximately 20 male chicks from a broiler breeder female line on clean pine shaving (5 cm deep) and a concrete floor. Seeder chicks were provided a starter diet that contained a 10 × dose of Coccivac B52 (Merck, Kenilworth, NJ) for the first 2 d of life. After removal of the treated diets, seeder birds were fed common starter and grower diets until 28 d. This 28-d period allowed to the generation of used litter, but also allowed the seeder birds to shed 3 to 4 cycles of coccidial spores into the litter. After 28 d, the seeder birds were removed and the litter was moved to the center of the house and mixed before approximately equal amounts used litter were returned to each NC and treatment pen. All feed was removed from the pens. The pens set up to start experimental chicks within 1 wk to allow for the experimental birds to start on fresh used litter. Basal diet formulation for starter (d 1−14), grower (d 14 −28), and finisher diets (d 28−42) are shown in Table 1. Birds were provided ad libitum access to experimental diets and water throughout the experiment. The temperature was set to 32 to 35˚C for the first 7 d, and then was reduced by 2.78˚C over approximate seven d periods until 21˚C was reached and maintained. Continuous lighting and supplemental heat were provided via heat lamps over the first 7 d, after which lighting was adjusted to 18 h of light per day. Birds were

Table 1. Formulation and nutrient profile of experimental diets fed to male broiler chickens provided various concentrations of coated butyric acid and raised on used pine shaving litter.[1]

Ingredient	Starter 1 to 14 d	Grower 14 to 28 d	Finisher 28 to 42 d
		%	
Corn	61.31	64.50	67.31
Soybean meal (48% CP)	30.95	26.00	23.77
Dried distillers grains with solubles	3.09	3.85	3.00
Soy oil	0.70	1.97	2.63
Salt	0.34	0.34	0.32
DL-Methionine	0.29	0.25	0.21
L-Lysine·HCl	0.27	0.26	0.20
L-Threonine	0.05	0.04	0.04
Limestone	1.15	1.11	1.04
Dicalcium phosphate	1.12	0.98	0.79
Choline chloride (60%)	0.10	0.10	0.10
Vitamin and mineral premix[2]	0.63	0.50	0.50
Phytase[3]	0.10	0.10	0.10
Nutrient profile[4]			
CP	21.3 (21.9)	19.4 (17.2)	18.2 (16.9)
ME (kcal/kg)	3,000	3,108	3,180
Calcium	0.90	0.84	0.76
Nonphytate phosphorus	0.42	0.39	0.35
Crude fat	3.77 (2.29)	5.13 (3.90)	5.76 (4.24)
Crude fiber	2.74 (2.76)	2.70 (3.62)	2.64 (3.69)
Digestible methionine + cysteine	0.98	0.89	0.82
Digestible lysine	1.31	1.17	1.06
Digestible histidine	0.57	0.52	0.49
Digestible tryptophan	0.24	0.21	0.20
Digestible threonine	0.86	0.77	0.72
Digestible arginine	1.38	1.22	1.14
Digestible isoleucine	0.89	0.80	0.75

[1]Treatments diets were obtained by replacement of 0.02; 0.04, and 0.08% corn with butyric acid in negative control (NC) + 200 ppm butyric acid, NC + 400 ppm butyric acid and NC + 800 ppm butyric acid, respectively.

[2]The vitamin and mineral premix was formulated to supply the following in the starter diets: vitamin A, 5,512 IU/kg; vitamin D3, 1,847 IU/kg; vitamin E, 1.38 IU/kg; menadione, 0.96 mg/kg; vitamin B12, 0.006 mg/kg; choline, 319 mg/kg; niacin, 16.5 mg/kg; D-pantothenic acid, 5.1 mg/kg; riboflavin, 3.45 mg/kg; Cu, 3.375mg/kg; Fe, 42.2 mg/kg; I, 0.84 mg/kg; Mn, 53.6 mg/kg; Zn, 40.6 mg/kg; Co, 0.21 mg/kg. As the vitamin and mineral premix was reduced in the grower and finisher diets, please multiply the above values by 80% to calculate the inclusion concentrations for those diets.

[3]Phytase was formulated to provide 0.10% Ca and nonphytate phosphorus, Quantum BLUE 5 G, AB Vista, Marlborough, UK.

[4]Values within parenthesis are analyzed values for complete diets

monitored at least twice daily for health, mortality, and to ensure access to feed and water.

Growth Performance

Group BW and feed intake (**FI**) were recorded on d 0, 14, 28, and 42 to correspond with the beginning and end of each dietary phase. Body weight gain was calculated for the 0 to 14, 0 to 28, and 0 to 42 d periods. Feed offered and refused was used to calculate FI for the 0 to 14, 0 to 28, and 0 to 42 d periods. Any chicks that died or were sampled or culled were weighed and record when they were discovered and used to correct feed conversion ratio for mortality. Total pen feed intake was divided by total pen BW gain plus mortality weight gain to calculate mortality corrected FCR (FCRm).

Oocyst Shedding and Lesion Scoring

On d 14, litter samples were collected from each pen and stored at 4˚C to determine oocyst shedding. A representative sample was taken from the center of the pen and the center of each of 4 quadrants of the pen and mixed. Oocysts were counted in duplicate using a counting chamber (McMaster; JA Whitlock & Company, New South Wales, Australia) and microscope (Olympus CX21-FS1, Tokyo, Japan) set a 10× magnification (Dalloul et al., 2003). On d 14, three birds per replicate pen were randomly selected, individually weighed, and euthanized. Lesions in the duodenum, jejunum, and ileum were scored (blinded to experimental treatment). In total 3 individuals scored lesions, with each individual scoring one bird per replicate pen so minimize any scoring bias across all treatments. Scores were assigned using a scale of 0 to 4. A score of 0 was given if lesions were not present, 1 if any lesions were present in a section, 2 if greater than 10 lesions per section were present, 3 if lesions began to overlap/coalesce, and 4 when lesions covered a large portion of the section, resulting in cells beginning to slough off the intestinal lining and to talk on a "terrycloth" appearance (Johnson and Reid, 1970).

Body Composition

At the end of the experiment, 5 birds from each pen were individually weighed and leg banded before being euthanized by cervical dislocation, scalded at 60°C (Brower AM 48, Houghton, IA), de-feathered (Ashley SP23, Greensburg, IN), frozen and stored at −20°C until analysis. After thawing, the 5 carcasses were scanned using dual energy X-ray absorptiometry (**DXA**, General Electric Healthcare, Madison, WI). After scanning, fat mass (g), lean mass (g), and total mass (g) was calculated and reported (Murugesan and Persia, 2013).

Statistical Analysis

All data were analyzed using a one-way analysis of variance (**ANOVA**) in the SAS program (SAS Institute Inc., Cary, NC). Statistical differences were considered significant at $P \leq 0.05$. If significant differences were noted, means were separated using Fisher's LSD test. All observations were made at the pen level for statistical analysis. In the case of performance data, experimental units were defined as all birds remaining within the pen at time of measurement. Lesion scores and body composition experimental units were defined as a sample of 3 and 5 birds, respectively, from within each of the 12 pens. One pooled litter sample was collected and generated from within each of the 12 pens and used as the experimental unit. Mortality data were collected daily, but due to low variation among observations, were not statistically analyzed and are presented for observation only.

RESULTS AND DISCUSSION

Supplementation of male broiler chickens with butyric acid was able to improve both BW and FCRm throughout the 42-d experimental period (Table 2). There were no differences in initial BW, with an overall average across all treatments of 37.7 g per chick ($P = 0.22$). Overall mortality was low. Over the 42-d period the PC, NC, NC + 200, NC + 400, and NC + 800 groups resulted in 3.6, 4.0, 0.7, 2.9, and 2.2% mortality, respectively. There were differences ($P \leq 0.05$) between the PC and NC fed broilers at 14 d as the NC fed birds resulted in a 14 g reduction in BW compared to PC fed birds. By 28 d, the NC fed broilers had a 41 g reduction in BW in comparison to the PC fed broilers, but the difference was no longer significant. When the experiment concluded at 42 d, the NC fed birds had fully recovered and resulted in a BW that was increased by 49 g in comparison to the PC fed broilers ($P > 0.05$). These results suggest an initial exposure to the used litter reduced body weight at 14 d of age, but did not result in overall reduced performance of the broilers as the NC and PC birds resulted in similar ending BW.

At 14 d of age, treatment of diets with all three concentrations of butyric acid increased BW above the NC treated broilers ($P \leq 0.05$). The NC + 400 resulted in the heaviest broilers on d 14 reaching 438 g and was significantly increased in comparison to the PC broilers. At 28 d of age, both the NC + 400 (1.48 kg) and NC + 800 (1.55 kg) were significantly increased in comparison to the NC (1.42 kg), and the NC + 800 treatments resulted in significantly

Table 2. Body weight and mortality corrected feed conversion ratio (FCRm) of male broilers raised on used pine shaving litter fed a control diet or the same control diet supplemented with various concentration of butyric acid.[1]

	BW			FCRm		
Treatments[2]	14 d	28 d	42 d	1 to 14 d	1 to 28 d	1 to 42 d
	(g)	(kg)	(kg)	(g feed/g gain)	(g feed/g gain)	(g feed/g gain)
PC	420[b]	1.46[bc]	2.95[d]	1.359	1.603[a]	1.713[a]
NC	404[c]	1.42[c]	3.01[cd]	1.359	1.609[a]	1.685[ab]
NC + 200	422[b]	1.47[bc]	3.06[bc]	1.340	1.597[a]	1.671[bc]
NC + 400	438[a]	1.49[b]	3.09[ab]	1.347	1.636[a]	1.687[ab]
NC + 800	428[ab]	1.55[a]	3.15[a]	1.335	1.538[b]	1.645[c]
Pooled SEM	4.5	0.019	0.026	0.0137	0.0187	0.0099
P-value	≤ 0.01	≤ 0.01	≤ 0.01	0.64	≤ 0.01	≤ 0.01

[1]Data are least square means of 12 pens of male broilers that begin with 25 chicks per pen. Initial BW was 37.7 g ($P = 0.22$).

[2]PC = positive control, nontreated diet with fresh pine shaving litter; NC = negative control, nontreated diet with used litter; NC + 200 = diet with 200 ppm butyric acid on used litter; NC + 400 = diet with 400 ppm butyric acid on used litter; NC + 800 = diet with 800 ppm butyric acid on used litter.

[a-c]Values in a column that do not share a common superscript are different, ($P \leq 0.05$).

increased BW in comparison to the PC treated broilers (1.46 g). At the conclusion of the experiment on day 42, all butyrate treated broilers had increased BW in comparison to the PC and both the NC + 400 and NC + 800 treatments had increased BW in comparison to the NC ($P \leq 0.05$). Although BW were generally increased with both the NC + 400 and NC + 800 treatments, FCRm was only improved in the NC + 800 fed broilers over both the 0 to 28 and 0 to 42 day periods ($P \leq 0.01$). These results agree with a meta-analysis that showed supplementation of mixed organic acids was able to improve growth performance of broiler chickens under challenge conditions (Polycarpo et al., 2017). This meta-analysis also suggested that butyric acid might be the most effective organic acid when supplemented as a single organic acid, although it appears that mixed acids were more effective. Similar to the current data with reused litter, 250 ppm, 350 ppm, and 450 ppm (Imran et al., 2018) and 200 ppm, 300 ppm, 400 ppm, and 500 ppm (Levy et al., 2015) of microencapsulated butyric acid supplemented to broilers without either infection or used litter increased BW gain and improved FCR. In contrast, Leeson and coworkers (2005) indicated that 2,000 and 4,000 ppm uncoated butyric acid did not affect the performance of unchallenged broilers during 0 to 20 d and 0 to 42 d whereas the 4,000 ppm dose reduced FI over the 0 to 20 d period. The uncoated butyrate might have been absorbed too quickly and did not remain in the lumen until reaching and being absorbed in the small intestine where positive effects have been reported. Another common model to assess feed additive performance is direct oral challenge with live *Eimeria* oocysts. This model generally results in high mortality, unlike *Eimeria* infection within the poultry industry, where birds are inoculated by the natural fecal-oral route (Blake and Tomley, 2014). Supplemental tributyrin ameliorated the negative effects of *Eimeria maxima* infection on broiler growth as 2,500 ppm tributyrin improved the weight gain and FCR 7 d postinfection (Hansen et al., 2021). In contrast, 400 ppm tributyrin failed to consistently increase growth performance of Chinese Yellow broilers exposed to a mixed *Eimeria* challenge (Wang et al., 2021). The current data and previous reports suggest that when broilers are raised in challenging conditions (direct challenge or reused litter) higher concentrations of a protected butyric acid may result in better broiler performance including BW gain and FCRm.

The effects of various concentration of butyric acid on lesion score and oocyst counts are presented in Table 3. No significant differences were observed in lesion scores observed in the duodenum or jejunum and overall lesion scores were low. The low lesion scores might be related to the mode of infection as birds ingesting oocysts from the litter might receive a smaller dose over a longer time period than

Table 3. Coccidiosis lesion scores of male broilers raised on used pine shaving litter fed a control diet or the same control diet supplemented with various concentration of butyric acid.[1]

Treatment[2]	Doudenum	Jejunum	Oocyst shedding
	(0−4)[3]	(0−4)[3]	(oocyst/g)
PC	0.22	0.08	6,009[c]
NC	0.45	0.06	81,687[a]
NC + 200	0.31	0.22	46,723[ab]
NC + 400	0.08	0.03	41,163[bc]
NC + 800	0.44	0.22	64,308[ab]
Pooled SEM	0.125	0.076	14,127
P-value	0.21	0.21	0.01

[1]Data are least square means of 12 pens of male broilers with 3 birds per pen sampled for lesion scoring and one day of litter sampling completed at 14 d of age.

[2]PC = positive control, nontreated diet with fresh pine shaving litter; NC = negative control, nontreated diet with used litter; NC + 200 = diet with 200 ppm butyric acid on used litter; NC + 400 = diet with 400 ppm butyric acid on used litter; NC + 800 = diet with 800 ppm butyric acid on used litter.

[3]Lesions were scored on a scale of 0−4, a score of 0 was given if lesions were not present, 1 if any lesions were seen in a section, 2 if greater than ten lesions per section were seen, 3 if lesions began to overlap/coalesce, and 4 when lesions covered a large portion of the section causing cells to begin to slough off the intestinal lining resulting in a "terrycloth" appearance to the intestinal lining.

[a-c]Values in a column that do not share a common superscript are different ($P \leq 0.05$).

models that use a direct acute gavage of concentrated sporulated oocysts. The presence of these low lesion scores are indicative of a mild or subclinical challenge, which was the aim of the natural infection model. Although lesions scores were low without much opportunity for the butyric acid to improve them, this response is consistent with previous reports where broilers fed 1,000 ppm microencapsulated butyric acid did not result in improved intestinal lesion scores for *E. acervulina, E. tenella*, or *E. maxima* (Ventura et al., 2021). In contrast, coccidiosis challenged Chinese Yellow broilers did result with reduced intestinal lesion scores 400 ppm tributyrin supplementation (Wang et al., 2021).

The results show that there were differences in oocysts shed into the litter among the treatment groups ($P = 0.01$). Raising broilers on the used litter resulted in a significant increase in oocysts shed into the litter (81,687 oocysts/g) in comparison to broilers raised on clean pine shavings (6,009 oocysts/g). The supplementation of 400 ppm butyric acid significantly reduced litter oocysts (41,163 oocysts/g) in comparison to the NC, while both 200 and 800 ppm butyric acid resulted in intermediate responses of 46,723 and 64,308 oocysts/g, respectively as they were not significantly different than either the NC + 400 or the NC treated broilers. The ability of butyric acid to reduce oocyst shedding has been previously reported as broilers challenged with a 10 × dose of Coccidia vaccine on d 5 and fed 400 ppm of tributyrin resulted in reduced excreta oocyst counts at 11 d, but not with subsequent counts at older ages (Wang et al., 2021). This inconsistency in response to oocyst shedding might indicate that the effects of butyric acid are not directly on the oocyst themselves, but might be more related to influence on the broiler.

The damage done to the intestinal mucosa due by coccidia infection in broilers can potentially alter the digestion and absorption of nutrients (Carbajal-Perez et al., 2010; Persia et al., 2006; Kettunen et al., 2001). Changes in nutrient digestion and absorption can result in alterations of broiler body/carcass composition as reduced amino acid digestibility resulted in decreased lean tissue and increased fatty tissue in broilers (Conde-Aguilera et al., 2013). In the current experiment, body composition of broilers fed various concentration of butyric acid resulted in significant differences among treatment groups in total mass, fat mass, and lean mass (Table 4). Similar to BW, there were no significant differences between the PC and NC broilers in total mass, fat mass and lean mass at 42 d. The NC + 800 fed broilers resulted in significantly increased total mass (2,811 g) in comparison to the NC broilers (2,667 g) with the NC + 200 and NC + 400 birds intermediate. Both fat mass and lean mass generally followed the same response with the exception of the fat mass of the NC + 200 fed broilers that was significantly increased in comparison to the NC broilers. The similar responses between both fat and lean mass tissues suggest that the ratio of lean to fatty tissue was unchanged (data not presented) and are in

Table 4. Body composition of male broilers raised on used pine shaving litter fed a control diet or the same control diet supplemented with various concentration of butyric acid.[1]

Treatment[2]	Total mass	Fat mass	Lean mass
	(g)	(g)	(g)
PC	2,646[c]	604[c]	2,005[c]
NC	2,667[bc]	599[c]	2,031[bc]
NC + 200	2,749[ab3]	643[a]	2,068[bc]
NC + 400	2,737[ab]	615[bc]	2,084[ab]
NC + 800	2,811[a]	631[ab]	2,142[a]
Pooled SEM	31.2	9.0	25.0
P-value	≤ 0.01	≤ 0.01	≤ 0.01

[1]Data are least square means of 12 pens of male broilers with 5 birds per pen sampled for body composition at 42 d of age.

[2]PC = positive control, nontreated diet with fresh pine shaving litter; NC = negative control, nontreated diet with used litter; NC + 200 = diet with 200 ppm butyric acid on used litter; NC + 400 = diet with 400 ppm butyric acid on used litter; NC + 800 = diet with 800 ppm butyric acid on used litter.

[a-c]Values in a column that do not share a common superscript are different ($P \leq 0.05$).

contrast to previous reports with a direct Coccidial challenge model (Ott et al., 2018). The differences in ratio of lean and fatty tissue between the current experiment using litter based exposure and previous experiments using oral gavage of higher doses suggest that different modes of action of performance reduction are potentially occurring. In the current experiment, the response is a general response that equally reduced both lean and fatty tissue, while the previous experiment resulted in a larger reduction in fatty tissue most likely associated with reduced energy and fat digestion and absorption due to acute coccidial infection (Rochell et al., 2016; Persia et al., 2006).

CONCLUSIONS AND APPLICATIONS

1. Coated butyric acid was able to increase the performance of 42-day-old broiler chickens raised on used litter as part of a natural challenge model. When compared to the NC fed birds, BW was increased at 400 ppm and 800 ppm of coated butyric acid and FCRm was improved with 800 ppm of coated butyric acid.
2. The used litter model was able to initially reduce BW, but as broilers aged, this response was diminished and lost by 42 d of age. Further refinement of the model is needed to maintain significant differences over the entire 42-d experiment.
3. The used litter model was able to show differences in litter oocysts and butyrate treatment did appear to have some ability to reduce litter oocyst counts at 400 ppm of coated butyric acid. Intestinal lesion scores were low regardless of clean or used litter treatment.
4. Neither the used litter model nor the coated butyric acid altered body composition outside of general growth responses.

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DISCLOSURES

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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