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Mitigating heat stress in broiler chickens using dietary onion (*Allium cepa*) and ginger (*Zingiber officinale*) supplementation

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Abstract

The objective of this study was to evaluate the effect of dietary onion (*Allium cepa*) and ginger (*Zingiber officinale*) supplementation on growth, carcass quality, antioxidant status, and immune response in broilers under heat stress. A total of 700 day-old Hubbard broiler chicks were weighed and assigned to five treatments and five replicates. Broilers were maintained in a thermoneutral (TN) environment or were exposed to heat stress (HS). For 35 days, HS birds were fed a control diet and three levels of onion and ginger powder as: 5 g/kg ginger + 1.5 g/kg onion (T1), 10 g/kg ginger + 2.5 g/kg onion (T2), and 15 g/kg ginger + 3.5 g/kg onion (T3). Body weight, feed conversion ratio (FCR), dressing percentage, and the weight of immune-related organs improved in T2 compared to the control. Blood concentrations of malondialdehyde (MDA) and paraoxonase-1 (PON1) were substantially higher in T2 than the control. Similarly, in the same dietary group, the antibody titre against Newcastle disease (ND) and total leucocyte count (TLC) were greater than the control diet. The findings of this research indicate that 10 g of ginger combined with 2.5 g of onion in the diet enhance broiler growth performance, carcass quality, antioxidant status, and immunological response under heat stress conditions.

Keywords: broiler, growth, redox balance, onion, ginger, heat stress
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Introduction

Thus far, heat stress has not been perfectly defined. Stress can be defined as forces that can potentially cause strain in the body (Marchini *et al*., 2016). According to some scientists, the general definition of heat stress is the impact of environmental temperature above the physical temperature of the body that results in poor performance and health of broilers (Khan *et al*., 2012a). For poultry, heat stress above 25 °C is considered detrimental. In most cases, for birds to suffer from heat exhaustion, the temperature should remain high for a few days to a few weeks and the temperature may reach up to 50 °C (Khan *et al*., 2021). When the bird is in a state of stress, physiological, behavioural, and neuroendocrine mechanisms enable them to acclimate to the adverse environmental conditions. Heat stress (HS) is usually considered to be acute HS, which refers to a higher environmental temperature and humidity over short period of time (Uyanga *et al*., 2022).

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Heat stress is one of the major predicaments to adversely impact the growth of the birds (Khan *et al*., 2011; Safiullah *et al*., 2019; Ahmad *et al*., 2020a; Hafeez *et al*., 2021). To enhance the performance of broilers under these conditions, different dietary supplements have been used. One such additive is the use of phytogenics, which have a wide range of beneficial impacts on the growth and health of broilers (Khan *et al*., 2012a; Ahmad *et al*., 2020a; Hafeez *et al*., 2020ab; Chand *et al*., 2021; Shuaib *et al*., 2021). These compounds play their role through a variety of mechanisms, such as antimicrobial activities, antioxidant capacity, and enhanced feed utilization (Khan *et al*., 2012b; Haq *et al*., 2020; Hafeez *et al*., 2021).

Onions (*Allium cepa*), in addition to saponins, flavonoids, and organo-sulphur compounds, also contain polyphenolic and phenolic acids, fructans, and fructo-oligosaccharides (Kothari *et al*., 2019). The major organic sulphur compounds found in *Allium* spp. in varying amounts are mainly: s-alk(en)yl-L-cysteine sulphoxides, allyl-cysteine, sulphoxides of sulphates of methyl–cysteines, and sulphoxides of sulphate of allyl-cysteines (Omar and Al-Wabel, 2010). Onions contain flavonoids (such as quercetin 4'-glucoside), catechin, and flavones (such as quercetin aglycone), as well as flavone di-glucosides (Miean and Mohamed, 2001; Nemeth and Piskula, 2007). An onion supplementation of 2.5% resulted in higher growth, improved immunity, and heat tolerance in broilers under heat stress (Al-Ramamneh, 2018).

Ginger (*Zingiber officinale*) is a well-known rhizome. There are numerous uses for it, including in cooking, as a delicacy, and even as a medicine. Nine ginger-derived compounds have been found to make a bond with serotonin, which may alter its gastrointestinal function (Botsoglou *et al*., 2002; Khan *et al*., 2012b). On degradation, shogaols, zingerone, and paradol have been isolated. Ginger compounds have been found to have pharmacological, antimicrobial, and antioxidant properties (Ali *et al*., 2008). Ginger extract (i.e., aqueous and acid) is known to have antioxidant properties and can therefore scavenge free radicals (Krishnakantha and Lokesh, 1993). Among the sesquiterpene hydrocarbons found in the volatile oils present in ginger are beta-sesquiphellandrene (27.2%), zingiberene (13.9%), caryophyllene (15.3%), alpha-farnesene (10.5%), and arcurcumin (10.5%) (El-Baroty *et al*., 2010). To the best of our knowledge, no information is available on the effect of ginger in heat-stressed livestock.

Despite the fact that onion and ginger contain numerous beneficial phytoconstituents and properties, little scientific research has been done on their use in broiler production and health when birds are exposed to heat stress. Thus, the purpose of this study was to determine the dietary effect of different onion and ginger concentrations on growth performance, immunity, and antioxidant status in broilers under heat stress.

Materials and Methods

The trial was approved by the Ethics Committee of the University of Agriculture, Peshawar (approval code 124 FAHVS/20).

Fresh onion and ginger were locally procured, and then washed with purified water before being dried in the shade for two weeks at room temperature. After a thorough drying process, onion and ginger were milled and stored in clean plastic bags until needed.

A total of 700 day-old Hubbard broiler chicks were weighed and allocated to four different feeding regimens (each with eight repetitions). Birds were divided into five treatments (five replicates). Each replicate had 30 birds. One group was thermoneutral (TN) and other four groups were heat stressed. Heat stressed groups were divided into a control (only basal diet) group and three levels of onion and ginger treatments. Chicks were raised in pens using wood shavings as bedding material. Each pen measured 160 × 240 cm and included round pen feeders and drinkers. Throughout the trial, birds had unrestricted access to feed and water. Standard vaccination schedules against infectious diseases, including Newcastle disease, were given to birds on a regular basis. For the first week, the birds were kept under fluorescent illumination for 23 h, followed by a 20 light: 4 dark cycle. As in Table 1 (Israr *et al*., 2021), broilers on day 8 were divided into either a temperature-controlled room at 24 ± 3 °C (thermoneutral group; TN) or were exposed to chronic heat stress.

Table 2 shows the ingredients and nutrient composition of the basal diet. In a completely randomized design, four diets were prepared and assigned to broilers, each of which contained a control (basal diet) as well as three levels of onion and ginger powder, supplied at rates of 5 g/kg ginger + 1.5 g/kg onion (T1), 10 g/kg ginger + 2.5 g/kg onion (T2), and 15 g/kg ginger + 3.5 g/kg onion (T3) for 35 days, with the first week serving as an adaptation period (28 trial days).

Table 1. Mean daily temperature and relative humidity during the experimental period for broilers

Hours	Temperature (°C)	Relative humidity (%)
08:00	34.22	80.23
12:00	36.57	89.54
16:00	38.76	72.77
20:00	36.14	74.86
24:00	34.42	76.12
04:00	30.52	78.15

In each pen, feed intake, body weight, and feed conversion ratio were monitored weekly, with the mean of each parameter recorded throughout the experiment. The performance parameters were studied under the starter (1–21 days) and the finisher (22–35 days) phases.

Five birds per pen were slaughtered on day 35, and 3 ml of blood was taken and split into two tubes for analysis, one containing EDTA and the other without. Blood not treated with EDTA was centrifuged for 10 min at 3000 rpm and kept at -80 °C until analysis.

The thiobarbituric acid (TBA) reaction was used to determine lipid peroxidation in serum samples (Ohkawa *et al.,* 1979). A spectrophotometric analysis of the colour generated by the reaction between TBA and MDA was performed at a wavelength of 532 nm. The presence of PON-1 in blood was determined using the Mackness *et al*. (1991) method, which utilized phenol as a substrate to quantify the concentration. The working reagents consisted of 1 mM phenylacetate, 1 mM calcium chloride, and 20 mM Tris HCl buffer. The absorbance was measured for 5 min at 270 nm after diluting the sample 1:3 with buffer and combining it with the substrate before measurement. Total leucocyte count (TLC) was determined in blood with EDTA following the method described by Chand *et al*. (2016). The hemagglutination inhibition test was used to examine the host's immune response to the ND virus.

A completely randomized design was used to analyse data using statistical software (Statistix 8.1). The Tukey's test was used to determine whether there was a significant difference among groups at the 5% probability level. To determine linear (L) and quadratic (Q) effects of the inclusion level of additives compared to the control, orthogonal contrasts were used.

Table 2. Ingredients and chemical composition of basal diet fed to broilers

Ingredients (%)	Starter (1–21 d)	Finisher (22–35 d)	Ginger	Onion
Corn	55.50	56.00		
Soybean meal, 44% CP	28.40	27.50		
Canola meal	6.06	5.51		
Sunflower meal, 28% CP	3.30	4.20		
Vegetable oil	2.10	2.10		
Molasses	1.00	1.00		
Dicalcium phosphate	1.90	1.90		
Limestone	1.00	1.00		
NaCl	0.01	0.01		
$NaHCO_3$	0.01	0.01		
DL-Methionine	0.20	0.10		
Lysine-HCl	0.22	0.37		
Vitamin-mineral premix[1]	0.30	0.30		
Chemical composition				
ME, kcal/kg	3,000	3,150		
Crude protein, %	23.50	21.30		
Methionine, %	0.55	0.44		
Lysine, %	1.42	1.23		
Sulphur amino acids, %	0.96	0.80		
Threonine, %	0.95	0.85		
Calcium, %	1.05	0.90		
Av. phosphorus, %	0.50	0.45		
Crude protein, %	-	-	8.83	11.53
Crude fibre, %	-	-	0.92	22.41
Crude lipid, %	-	-	5.60	4.81

[1]Vitamin–mineral premix contains per kg: vitamin A, 2400000 IU; vitamin D, 1000000 IU; vitamin E, 16000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B_2, 1600 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg, and zinc, 14000 mg

Results

The mean body weight gain, feed intake, and FCR of birds supplemented with onion and ginger during the starter, finisher, and overall period, as well as when subjected to chronic heat stress is shown in Table 3. At all phases, the TN and T2 groups gained more weight (P <0.05), whereas the control group gained the least weight. Weight gain, feed intake, and FCR were similar in the T1 and T3 groups.

Table 3. Growth performance of broilers supplemented with onion and ginger during the starter (1–21 days) and finisher (22–35 days) phases exposed to chronic heat stress conditions

Item		Body weight gain (g)			Feed intake (g)			Feed conversion ratio (g/g)		
		Starter	Finisher	Overall	Starter	Finisher	Overall	Starter	Finisher	Overall
TN		975^{a}	1450^{a}	2445^{a}	1823^{a}	2221^{a}	3876^{a}	1.86	1.53	1.58^{b}
Control		735^{c}	1095^{d}	1830^{c}	1338^{c}	1697^{c}	3035^{c}	1.81	1.55	1.65^{a}
T1		822^{b}	1308^{b}	2131^{b}	1503^{ab}	1875^{ab}	3349^{b}	1.81	1.43	1.56^{b}
T2		955^{a}	1379^{a}	2318^{a}	1613^{a}	1975^{a}	3589^{a}	1.68	1.41	1.52^{b}
T3		848^{b}	1248^{c}	2096^{b}	1483^{b}	1842^{b}	3325^{b}	1.74	1.56	1.58^{ab}
SEM		1.04	2.03	2.09	2.73	2.61	1.51	0.101	0.212	0.091
P-value		0.031	0.001	0.042	0.023	0.001	0.001	0.873	0.920	0.044
Orthogonal contrast	L	0.01	0.001	0.03	0.02	0.02	0.01	0.54	0.81	0.01
	Q	0.02	0.002	0.04	0.01	0.01	0.04	0.51	0.12	0.03

Mean values in the same column with different superscripts are statistically different (P <0.05). T1: 5 g/kg ginger + 1.5 g/kg onion, T2: 10 g/kg ginger + 2.5 g/kg onion; T3: 15 g/kg ginger + 3.5g/kg onion; TN: thermoneutral; L: linear; Q: Quadratic

The TN and T2 groups had the greatest (P <0.05) feed intake and weight gain during the starter and finisher phases, while the control group had the lowest (P <0.05) feed consumption and weight gain throughout these phases. Groups T2 and TN had greater overall feed intake and weight gain than the control (P <0.05). Feed intake and weight gain in T3 were lower compared to TN and T2. During the starter and finisher phases, there was no significant difference among the four groups in terms of FCR. Overall, the control treatment had the greatest FCR, which was not statistically different from T3. The FCR of groups T1, T2, and T3 were found to be similar. Further, a linear and quadratic response was observed for weight gain, feed intake, and efficiency.

Table 4 shows the mean dressing percentage of broilers fed different amounts of onion and ginger. The T1 and T2 treatments had a greater dressing yield (P <0.05) than T3. The use of ginger and onion supplements resulted in a substantial impact on the weight of lymphoid organs. The bursa of Fabricius was found to have the highest (P <0.05) weight in T2 and TN, while the control had the lowest (P <0.05) weight. The thymus of TN and T2 groups resulted in the highest weight. T1 and T3 had similar bursa of Fabricius and thymus weights. When comparing T1 and T3, it was found that T2 had the greatest (P <0.05) spleen weight.

Table 4. Mean dressing percentage (%), percentage weight of bursa, thymus, and spleen in broiler chicks supplemented with various combinations of ginger and onion under chronic heat stress

Treatments		Dressing percentage	Bursa of Fabricius	Thymus	Spleen
TN		67.43^{a}±0.13	0.141^{a}±0.001	0.235^{a}±0.01	0.142^{a}±0.001
Control		60.50^{c}±0.5	0.103^{c} ±0.002	0.214^{c}±0.004	0.107^{d}±0.004
T1		62.0^{c}±1.34	0.115^{b} ±0.006	0.225^{b}±0.005	0.123^{c}±0.004
T2		66.83^{a} ±0.77	0.13^{a} ±0.006	0.239^{a}±0.003	0.149^{a}±0.002
T3		64.98^{b} ±0.11	0.12^{b} ±0.003	0.224^{b}±0.004	0.133^{b}±0.005
P value		0.01	0.03	0.04	0.04
Orthogonal contrast	L	0.001	0.03	0.02	0.02
	Q	0.01	0.02	0.01	0.03

Mean values in the same column with dissimilar superscripts are statistically different (P <0.05); T1: 5g/kg ginger + 1.5g/kg onion; T2: 10g/kg ginger + 2.5g/kg onion; T3: 15g/kg ginger + 3.5g/kg onion

Table 5 shows the mean serum MDA, PON-1, and antibody titres against ND as well the TLC of broiler chicks subjected to heat stress and fed different amounts of ginger and onion. The control group had the highest (P <0.05) MDA level, which was not different (P >0.05) from T1. The MDA levels were lowest (P <0.05) in T2 and TN. The PON1 levels were different among groups: the control had the lowest (P <0.05) PON1 value, whereas groups T2 and TN had the highest (P <0.05). Reductions (P <0.05) in MDA and PON1 levels were observed in birds treated with ginger and onion. Further, the control group had the lowest (P <0.05) antibody titre against ND, whereas groups TN, T2, and T3 had the greatest (P <0.05) titres. Concentrations of MDA, PON-1 and ND was statistically similar in T1 and T3; the TLC level was similar in T2 and T3.

Table 5. Serum malondialdehyde (MDA), paraoxonase-1 (PON-1), and antibody titre against Newcastle disease (ND) and blood total leucocyte count (TLC) in broilers supplemented with onion and ginger exposed to chronic heat stress conditions

Item		MDA (nmol/ ml)	PON-1 (U/ml)	ND titre (Log_{10})	TLC ($10^3/mm^3$)
TN		6.03^{c}	12.34^{a}	6.32^{a}	20.11^{a}
Control		7.85^{a}	9.18^{d}	3.67^{c}	17.82^{c}
T1		7.45^{ab}	10.71^{c}	5.33^{b}	19.10^{b}
T2		6.18^{c}	13.19^{a}	6.67^{a}	20.14^{a}
T3		6.97^{b}	11.82^{b}	5.00^{b}	19.70^{a}
SEM		0.272	0.382	0.561	0.198
P-value		0.01	0.02	0.01	0.05
Orthogonal contrast	L	0.001	0.01	0.01	0.03
	Q	0.001	0.03	0.02	0.02

Mean values in the same column with different superscript are statistically different (P <0.05). T1: 5 g/kg ginger + 1.5 g/kg onion; T2: 10 g/kg ginger + 2.5 g/kg onion; T3: 15 g/kg ginger + 3.5g/kg onion; TN: thermoneutral; L: linear; Q: Quadratic

Discussion

At all levels of dietary supplementation, the ginger and onion combination boosted broiler feed intake in comparison to the unsupplemented groups under HS. The T2 had the greatest overall feed consumption, and the best outcomes were achieved when broilers were fed a combination of 10 g/kg ginger and 2.5 g/kg onion in the diet. Ginger contains bioactive chemicals that bind to serotonin receptors and have an effect on gastrointestinal function (Botsoglou *et al*., 2002). In a previous study, Ademola *et al*. (2006) reported similar findings, indicating that broilers given a ginger-supplemented diet consumed more feed. Furthermore, ginger contains zingibain, which aids in feed digestion and targets parasites and their eggs in the gastrointestinal tract (Mohammed and Yusuf, 2011). A higher feed intake was observed by Karangiya *et al*. (2016) in broilers fed 1% ginger, compared to the control birds, indicating that ginger inclusion in diet had no effect on feed palatability. Slyranda *et al*. (2011) reported an increase in broiler feed intake when 100 mg onion was added in the diet, which is consistent with the current experiment's findings. The organo-sulphur components found in onion are thought to be responsible for its beneficial effect on feed intake (An *et al*., 2015). In this regard, Farahani *et al*. (2015) and Goodarzi *et al*. (2013) also observed an increase in feed consumption when onion extract was added to drinking water. In the current study, the T2 group produced the best results in term of improved growth performance compared to T1 and T3. It seems that the dose in T2 was conducive to growth in broilers under HS.

In the present study, the findings indicated that treatments including ginger and onion enhanced body weight growth in all broiler groups (T1 to T3). Ginger has a variety of active chemicals, including thiol proteinase, humulen, borneol, kamfen, atsiri oil, limonen, zingiberene, and zingiberol (Rismunandar, 1988), all of which aid in feed digestion and body weight growth. An addition of 2% ginger in the broiler diet was observed to result in an increase in weight gain (Onimisi *et al*., 2005). This is because ginger acts as a natural growth promoter due to its antibacterial, antioxidant, and pharmacological properties. As a result, it aids in overall digestion (Ali *et al*., 2008). When ginger is supplemented in the diet, it increases the production of digestive enzymes such as maltase, lipase, and disaccharidase (Zhang *et al*., 2009). Herawati (2010) found a substantial rise in the final body weight of the ginger-fed groups compared to a control treatment. Moreover, using ginger has been shown to increase the production of lactic acid bacteria while decreasing the production of others, such as mesophilic, aerobic, and coliform bacteria, and *E. coli* (Tekeli *et al*., 2011). Accordingly, Arshad *et al*. (2012), Mohamed *et al*. (2012), and Sadeghi *et al*. (2012) reported similar findings, in which the addition of ginger to the diet resulted in an increase in broiler weight gain.

The FCR of birds in the treatment groups improved markedly compared to those in the control group. The improved FCR may be attributed to the bioactive compounds found in both ginger and onion, which include antibacterial, antioxidant, and pharmacological activities (Nemeth and Piskula, 2007; Ali *et al*., 2008). Additionally, the presence of phenolic and organo-sulphur compounds in onion enhances growth performance and, eventually, the FCR (An *et al*., 2011). The current study's results are consistent with those of Onimisi *et al*. (2005), Moorthy *et al*. (2009), and Arshad *et al*. (2010), who found that ginger-supplemented birds had a higher FCR. Ginger promotes the development of a stable microbiota, thus improving the feed efficiency (Tekeli *et al*., 2011). A positive influence of the inclusion of onion on FCR was also observed by Aji *et al.* (2011) and Goodarzi *et al.* (2013), which is similar to the findings of the present study. The treated groups demonstrated a substantial improvement in dressing percentage when compared to the control group. These results are explained by the fact that the relevant groups gained more weight. The groups treated with ginger and onion had a markedly higher lymphoid organ weight. Ibrahiem *et al*. (2004), Goodrazi *et al*. (2013), Golshan *et al*. (2015), and Shewita *et al*. (2018) showed increased immune-related organ weight in domestic birds in response to ginger and onion supplementation.

The current research found that ginger and onion supplementation substantially decreased MDA levels and increased PON1 values. Although paraoxonase neutralises free oxygen radicals, its concentration decreases as the temperature exceeds the thermoneutral zone (James *et al*., 2000). It has been observed that as the temperature rises, the respiratory chain activity in mitochondria increases, perhaps resulting in increased blood MDA levels (Khan *et al*., 2014). Malondialdehyde is the end product of peroxidation of polyunsaturated fatty acids and serves as a marker of oxidative stress (Rehman *et al*., 2018). Zhang *et al*. (2009) found that birds given ginger had a higher antioxidant status and lower MDA levels, which agrees with the current study's findings. Further, Habibi *et al*. (2014) discovered lower MDA concentrations in the serum after ginger supplementation, which is consistent with the current experiment's results. Recently, An *et al*. (2019) revealed comparable findings to ours, reporting reduced MDA levels in birds fed ginger. This is because of ginger has been shown to neutralise free radicals and superoxide ions (Krishnakantha and Lokesh, 1993), thus enhancing the antioxidant state of the birds. Similarly, Yuanita *et al*. (2019) revealed comparable results to the present research, where broilers fed Dayak onion had reduced MDA levels.

The present research revealed that when ginger and onion were added to diet, the antibody titre against ND was increased in treated groups compared to the control. Ginger supplementation increases the number of lactic acid bacteria in the jejunum (Tekeli *et al*., 2011); additionally, ginger rhizome extract in water acts as an immunostimulant against ND and other illnesses (Nidaullah *et al*., 2010). It was found by Arshad *et al*. (2012) that ginger extract had a beneficial effect by increasing antibody titres against ND, which is consistent with the current study. Due to the presence of bioactive components such as shogaols and gingerols, ginger has been demonstrated to display antioxidant activity (both *in vivo* and *in vitro*) and to improve immunity (Khan *et al*., 2012). Recently, Safiullah *et al*. (2019) found a rise in antibody titre in birds given ginger and selenium, which corresponds to the present experiment's findings. Onion contains a potent antiviral compound, quercetin, and onion extract is efficient in preventing viruses from attaching to cells (Harazem *et al*., 2019). The blood TLC and lymphocyte concentrations were substantially increased in the ginger and onion supplemented groups compared to control in the current study. In a previous study, Oleforuh *et al*. (2012) found comparable findings to the current research, stating that broilers given ginger and garlic infusion had a substantial increase in white blood cells. The current findings are consistent with those of Agu *et al*. (2017), who found that broilers fed ginger meal had greater total white blood cells than those in the control group. Accordingly, Bilal *et al*. (2018) observed comparable findings to our research, with a significant difference in total leukocyte and lymphocyte counts in ginger- and garlic-supplemented birds. This suggests that ginger enhances immunity by increasing the number of white blood cells.

Conclusions

In conclusion, ginger and onion dietary supplementation at a rate of 10 and 2.5 g/kg, respectively, improves the growth, carcass quality, antioxidant potential, and immune response of birds under heat stress conditions.

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Author contribution s

All the authors approved the final version of the manuscript. MS, NC, MS, RUK, MR, ST, VL, and VT: conceptualization, formal analysis, methodology, validation, writing-review & editing, writing-original draft.

Data availability

Data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Declaration

The authors declare no conflicts of interest.

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