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Effect of red tea (*Hibiscus sabdariffa*) powder and selenium on growth performance, intestinal morphology and some blood parameters in heat-stressed broiler chickens

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Abstract This study was carried out to determine the influence of red tea (*Hibiscus sabdariffa*) powder and selenium (Se) on growth performance, intestinal morphology, and some blood constituents in heat-stressed broiler chickens. In this experiment, 240 one-day-old male broiler chickens (Ross 308) with a mean weight of 40±2 g were used in a completely randomized design, as a 2×3 factorial arrangement consisting of three levels of red tea (0, 0.5, and 1.0 g/kg) and two levels of Se (0, 0.4 mg/kg). Broiler chickens received the diets from days 25 to 42 of age. Heat stress was applied from days 25 to 42 of the experiment for eight hours, 09:00 am to 05:00 pm (34±2 °C). On d 42, two birds from each pen were randomly chosen, blood sampled, and slaughtered. Inclusion of red tea powder in the diet increased daily weight gain (DWG) and improved feed conversion ratio (FCR) (P<0.05). The interaction effect of Se and red tea powder on feed intake (FI), DWG, and FCR was significant (P<0.05). Broiler chickens that received a diet containing 1.0 g/kg red tea and no Se had the greatest BWG and the lowest FCR. Maximum FI was observed in broiler chickens that received a diet containing 0.4 mg/kg Se and no red tea powder. The interaction effect of Se and red tea powder improved the intestinal morphology and increased glutathione peroxidase activity in broiler chickens (P<0.05). According to the findings of this research, diet supplementation with 1.0 g/kg of red tea powder and 0.4 mg/kg of Se may improve growth performance, intestinal morphology and increase glutathione peroxidase activity in broilers under heat-stress conditions.

Keywords: broiler, growth performance, heat stress, red tea, selenium

Introduction

One of the main features that adversely affect the production performance of animals is heat stress (Wasti et al., 2020). Poultry producers, especially in tropical regions, have to mitigate the harmful impacts of heat stress (Azad et al., 2010). Heat stress usually occurs within the last weeks of the rearing period while broilers require an optimum heat between 18 and 22°C (Lin et al., 2006). When broilers were exposed to high ambient temperature, their feed intake and growth performance decreased (Goo et al., 2019). Besides nutritional impacts, heat stress also leads to lipid oxidation which generates cytotoxic and genotoxic compounds that are deleterious for humans' health (Botosglou et al., 2014). Studies have reported that heat stress in broilers can induce heightened levels of oxidative stress, leading to lipid oxidation and the formation of reactive oxygen species. Consequently, meat may experience discoloration, appearing darker and losing its natural pink hue (Gonzalez-Rivas et al., 2020). Heat stress may impair the immune system which negatively affects animal growth performance and increases mortality. Moreover, immunoglobulins regulate the immune functions and stimulate immune responses (Ulfman et al., 2018). Immunoglobulin levels are also modified by heat stress (Calefi et al., 2016). Fortunately, over the past decades, there has been a lot of progress in improvement of the poultry houses in hot areas. On the other hand, some researchers proposed different nutritional approaches including feed restriction or supplementation of diets with vitamins and minerals, the addition of ammonium chloride or sodium bicarbonate to diet or drinking water, reducing heat increment of the diet by replacing part of its carbohydrate with lipids, and reducing the amount of protein along with addition of some essential amino acids to the diet (Windish et al., 2008). Recently, the role of natural antioxidants, like medicinal herbs, has also been considered (Surai et al., 2019). In poultry farming, numerous researches have been carried out on herbs and herbal extracts as growth enhancers or for disease prevention. Red tea, also called rooibos tea, comes from the *Aspalathus linearis* plant, and is completely free of caffeine, contrasting the other varieties. Rooibos tea is rich in antioxidants and a good source of some unique polyphenols, including aspalathin, which is effective against free radical damage and conditions like diabetes, heart disease, and potentially cancer (Piek et al., 2019). Aspalathin acts through numerous mechanisms, including direct scavenging of reactive oxygen species (ROS), chemical dropping activity, stimulation of antioxidant enzymes, and prevention of oxidases (Mannan et al., 2020). Red tea contains compounds such as alkaloids, anthocyanins, beta-carotene, ascorbic acid, and other useful compounds that have been used for treatment of several illnesses in the past. Moreover, tea powder contains vitamin C and has antioxidant properties, which could be useful in heat-stress conditions (Ismail et al., 2008).

Selenium (Se) is an essential trace element with important roles in immune and reproductive functions, reproductive health, gonadal development, gametogenesis and fertilization, and maintenance of homeostasis; it is also involved in cancer chemoprevention. Selenium is an important antioxidant because of its role in the building of Se-reliant enzymes, especially glutathione peroxidase. The main role of glutathione peroxidases in the antioxidant system is to eliminate radical hydro-peroxide and hydrogen peroxide (Duntas and Benvenga, 2015). Addition of selenium to feed improves the performance of broilers where it can function an antioxidant in the intercellular space, cytosol, and in conjunction with membranes of cells (Li et al., 2021).

As antioxidants, both red tea and selenium may act synergistically; therefore, the current research aimed to evaluate the influence of adding red tea powder and Se in the diet on performance, intestinal morphology, and some blood components in heat-stressed broilers.

Materials and methods

Bird management, diets and experimental design

Two hundred forty (1-d-old) male broilers (Ross 308) with a mean weight of 40±2 g were purchased from a hatchery in Kerman, and raised according to the Ross 308 manual guidelines until 25 days of age. Then, they were randomly allocated to 24 floor pens (1×1.5 m) in a 2×3 factorial experiment with 3 levels of red tea powder (0, 0.5, and 1.0 g/kg of diet) and 2 levels of Se (0, 0.4 mg/kg of diet) and 4 replicates (10 chicks each). Diet were fed from 25 to 42 days of age and heat stress (34 ± 2 °C) was applied for eight hours (0900 am to 0500 pm) during this period. Feed intake (FI) and body weight were recorded to calculate feed conversion ratio (FCR) and body weight gain (BWG) at the end of the trial. On d 42, two birds from each pen were randomly chosen, blood sampled, and slaughtered. The trial was conducted according to the approved protocol by the University of Jiroft Animal Care Committee. Feed ingredients and nutrient composition of the basal diet are presented in Table 1. The basal diet was formulated according to the dietary requirements following the Ross Manual. Selenium was purchased from BASF Company (Ludwigshafen, Germany). Red tea was collected from a city in the southeast of Kerman, Iran. The dried tea was milled and added to the diet. Water and feed were offered *ad libitum*. The light program was according to the Ross Manual. The relative humidity in the rearing house was 50 to 70%.

Table 1. Ingredients (as-fed) and chemical composition (calculated analysis) of the basal diet

Ingredients (%)	From 25 to 42 d of age
Corn	60.63
Soybean meal	30.85
Sunflower oil	4.85
Limestone	1.30
Dicalcium phosphate	1.21
Vitamin and mineral premix[b]	0.50
Sodium chloride	0.29
DL-Met	0.25
L-Lys	0.13
Composition	
ME (kcal/kg)	3200
CP (%)	19.0
Lys (%)	1.09
Met + Cys (%)	0.86
Thr (%)	0.82
Arg (%)	1.43
Ca (%)	0.85
Available P (%)	0.42
Se (%)	0.048

[a] Selenium (0, 0.4 mg/kg) and Red tea (0, 0.5 and 1.0 g/kg) were included on top of the basal diet.

[b] Vitamin and mineral premix supplied the following per kilogram of diet: vitamin A, 9000 IU; vitamin D3, 215 IU; vitamin E, 18 IU; vitamin K3, 2 mg; vitamin B1, 18 mg; vitamin B2, 6.6 mg; vitamin B6, 3 mg; vitamin B12, 0.015 mg; nicotinic acid,10 mg; folic acid, 1 mg; pantothenic acid, 12 mg; choline chloride (60%), 5000 mg; Mn, 100 mg; Cu,10 mg; I, 1 mg; and Fe, 50 mg.

Growth performance

On days 25 and 42 of the trial, the birds were weighted, FI was measured, and BWG and FCR were calculated. The chicks were daily monitored and mortality and the date of death were documented, for correcting the FCR, and BW.

Blood features

On d 42, two birds per pen were randomly chosen. Blood samples were taken from the wing vein using a 5 mL syringe. A portion of blood samples (2 mL) was carefully transferred into tubes containing ethylenediaminetetra acetic acid (EDTA) as the coagulant for determination of glutathione peroxidase enzyme (GPX), and the remaining portion was transferred into non-heparinized tubes to measure RBC, WBC, heterophil and lymphocyte count. An automated hematology analyzer (Sysmex K-1000, Sysmex Corp., Kobe, Japan), which had been calibrated to for analysis of chicken blood, was used to define the deliberation of total WBC (cells/μL). Glutathione peroxidase was measured by a commercial kit (sensitivity 12.65 U, a precision of <4.86% CV, correlation coefficient of r=0.9829; Randox, Co. Antrim, United Kingdom).

Internal organs and intestinal morphology

At the end of the trial, two chicks with the nearest weight to the average of each pen, were chosen and slaughtered to measure the weight of spleen, Bursa of Fabricius, and liver. The values were stated as a percentage of the fasting body weight. To measure the villus height and crypt depth, sections of approximately 5cm of ileum, between Meckel's diverticulum and ileocecal junction, were obtained. The cut pieces were dipped in a phosphate-buffered formalin solution. Two segments per sample were cut and fixed in paraffin wax. For morphometric study, transverse sections (6 μm) were stained and examined under a light microscope (OLYMPUS Germany, BX51 model) (Brudnicki et al., 2017). The height of 10 villi and their associated crypts were measured on each section, the means of which were used for data analysis.

Statistical analysis

The GLM procedures (Minitab, 2009) was used for data analysis. The effects of Se and tea powder inclusion were evaluated, and P≤0.05 was considered significant.

Results

Body temperature

The effects of Se and red tea powder on production performance of the chickens under heat stress are shown in Table 2.

Table 2. Effects of red tea powder and Se on performance of broilers reared under heat stress (n=10 birds per replicate)

Treatment		Daily BWG (g/b/d)	FI (g/b/d)	FCR (g/g)
Se (mg/kg)				
0		81.09	132.7[a]	1.64
0.4		78.48	138.0[b]	1.75
SEM		1.053	1.074	0.022
P-value		0.096	0.002	0.091
Red tea powder (g/kg)				
0		77.26[b]	136.9	1.77[a]
0.5		78.85[ab]	135.7	1.72[a]
1		83.25[a]	133.4	1.60[b]
SEM		1.290	1.315	0.028
P-value		0.011	0.185	0.000
Se (mg/kg)	Red tea powder (g/kg)			
0	0	75.01[b]	130.3[b]	1.893[a]
0	0.5	82.10[ab]	136.4[ab]	1.673[bc]
0	1	86.16[a]	131.3[b]	1.524[c]
0.4	0	79.50[ab]	143.5[a]	1.767[ab]
0.4	0.5	75.59[b]	135[b]	1.787[ab]
0.4	1	80.34[ab]	135.5[ab]	1.708[b]
SEM		1.824	1.860	0.039
P-value		0.012	0.004	0.003

[a-c]In each column, means with common superscript(s) do not differ (P>0.05).

There was no effect of Se on BWG but red tea powder and its interaction significantly affected BWG. The highest BWG was obtained by feeding one g per kg red tea powder vs. the control group (P≤0.05). Red tea powder did not affect FI (P>0.05) but selenium increased FI (P≤0.05) while the interaction effect of Se and red tea powder on FI was also significant. The greatest amount of FI was found in broilers feeding on the diet containing 0.4 mg Se without red tea powder compared to the control group. Feeding one g red tea powder per kg diet improved FCR but Se did not impact on FCR. The interaction effect of red tea powder and Se on FCR was also significant; the best FCR was recorded in chickens which were fed with the diet containing one g of red tea powder per kg diet without Se. There was no significant effect of red tea powder, Se or their interaction on the liver and spleen weights (Table 3).

Table 3. Effects of red tea powder and Se on relative weights of internal organs (g/100 g of live weight) in broilers reared under heat stress (n=2 birds per replicate)

Treatment		Liver	Spleen	Bursa of Fabricius
Se (mg/kg)				
0		2.071	0.1127	0.070
0.4		2.004	0.1182	0.079
SEM		0.062	0.006	0.005
P-value		0.459	0.459	0.247
Red tea powder (g/kg)				
0		2.079	0.1152	0.059 [b]
0.5		1.981	0.1175	0.078 [ab]
1		2.053	0.1138	0.086 [a]
SEM		0.076	0.007	0.007
P-value		0.653	0.653	0.032
Se (mg/kg)	Red tea powder (g/kg)			
0	0	2.003	0.1112	0.069[ab]
0	0.5	2.050	0.1055	0.072[ab]
0	1	2.159	0.1215	0.067[ab]
0.4	0	2.154	0.1192	0.049[b]
0.4	0.5	1.912	0.1294	0.083[ab]
0.4	1	1.947	0.1060	0.1040[a]
SEM		0.108	0.010	0.007
P-value		0.2	0.234	0.024

[a-c]In each column, means with common superscript(s) do not differ significantly (P>0.05).

The relative weight of the Bursa of Fabricius was not affected by Se feeding alone but it was increased by adding one g red tea power per kg to the diet; further increase was recorded when the diet contained 0.4 mg Se and one g red tea powder (per kg). The influence of adding Se and red tea powder on the blood components of broilers is shown in Table 4.

Table 4. Effects of red tea powder and Se on blood attributes in broilers reared under heat stress (n= 2 birds per replicate)

Treatment		WBC ($\times 10^3$µl)	RBC ($\times 10^6$µl)	Hematocrits %	Heterophil %	Lymphocyte %	Glutathione peroxidase (U/mL)
Se (mg/kg)							
0		13573	2.651	38.14	9.333	90.67	214.4 [a]
0.4		14990	2.788	39.04	10.250	89.70	258.6 [b]
SEM		711	0.055	0.784	0.460	0.487	6.635
P-value		0.176	0.095	0.425	0.225	0.177	0.001
Red tea powder (g/kg)							
0		13031	2.666	38.59	9.000[b]	91.00	207.4[b]
0.5		13875	2.648	37.30	9.100[ab]	90.67	251.7 [a]
1		15938	2.844	39.87	11.120[a]	88.88	250.3 [a]
SEM		870.7	0.067	0.961	0.563	0.596	8.126
P-value		0.078	0.102	0.196	0.025	0.096	0.001
Se (mg/kg)	Red tea powder (g/kg)						
0	0	11844	2.619	39.06	9.250	90.75	164.9 [b]
0	0.5	14625	2.622	36.97	9.250	90.75	241.1 [a]
0	1	14250	2.713	38.37	9.500	90.50	237.1 [a]
0.4	0	14219	2.712	38.13	8.750	91.25	250.0 [a]
0.4	0.5	13125	2.675	37.63	8.950	90.59	262.3 [a]
0.4	1	17625	2.975	41.37	12.750	87.25	263.6 [a]
SEM		1231.4	0.095	1.359	0.796	0.843	11.49
P-value		0.141	0.517	0.366	0.052	0.086	0.02

[a-c]In each column, means with common superscript(s) do not differ significantly (P>0.05).

White and red blood cells and hematocrit, neutrophils, and lymphocytes were not affected by adding Se and/or red tea powder to the diet, but heterophil count significantly increased by adding one g of red tea powder per kg diet. Chicks which received the diet comprising 0.4 mg Se per kg had a greater glutathione peroxidase activity than the control group (P<0.01). Inclusion of red tea powder alone in the diet (at both levels) increased the glutathione peroxidase activity compared to the control group (P<0.01). The interaction effect of Se and red tea powder was also significant, so that, the control group had the lowest level of glutathione peroxidase activity compared to other groups.

Dietary inclusion of red tea powder or Se affected the villus height, width, crypt depth, and villus height to crypt depth ratio (Table 5).

Red tea powder at one g/kg diet increased the villus width (P<0.05) and crypt depth (P<0.01), but reduced the villus height to crypt depth ratio (P<0.01). Adding 0.4 mg Se per kg diet increased the villus width (P<0.01). The interaction effect of red tea powder and Se on all measured morphometric parameters was significant

(P<0.01). Birds receiving the diet comprising one g/kg red tea and 0.4 mg/kg Se had the greatest villus height and width.

Table 5. Effects of red tea powder and Se on intestinal morphology in broilers reared under heat stress (n= 2 birds per replicate)

Treatment		Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus height:crypt depth ratio
Se (mg/kg)					
0		1686	134.0[a]	93.58	18.02
0.4		1692	141.9[b]	91.00	18.59
SEM		8.125	1.162	2.689	0.423
P-value		0.628	0.001	0.506	0.837
Red tea powder (g/kg)					
0		1676	134.1[b]	76.13[b]	22.50[a]
0.5		1687	138.9[ab]	101.75[a]	16.94[b]
1		1704	140.8[a]	99.00[a]	17.42[b]
SEM		9.951	1.423	3.293	0.518
P-value		0.162	0.011	0.001	0.001
Se (mg/kg)	Red tea powder (g/kg)				
0	0	1677[bc]	122.8[c]	76.25[c]	22.92[a]
0	0.5	1722[ab]	145.9[a]	115.00[a]	15.21[c]
0	1	1659[c]	133.3[b]	89.50b[c]	18.54[b]
0.4	0	1675[bc]	145.5[a]	76.00[c]	22.08[a]
0.4	0.5	1651[c]	132.0[b]	88.50[bc]	18.67[b]
0.4	1	1749[a]	148.2[a]	108.50[ab]	16.30[bc]
SEM		14.073	2.012	4.657	0.733
P-value		0.001	0.001	0.001	0.003

[a-c]In each column, means with common superscript(s) do not differ significantly (P>0.05).

Discussion

Growth performance

The results of this research revealed that red tea powder enhanced BWG and improved FCR. In contrast, another research showed that green tea extract had no effect on broiler production under heat stress environment (Son et al., 2023). In the current experiment Se improved FI of heat-stressed broiler chickens; in contrast, another report stated that body weight and feed intake of heat stressed broilers were not influenced by dietary Se, while feed conversion was significantly improved by Se-supplementation at 0.2 mg/kg (Niu et al., 2009a). Lin et al. (2005) reported that the growth rate decreased in broiler chickens from the fourth week of rearing, by increasing temperature from 18 to 35 °C. It has been proposed that a decrease in growth rate is due to the effect of thermal stress on appetite and FI (Bartlett and Smith, 2003; Niu et al., 2009b; Frei et al., 2010). These researchers reported that growth performance was negatively affected by heat stress due to a reduction in the availability of nutrients, rise in energy waste as heat during the metabolism of nutrients, decline in protein synthesis, and enhancement in lipogenesis. Moreover, chronic exposure to heat stress leads to oxidative stress as excessively induced reactive oxygen species damage and imbalance to the body's antioxidant system. The antioxidants such as green tea constituents maintain the equilibrium of oxidation–reduction and modulate antioxidant enzymes to alleviate damage caused by heat stress, such as improving poultry growth performance (Hu et al., 2019). Many researchers have investigated the alleviating effect of adding green tea to broilers diet in heat stress condition, but the effect on production has been varied (Imik et al., 2012; Kim et al., 2021). Fischer et al. (2008) stated that FI and BWG decreased in turkeys fed with low Se diet. In agreement with the findings of the current research, Payne and Southern (2005) and Yoon et al. (2007) also reported that adding additional amounts of Se in chicken diets did not affect their BWG. The decrease in feed consumption of broiler chickens in heat stress situation is part of their natural responses to alleviate the excess heat production in the body (Teyssier et al., 2022). At high temperatures, this heat dissipation will aggravate the effect of heat stress. In such situations, the bird should reduce its basal metabolism to maintain life, and therefore will have to reduce the voluntary feed intake (Onagbesan et al., 2023).

In contrast to our findings, Payne and Southern (2005) and Yoon et al. (2007) reported that Se supplementation to the diet did not affect FI in broilers. Sahin et al. (2008) described that the use of 0.15 or 0.3 mg/kg organic or inorganic Se in the diet of heat-stressed laying quails (34 °C for 8 hours per day) increased FI compared to the control group. Supplementation of the diet with different amounts of organic or inorganic Se had no effect on broiler performance (Edens et al., 2001, Spears et al., 2003 and Biswas et al., 2006). Instead, Denise et al. (2005) described that feeding a diet containing 0.3 mg/kg of Se yeast improved the FI in broiler chickens, but similar level of sodium selenite did not affect the FCR. The reason for the difference between the current research and prior studies could be attributed to the method of application, the duration of feeding, distribution in different parts of the diet, the age of bird, hygienic condition and environmental stress.

Blood constituents

The blood constituents are sensitive to temperature changes and the physiological reaction of the birds under heat-stressed conditions. Borges et al. (2007) reported quantitative variations in the number of white blood cells, erythrocytes, and hemoglobin during heat stress conditions. A reduction in hemoglobin concentration and a rise in leukocyte counts under thermal stress conditions were reported by Kirk et al. (2002). Heterophil to lymphocyte ratio is one of the best indicators of stress assessment in poultry (Zhang et al., 2023).

As discussed previously, oxidative stress not only increases the creation of free radicals but also modifies the ability of the antioxidant system including scavenging of free radicals (Lin et al., 2008). Selenium plays a role in the cytoplasm as a part of the glutathione peroxidase in scavenging peroxides (Sahin et al., 2002). It has been stated that feeding black tea significantly slowed down the decrease in glutathione peroxidase in rat muscles. Furthermore, Haidari et al. (2013) reported that green tea extract lowered the lipid peroxidase activity in heart and aorta of the diabetic rats. Frei and Hugdon (2010) stated that tea polyphenols act as antioxidants, and the addition of green or black tea in laboratory conditions prevented the reduction of superoxide dismutase. They also stated that, to some extent, catechins of tea prevented the oxidation of proteins. These studies confirmed the antioxidant properties of Se and red tea powder, and it is expected to increase the glutathione peroxidase concentration.

Internal organ

Heat stress decreased the relative weight of lymphatic organs and liver in broilers (Bartlett and Smith, 2003; Anwer et al., 2004; Niu et al., 2009). It is suggested that degeneration of lymphatic organs may be a result of reduced FI. In line with our findings, Biswas et al. (2006) reported that adding 0.5 and 1.0 mg/kg Se to the diet of Japanese quail did not affect the liver and spleen weights. Moreover, the oxidative stress induced by the thermal stresses will increase the peroxidation of lipids (Pamok et al., 2009). It can be suggested that the use of additional levels of antioxidants, such as Se and tea powder, while decreasing the oxidative influences of heat stress, could prevent the damages to these organs.

Intestinal morphology

Inclusion of antioxidants like red tea powder and Se to the diet increased the villus height (VH) and width (VW) while decreased villus height to crypt depth (CD) ratio in heat-stressed broiler chickens which concurs with the findings of the other researchers (Rebel et al., 2004; Read-Snyder et al., 2009). Intestinal morphology, especially VH and VH/CD ratio, affects the digestion and absorption of nutrients in the small intestine (Lee et al., 2022). It has been reported that heat stress negatively affects the intestinal morphology and villi parameters, such as decreased VH and increased CD, and induces intestinal ischemia leading to reduced intestinal integrity and lowered digestibility (Bahrampour et al., 2021). Several studies have demonstrated that the addition of antioxidants including vitamin E and C, and minerals to feeds mitigates the negative effects of heat stress on nutrient digestibility (El-Senousey et al., 2018; Bahrampour et al., 2021). It has been shown that vitamin C supplementation has a positive effect on intestinal health by improving intestinal histology, and epithelial growth, and preventing oxidative protein degeneration (Amer et al., 2021). Bahrampour et al. (2021) revealed that when antioxidants and minerals, including vitamin C, were added to feed, the negative effects on nutrient digestibility were mitigated and damage caused by heat stress was prevented. Owulade et al. (2004) described that *Hibiscus sabdariffa* improved intestinal function in rats. It reduced transport rate indicating a decrease in intestinal motility and augmenting the passage time. They proposed that *Hibiscus sabdariffa* could therefore be a constipating agent at a proper dose.

Conclusions

The present experiment showed that adding Se and red tea powder to the diet of broiler chickens had a positive effect on BWG, FI, and FCR. Broilers that received a diet comprising 0.4 mg/kg Se had the greatest amount of glutathione peroxidase activity. The treatment including 1.0 g/kg red tea powder and 0.4 mg/kg Se increased the villus height and width; this combination may improve growth performance, intestinal morphology, and some blood attributes in broilers under heat-stress conditions.

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Conflict of interest statement

The authors indicated no conflict of interest.

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