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Impact of Different Levels of Ginger Derived Phyto-Protease (Zingibain) on Broiler Production Performance and Nutrient Digestibility Fed Diet With Reduced Level of Protein and Amino Acids

Muhammad Shahkar Uzair*, Asad Sultan, Sar Zamin Khan and Sher Bahadur Khan

Department of Poultry Science, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan

ABSTRACT

This study was planned to assess the efficiency of ginger-derived phyto-protease enzyme on broiler production performance and nutrient digestibility fed with protein and amino acids deficient diets. A total of 200 (day-old) broiler chicks (ROSS-308) were acquired from a commercial hatchery and randomly assigned to one of the four treatments in this experiment and the trial lasted for 35 days. Birds in the T1 group were offered a starter (1-21 days) and finisher (22-35 days) corn-soybean meal-based diet. The birds in the T2 group were fed a diet in which crude protein and digestible amino acids were reduced by 3%. The other groups like T3 and T4 were fed the same diet as T2 however; they were supplemented with 0.01 and 0.02% of phyto-protease, respectively. The supplementation of phyto-protease enzyme into the broiler diet with reduced crude protein and digestible amino acid levels significantly improved the production performance of the broiler during the finisher and overall production period from day 1-35. Body weight gain (BWG) and feed conversion ratio (FCR) were improved by the supplementation of phyto-protease enzyme in broilers fed diet in which crude protein and amino acid level were reduced by 3%. Carcass yield increased significantly improved by the phyto-protease enzyme. Analysis of the data indicates that supplementation of protease enzyme has non significantly affected digesta pH in different gut sections. All dietary treatments significantly improved ($p<0.05$) nutrient utilization and apparent metabolizable energy of the treated group compared with the control group. The digestibility of dry matter (DM) and crude protein (CP) was improved by the supplementation of protease enzyme compared to birds in the T1 and T2. The highest value for AME was observed in the T4 group followed by T3 respectively. The highest mean value for AME was noted in-group T4 and least in the T1 fed with a diet deficient in 3% crude protein and amino acid. These findings of the present study revealed that supplementation of phyto–protease is effective in improving the adversative effects associated with feeding protein and amino acid-deficient diet.

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Deficient diet, protein, amino acid, growth performance, nutrient absorption, apparent metabolizable energy

INTRODUCTION

The cost of feeding is increasing day by day due to escalation of soybean prices throughout the world. Therefore, to compensate high feed prices, various strategies are being practiced in broiler production with no compromise on the growth performance index. Different levels of crude protein (CP) have been studied to find the economic impact on the poultry industry. Dietary CP is crucial for proper growth of broilers and a major concern of the poultry industry since it represents the main component of poultry feed (Laudadio *et al*., 2012). Protein is an essential ingredient in broiler diets and is used for various physiological processes, particularly muscle development. Broiler diets high in crude protein (CP) are both economically and physiologically costly since it must catabolize an excess of amino acids and excrete excess nitrogen as uric acid (Kidd *et al*., 2001). If enough knowledge is available regarding the specific amino acid requirements for growth, the level of dietary protein can be lowered (Firman and Boling, 1998). The adoption of low CP diets can result in lower feed costs and greater feed formulation flexibility. Previous study, however, has demonstrated that growth performance is impaired when broilers are fed low CP diets rather than high CP diets (Aletor *et al*., 2000). It was also noted that a fall in CP concentration of three or more points could have a negative impact on bird performance. To achieve

* Corresponding author: shahkaruzair362@gmail.com
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equivalent broiler performance, low CP broiler diets were frequently supplemented with crystalline amino acids (Cho *et al*., 2020; Aletor *et al*., 2000), although different results were observed. A variety of proteolytic enzymes is produced that are said to be sufficient for protein usage (Nir *et al*., 1993). However, a significant amount of the protein excreted undigested from the bird's gastro intestinal tract due to incomplete digestion (Lemme *et al*., 2004). This undigested fraction allows for the inclusion of exogenous proteases in chicken diet. Furthermore, exogenous proteases can increase amino acid consumption, leading in improved growth performance (Wang *et al*., 2006). In investigations on the addition of exogenous proteases in broiler diets, there is variability (Marsman *et al*., 1997). Ghazi *et al*., (2003), on the other hand, observed that exogenous protease supplemented diets increased growth performance and protein utilization in broilers. Similarly, Freitas *et al*. (2011) found that adding of protease enzyme to low CP and low energy diets enhanced feed efficiency in broilers. However, additional research is needed on the impact of exogenous protease in low CP broiler diets, particularly by incorporating feedstuffs with low CP digestibility, such as animal protein-based diet. Therefore, this study was designed to evaluate the impact of individual and combined effect of ginger derived phyto-protease (zingibain) of protein and amino acid deficient diet (i) on production performance at day 21 and 35, (ii) gut pH, (iii) total tract nutrient retention and (iv) apparent metabolizable energy of broiler chickens.

MATERIALS AND METHODS

Experimental design and treatments

A total of 200 day-old broiler chicks (ROSS-308) was acquired from a commercial hatchery and reared in an open-sided house bedded with softwood shavings. Birds were randomly allotted to one of the four treatments in this experiment and trial lasted for 35 days. Each experimental diet was assigned to five replicates with 50 birds under each treatment. Birds in the T1 group was offered a starter (1-21 days) and finisher (22-35 days) corn-soybean meal-based diet fulfilling all their nutritional requirements as per ROSS 308 nutrients specification guide. While the birds in T2 group was fed with diet, in which crude protein and digestible amino acids were reduced by 3%. The other groups like T3 and T4 was fed same diet as T2 however; they were supplemented with 0.01 and 0.02% of phyto-protease respectively. The birds were vaccinated according to the local vaccination schedule. Optimum environmental conditions of temperature, humidity, ventilation and light was maintained as needed at different stages of rearing.

Experimental diets and its composition

Three diets were formulated in local feed mill for this experiment and fed to the birds throughout the trial from day 1 to 35 post hatch. The experimental diets were designed as positive control diet (standard diet with corn-soybean meal), and fed to birds in group T1, negative control diet in which crude protein and digestible amino acids was reduced by 3% and fed to the birds in treatment group T2, birds in treatments groups T3 and T4 were fed with same diet as T2 but supplemented with 0.01 and 0.02% of phyto-protease enzyme, respectively. All the diets were iso-caloric (ME 2850 kcal.kg^{-1}). The celite® was used as digestibility marker (acid insoluble ash) and was added at 1% of the feed using Brill Formulation® software (Table I).

Estimation of production performance parameters

Body weight of chicks was measured per cage on arrival (day 0), as well as on a weekly basis (7, 14, 21, 28 and 35). Data obtained from weekly weight gain was used to calculate the starter and finisher weight gain. Individual weights was calculated based on the average of each cage. Feed intake was measured on weekly basis according to the total feed consumed per week per cage. Weekly feed intake was used to calculate overall feed intake. Data obtained from weekly weight gain and feed intake was used to calculate feed conversion ratio (FCR) (Sultan *et al*., 2023).

Carcass traits analysis

During finisher phase at day 35, three birds from each replicate was randomly selected and body weight was recorded. After removal of feathers and other organs, the dressing percentage was calculated. Other organ weight including breast and thigh weight was determined as percent of the dressed weight.

Digesta pH measurements

On day 28 randomly three birds were selected from each replicate in all different treatments. Birds was slaughtered, gastrointestinal tract was removed and fragmented into different parts. pH of different sections was determined using a benchtop pH meter (Baxlo, USA) as per standard protocol (Islam *et al*., 2022).

Immune response against ND, IB and IBD

Blood samples was collected from each group on day 35 and centrifuged for 20 min at 400 rpm for serum collection. Hem-agglutination (HA) and hem-agglutination inhibition blood samples (HI) was used to determine the antibody titter against infectious bursal bronchitis (IBD), infectious bronchitis (B) bursal disease and Newcastle disease (ND).

Table I. Composition and estimated nutrient values of experimental diets.

Ingredients mixed %	T1		T2	
	Starter (0-21 days) %	Finisher (22-35 days) %	Starter (0-21 days) % (-3% CP and dAA)	Finisher (22-35 days) % (-3% CP and dAA)
Corn	55.96	60.25	57.62	62.04
Soybean meal (48%)	36.52	31.43	35.11	29.92
Animal fat	3.71	4.62	3.44	4.34
Dicalcium phosphate	1.51	1.31	1.52	1.32
Limestone	1.10	1.02	1.10	1.03
DL-methionine	0.25	0.37	0.24	0.35
L-lysine (HCl)	0.21	0.26	0.20	0.26
L-threonine	0.01	0.00	0.01	0.00
Common salt (NaCl)	0.40	0.40	0.40	0.40
Vitamin premix	0.25	0.25	0.25	0.25
Mineral premix	0.09	0.09	0.09	0.09
Total	100	100	100	100
Calculated nutrients				
AME (Kcal/Kg)	3100	3200	3100	3200
Crude protein (%)	22	20	21.44	19.40
Ca (%)	0.87	0.78	0.87	0.78
Avail. P (%)	0.44	0.39	0.44	0.39
Na (%)	0.18	0.18	0.18	0.18
Cl (%)	0.27	0.27	0.27	0.27
Lysine (%)	1.28	1.19	1.24	1.15
Methionine (%)	0.57	0.43	0.54	0.41
Total sulphur amino acids (%)	0.87	0.94	0.85	0.91
Threonine (%)	0.77	0.68	0.75	0.66
Tryptophan (%)	0.26	0.16	0.25	0.22
Arginine (%)	1.43	1.09	1.39	1.03
Valine (%)	1.08	0.78	1.06	0.75

T1, positive control (standard diet; recommended CP and amino acid level); T2, negative control (level of CP and amino acid reduced by 3%).

Nutrient digestibility analysis

The chicken was selected (n=10) from all replicates on day-35 and shifted to metabolic cages for total excreta collection for the final four days until day-42. Feed intake and excreta were collected and weighed daily morning for four days. For the determination of ileal nutrients, digestibility 0.2% indigestible marker was used. Dried samples of excreta, ileal digesta, and feed ground to pass through a 1-mm screen. The gross energy of feed and fecal samples was measured using an adiabatic bomb calorimeter and AME was determined. Proximate analyses of feed and excreta samples was done as outlined in (AOAC, 2005). The concentration indigestible marker was determined with a UV absorption spectrophotometer (Williams *et al*., 1962). Apparent ileal metabolizable energy and nutrient digestibility was calculated by using the following formulas (Islam *et al*., 2024).

$$\text{AME}\left(\frac{\text{kcal}}{\text{kg}}\right) = \left\{\text{GE}_i - \left(1 + \text{GE}_0 \times \frac{C_i}{C_o}\right)\right\}$$

GEi and GEo are the gross energy (kcal/kg) in the diet and digesta respectively; Co and Ci are the concentration of indigestible markers in diet and digesta, respectively.

$$\text{Digestibility (\%)} = \left\{1 - \left(\frac{C_i}{C_o} \times \frac{N_o}{N_i}\right)\right\} \times 100$$

Co and Ci= represent the amount of indigestible marker in digesta and diet respectively; Where Ni and No represent the nutrient content in the diet and digesta, respectively.

Statistical analysis

The mean and standard error means values were calculated for all studied parameters, which presented significant difference among treatments. All the data was subjected to analysis of variance as a completely randomized design in the General Linear Model procedure of the SAS 9.3 package (Guide, 2010) where statistical differences between means were determined using the Tukey test and significance was determined at $p<0.05$.

RESULTS

Effect of phyto-protease enzyme on growth performance

The growth performance data i.e., feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) are shown in Table II. The enzyme supplementation increased BWG and FCR significantly. Similarly, enzyme supplementation during day 1-35 increased FI and BWG. FCR was improved by enzyme addition. Reducing the level of crude protein and digestible amino acid level in broiler diet at rate of 3% reduced the BWG and feed conversion ratio. The supplementation of phyto-protease enzyme in to the broiler diet with reduced crude protein and digestible amino acid level significantly improved the production performance of broiler during finisher and overall production period from day 1-35.

Effect of phyto-protease enzyme on carcass yield

Data regarding carcass yield and traits are presented in Table III. Carcass yield and breast meat yield BMY % was significantly ($p < 0.05$) increased when phyto-protease was supplemented in broiler diet both at rate of 0.01 and 0.02 % in broiler diet contains reduced CP and amino acid level. Abdominal fat was reduced by the addition of phyto-protease enzyme in broiler diet.

Table II. Effect of ginger derived phyto-protease enzyme on broiler production performance.

Production traits	Treatments				p value
	T1	T2	T3	T4	
Day 1-21					
BWG (g)	758.45^{b}±0.16	741.08^{c}±0.15	787.51^{a}±0.13	790.47^{a}±0.12	0.000
FI (g)	1128.01± 0.01	1123.41±0.04	1125.61±0.02	1123.60±0.05	0.692
FCR	1.48^{b}±0.02	1.65^{a}±0.03	1.42^{c}±0.01	1.42^{c}±0.01	0.049
Day 22-35					
BWG (g)	1043.51^{c}± 0.33	1025.01^{d}±0.31	1056^{b}.21±0.33	1069.71^{a}±0.34	0.006
FI (g)	2232.30± 0.66	2240.31± 0.65	2238.60± 0.63	2239.30± 0.63	0.321
FCR	2.13^{b}± 0.02	2.18^{a}±0.03	2.12^{b}±0.02	2.09^{c}±0.02	0.007
Day 1-35					
BWG (g)	1801.52^{b}±0.15	1766.08^{c}±0.13	1843.70^{b}±0.15	1860.18^{a}±0.16	0.000
FI (g)	3360.31±0.42	3363.72±0.40	3364.20±0.41	3363.00±0.40	0.358
FCR	1.86^{b}± 0.23	1.90^{a}± 0.25	1.82^{c}± 0.22	1.81^{c}± 0.22	0.000

T1, positive control (standard diet; recommended CP and amino acid level); T2, negative control (level of CP and amino acid reduced by 3%); T3, negative control + 0.01 % Phyto protease; T4, negative control + 0.02 % Phyto protease.
BWG, body weight gain; FI, feed intake; FCR; food conveersion ratio.

Table III. Effect of ginger derived phyto-protease enzyme on carcass traits at day 35.

Carcass traits	Treatments				p- value
	T1	T2	T3	T4	
Carcass yield (%)	69.85^{b}±0.22	69.46^{c}±0.24	69.93^{b}±0.22	70.60^{a}±0.21	0.039
Thigh (%)	24.33± 0.13	24.60± 0.14	24.90± 0.16	24.66±0.14	0.671
Breast (%)	20.71^{c}±0.11	19.26^{d}±0.10	21.58^{b}±0.12	22.63^{a}±0.14	0.002
Drumstick (%)	7.65± 0.05	7.68±0.06	7.56±0.03	7.68±0.06	0.697
Abdominal fat (%)	1.13^{b}± 0.03	1.20^{a}±0.05	0.91^{c}±0.01	0.92^{c}±0.01	0.041

For details of treatment group, see Table II.

Digesta pH in a different section of the digestive tract

The effects of protease enzyme on digesta pH in a different section of the gastrointestinal tract of broiler chickens fed with reduced CP and amino acid level diet are presented in Table IV. Analysis of the data indicates that supplementation of protease enzyme have non significantly affected digesta pH in different gut section. Mean digesta pH in all sections of the gut were non significantly affected by the treatments, however, it was numerically decreased in groups supplemented with protease enzyme.

Table IV. Effect of ginger derived phyto-protease enzyme on pH of different gut section of broiler chickens.

Gut region	Treatments				p value
	T1	T2	T3	T4	
Proventriculus	2.74±0.06	2.73±0.04	2.79±0.08	2.72±0.06	0.080
Duodenum	5.75±0.02	5.72±0.04	5.78±0.05	5.77±0.05	0.090
Jejunum	6.45 ±0.05	6.35±0.06	6.83±0.01	6.18±0.03	0.090
Ileum	7.56 ±0.03	7.46±0.04	7.71±0.06	7.73±0.06	0.070

For details of treatment group, see Table II.

Immune response against ND, IB and IBD

The immune response was checked by employing the HI test to detect the antibody titer against ND and ELISA for IB and IBD on days 35 (Table V). The dietary treatments significantly ($p<0.05$) affected the antibody titer against ND, and IB on days 35. The HI titer (log2 values) against ND were significantly ($p<0.05$) improved by the supplementation of protease as compared to T1 and T2. Mean serum antibody titer against infectious bronchitis (IB) disease virus was also significantly improved the antibody titer on day 35. Mean serum antibody titer against IBD was non significantly ($p>0.05$) affected by the treatments however the titer was numerically improved by protease supplemented treatment group compared with T1 and T2 treatment groups.

Table V. Effect of ginger derived phyto-protease enzyme on immunity of broiler chickens.

Antibody titer	Treatments				P value
	T1	T2	T3	T4	
ND	4.20^{b}±0.05	4.40^{b}±0.05	4.92^{ab}±0.07	5.45^{a}±0.08	0.0058
IBD	3.20±0.03	3.20±0.03	3.22±0.04	3.23±0.04	0.1246
IB	5.00^{b}±0.04	4.87^{c}±0.03	5.02^{b}±0.04	5.42^{a}±0.05	0.0053

For details of treatment group, see Table II.
ND, Newcastle Disease; IB, infectious bronchitis; IBD, infectious bursal disease.

Ileal nutrient utilization and apparent metabolizable energy

The nutrient utilization and apparent metabolizable energy in broiler fed with a 3% Crude protein and amino acid deficient diet at day 35 are given in Table VI. All dietary treatments significantly improved ($p<0.05$) nutrient utilization and apparent metabolizable energy of the treated group compared with control group. It was observed that the dietary treatments T3 and T4 significantly increased ($p<0.05$) ileal dry (DM), and crude protein (CP), compared with the T1 and T2 group. Apparent metabolizable energy was A significantly improved ($p<0.05$) by the protease enzyme supplementation in-group T3 and T4 compared with T1 and T2. The highest value for AME was observed in the T4 group followed by T3, respectively. The highest mean value for AME was noted in the group T4 and least in the T1 fed with a diet deficient in 3% crude protein and amino acid.

DISCUSSION

Protein is the most expensive primary ingredient in poultry feed formulation, a considerable amount (18–20%) of protein passes through the digestive tract undigested and unabsorbed (Applegate and Angel, 2008), which drives up feed costs. It is imperative to increase the protein's capacity for digestion and look for

Table VI. Effect of ginger derived phyto-protease enzyme on nutrients digestibility of broilers at finisher phase of production.

Digestibility %	Treatments				p value
	T1	T2	T3	T4	
Dry matter	76.50^{b}±0.03	75.36^{c}±0.02	78.30^{a} ± 0.05	79.70^{a} ±0.06	0.000
Crude protein	76.50^{c}±0.06	76.66^{c} ±0.05	79.60^{b} ±0.06	82.70^{a} ±0.08	0.001
Ether extract	88.40^{b}±0.06	86.68^{c} ±0.03	89.36^{b} ±0.06	91.70^{a}±0.07	0.000
AME	2688.2^{c}±0.35	2689.2^{c}±0.35	2756.2^{b}±0.37	2821.7^{a}±0.39	0.000

For details of treatment group, see Table II.

inexpensive local protein sources. Animal protein source can be utilized as a source of protein to reduce feeding costs and promote environmental sustainability. Due to high crude protein content (>50%), animal protein sources the feeding value may be increased by enzymatic valorization using exogenous enzymes, which would complement the body's natural enzyme system and improve the breakdown of certain proteins while also increasing digestibility (Classen *et al*., 1991). Enzyme supplementation has been documented nutritionally, environmentally, and economically. More digestible protein are required for the optimal growth of birds. The protein digestibility of soybeans may be greatly impacted by the protein's complex structure, quick transit through the digestive system, and lack of innate enzymes. As a result, adding protease enzymes to the broiler's diet is crucial. The purpose of the current study was to determine the impact of phyto-protease enzyme derived from ginger on broilers fed diets with deficient protein and amino acid content. In the present study, feed intake was none significantly affected by the supplementation of ginger dervieved phyto protease enzyme. These results are consistent with the findings of some previous researchers who observed a non-significant effect of ginger derived phyto protease enzyme on feed intake. Duwa *et al*. (2020) find out that there is no significant effect of ginger supplementation on daily feed intake. Similarly, Onu (2010) also reported no significant changes in the feed intake of birds fed with different ginger treatments compared to control. In contrast to the findings of the present study Safiullah *et al*. (2019) work shows that ginger supplementation along with selenium increase feed intake and palatability. Similarly, Ademola *et al*. (2006) witnessed significantly higher feed consumption ratio in broilers fed with ginger-supplemented diet compared to the control group.

Significant increase in body weight gain was recorded in the present study is in agreement with some earlier studies that examined the same variable (Herawati, 2010). However, some other researcher reported a non-significant effect on body weight gain when broiler fed with a diet contains ginger (Omage *et al*., 2007). Improvement in body weight gain was recorded in broiler fed with dried ginger root (Zhang *et al*., 2009). In present study, better-feed efficiency was recorded in the birds supplemented with ginger derived powder protease enzyme. This finding are in agreement with some studies that have investigated the same variable (Saaci *et al*., 2018; Risdianto *et al*., 2019). Onimisi *et al*. (2009) reported significantly better-feed conversion efficiency in the birds supplemented with ginger compared to the control group. Similarly, Ademola *et al*. (2006) reported a significant increase in feed efficiency in birds supplemented with ginger compared to control birds. The improvement in feed efficiency might be due to enhanced gut micro-flora, which inhibited microbial fermentation and improved feed efficiency.

Positive effect of ginger derived protease enzyme was noticed on carcass yield and breast meat percentage. These results are in agreement with findings of previous researcher who observed positive effect of ginger derived phyto protease enzyme on carcass traits and organ weight carcass trait (Mahmood *et al*., 2018; Asghar *et al*., 2021). Mahmood *et al*. (2018) also noted an improvement in carcass yield in animal protein based diets with the addition of phyto-protease generated from ginger; however, this addition had no influence on any other carcass metrics. Our results, which showed an improvement in dressing % in birds fed diets with additional enzymes, were in line with those of Espino *et al*. (2000). In contrast to our findings, Barazesh *et al*. (2013) observed that varied ginger concentrations had no effect on carcass features. This study, which reported an improvement in the carcass output of broilers fed protease in low-protein diets, corroborated the data of Ajayi *et al*. (2015). This investigation supported the results of Asghar *et al*. (2021), who noted improvements in the carcass features of broilers and noted a numerical rise in the weight of the giblets. A possible explanation for the higher carcass yield in the phyto-protease supplemented groups could be an increased ability to use and deposit protein. Improved utilization and deposition of protein might be responsible for improved carcass yield in ginger derived powder protease-supplemented groups. Gut health is an important indicator of bird health and performance (Kawalilak *et al*., 2010). There was a significant effect of ginger derived powder protease enzyme supplementation on nutrient digestibility in the chickens. Similarly, Saaci *et al*. (2018) study the effect of aqueous extract of ginger on nutrient digestibility, FCR, and economy of broiler chicks and find out that it was significantly increase nutrient digestibility. Duwa *et al*. (2020) study the effect of ginger on growth performance, nutrient digestibility of finisher broiler chicks and reported increased digestibility of nutrients. Increased in digestibility is linked with phenolic compound present in ginger which activate endogenous digestive enzyme in the gut of broiler chicken (Wafaa *et al*., 2012). Protease enzyme in ginger increases protein digestibility, and nutrient absorption, and hence increased digestibility of dry matter. Digestibility and absorption of ash is increased due to improvement of gut morphology. Crypt cells are responsible for the secretion of electrolytes along with release of water increase nutrient digestibility. These results might be due to the active compound gingerol present in ginger, which stimulates the secretion of digestive enzyme, which aids in digestion of nutrients untimely improve the nutrient utilization and growth

(Risdianto *et al*., 2019).

CONCLUSION

It can be concluded that Ginger derived powder protease enzyme can be safely used to enhance the production performance, gut health and nutrient digestibility in broiler chickens.

DECLARATIONS

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IRB approval

The departmental board of studies approved the experimental protocols (Approval number: 621-2023/PS/UAP

Ethical approval

The experiment was carried out in a poultry research unit at the University of Agriculture Peshawar. The departmental board of studies and the Animal Research and Ethics Board of the University of Agriculture Peshawar approved all experimental procedures adopted.

Statement of conflict of interest

The authors have declared no conflict of interest.

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