



The effect of purslane hydroextract and zinc on performance, antioxidant capacity and immunity of broiler chickens during summer conditions

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Abstract. Due to negative effects of high ambient temperature, the present study was conducted to investigate the effects of *Portulaca oleracea* hydroextract (PHE) and zinc (Zn) on the growth performance and immunity of broiler chickens reared under high summer ambient temperature conditions. A total of 420 day-old male broiler chickens (Ross 308) were randomly allocated to seven dietary treatments each replicated four times with 15 birds per replicate. The basal diet as the control diet was supplemented with 100 mg kg⁻¹ butylated hydroxytoluene (BHT), or 300 and 600 mg kg⁻¹ of PHE, 100 mg kg⁻¹ of zinc and 100 mg kg⁻¹ of zinc plus 300 or 600 mg kg⁻¹ of PHE (ZP300 and ZP600). The result of the present study showed that Zn, PHE and their combination did not alter daily feed intake and feed conversion ratio. Although there was a tendency toward an increase in broiler body weight at 24 and 44 days of age ($P = 0.059$ and $P = 0.061$), the body weight of birds fed the basal diet supplemented with Zn reduced at day 10. The birds fed diets supplemented with BHT or ZP600 showed lower aspartate aminotransferase (ASAT) and lactate dehydrogenase activity at 24 days of age ($P < 0.05$), but it remains lower in the ZP600 group only for ASAT activity level at day 44. The results of the present study suggest that dietary PHE and Zn supplementation partially improved body weight and serum antioxidant capacity, superoxide dismutase activity and antibody titer against sheep red blood cells and Newcastle diseases in the broiler chickens during summer conditions.

1 Introduction

One of the prominent environmental elements influencing broiler performance and health is high ambient temperature (Song et al., 2012; Sun et al., 2015). In most middle eastern countries, the incidences of heat stress commonly happen during the summer conditions and when environmental temperature exceeds the comfort zone of the broiler chickens. In addition to the impairment of feed intake and growth rate, high ambient temperature can cause oxidative stress in farm animals (Mager and Kruijff, 1995; Iwagami, 1996; Lin et al., 2006). Animal cells have an antioxidant defense system which is responsible for protection and/or repair of cell damage caused by reactive oxygen species (ROS). Antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) play a critical role in neutralizing ROS and protect cells from damage.

Zinc is known as a biologically important trace mineral which acts as a cofactor and activator of more than 300 enzymes in different metabolic pathways (Vallee and Falchuk, 1993; Golding, 2002; Takkar, 2011). Zinc is the cofactor of copper–zinc SOD, as a key enzyme of the antioxidant defense system (Perry et al., 2010). Zinc supplementation (20 to 60 mg kg⁻¹ diet) ameliorated oxidative stress in broiler chickens by increasing the activity of copper–zinc SOD and finally decreased lipid peroxidation (Bun et al., 2011). Furthermore it is reported that the serum malondialdehyde (MDA) concentration, an indicator of lipid peroxidation, decreases with dietary zinc supplementation in heat-stressed quails (Sahin et al., 2006; Kucuk, 2008). Sunder et al. (2008) demonstrated that antibody titers against sheep red blood cells (SRBC) significantly increased in broiler chickens fed diets supplemented with zinc up to 80 mg kg⁻¹. Also, different medicinal herbs, containing antioxidant-rich flavonoids

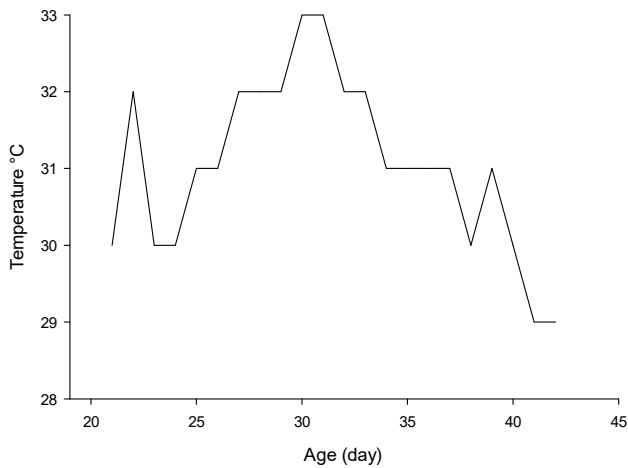


Figure 1. The average daily temperature of the chicken house from 10:00 to 18:00 local time (GMT + 4:30).

and polyphenols, have been receiving much attention as natural antioxidants in the poultry industry. *Portulaca oleracea* L. (commonly called purslane) is a herbaceous weed rich in antioxidants, including phenolic compounds, β carotene, ascorbic acid, tocopherol and glutathione (Xiang et al., 2005; Yang et al., 2009). Dietary supplementation of purslane (extract or powder) in broiler chickens improved their performance and antioxidant status under thermoneutral conditions (Zhao et al., 2013; Sadeghi et al., 2016). To the best of our knowledge, there is no previous research considering a combination of zinc and purslane hydroextract (PHE) on the performance, immune system and antioxidant status of broiler chickens under natural summer high ambient temperature. Although there are dozens of studies conducted on the effect of heat stress in broiler chickens, most of them used artificial heat stress. We hypothesize that the administration of PHE in the diet of broiler chickens during summer alone or in combination with zinc could contribute to improve growth performance, immunity and antioxidant status of broiler chickens. Therefore, the present study was conducted to investigate the effect of PHE (300 and 600 mg kg⁻¹ of diet) and supplemental zinc (100 mg kg⁻¹ of diet) on the performance, immunity and antioxidant status of broiler chickens under summer high ambient temperature.

2 Material and methods

2.1 Experimental design and purslane extract preparation

Four hundred and twenty day-old male broiler chickens (Ross 308) were randomly allocated to seven dietary treatments each replicated four times with 15 birds per replicate. The basal diet as the control diet was supplemented with 100 mg kg⁻¹ butylated hydroxytoluene (BHT), or 300 and

Table 1. Ingredient composition and analysis of the basal diets.

Item (% unless noted)	Days 0–10	11–24	25–44
Ingredients			
Corn	51.14	54.58	56.01
Soybean meal	40.33	36.45	34.04
Soybean oil	4.19	5.04	6.28
Dicalcium phosphate	1.94	1.69	1.55
Limestone	1.01	0.95	0.89
Salt	0.37	0.37	0.39
DL-Methionine	0.22	0.19	0.17
L-lysine	0.30	0.23	0.17
Vitamin and mineral premix	0.5	0.5	0.5
Calculated analysis			
Metabolizable energy (ME) (Mcal kg ⁻¹)	3	3.1	3.2
Crude protein (CP)	22.5	21.0	20
Calcium	0.96	0.87	0.81
Available phosphorus	0.48	0.43	0.40
L-lysine HCL	1.44	1.29	1.16
Methionine	0.56	0.51	0.47
Calculated Zn (mg kg ⁻¹)	72.27	71.09	70.52
Analyzed Zn (mg kg ⁻¹)	68.62	69.10	68.24

600 mg kg⁻¹ of PHE, 100 mg kg⁻¹ of zinc and 100 mg kg⁻¹ of zinc plus 300 or 600 mg kg⁻¹ of PHE (ZP300 and ZP600). The experiment was conducted during July and August 2014. No artificial cooling system was provided to the chickens during the whole experiment period. The average temperature of the poultry house is shown in Fig. 1. Locally harvested whole plants of purslane have been dried (shade dry), powdered and then used for preparing PHE. The antioxidant properties of the PHE have been analyzed and reported previously by Habibian et al. (2017). The content of zinc in the basal diet was determined by a flame atomic absorption spectrophotometer (Phoenix-986, AA spectrophotometer, UK) after wet ashing (Favero et al., 2013).

2.2 Sample collection

On 10, 24 and 44 days of age, body weights and feed intake were recorded to calculate daily feed intake (DFI) and feed conversion ratio (FCR). At 24 and 44 days, two birds per replicate were randomly selected, weighted and then a blood sample was drawn from a wing vein by using a syringe within 30 s and collected in tubes. Serum was obtained after centrifugation (Hettich EBA 21, USA) at 400 g for 10 min at 4°C and was stored at -20°C for further analysis of triglycerides (TG), aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH), malondialdehyde (MDA), total antioxidant capacity (TAC) and total SOD (T-SOD) activity. On day 44, after blood sampling, the birds were sacrificed and abdominal fat, liver, spleen and bursa of Fabricius were weighted.

2.3 Newcastle virus and SRBC antibody response

At 27 days of age, all the selected chickens were injected in the right wing vein with 1 mL of 9% SRBC suspension. Pre-challenge antibody titers were found to be negative. At 7 and 14 days after the first challenge, the birds received a booster injection of 1 mL 9% SRBC. Three days after the last booster injection (on day 44), blood samples were obtained and harvested serums stored at -20°C until assay of the primary antibody response to SRBC. All birds were immunized intramuscularly with a killed vaccine of Newcastle virus at 8 days of age (Clone 30, Nobilis AI + ND). Live Newcastle disease vaccine was administered orally (La Sota strain, Cevac New L) at 18 days of age. At 34 days of age, double blood samples were collected from two birds per replicate. Serum antibody titers against Newcastle virus were determined by a hemagglutination inhibition test and were expressed as the logarithm to base 2 (Allan and Gough, 1974). The experiments were undertaken following the guidelines of the Animal Ethics Committee in Kurdistan University.

2.4 Serum biochemical and antioxidant parameters

The concentration of TG and activity of ASAT and LDH in serum were determined by commercial kits (Pars Azmun, Tehran, Iran) using a Jasco V-570 spectrophotometer (Jasco, Tokyo, Japan). The concentrations of MDA and TAC in the serum and T-SOD activity were detected spectroscopically (SHIMADZU UV-1800, Kyoto, Japan) by biochemical methods following the instructions of the corresponding reagent kits (Nanjing Jiancheng Bioengineering Institute of China, Nanjing, China).

2.5 Statistical analysis

The experiment was conducted using a completely randomized design. All data were subjected to one-way ANOVA using SAS software (version 8). Duncan's multiple-range analysis was used to compare differences between significant means. Means with $P < 0.05$ were considered statistically significant.

3 Results

3.1 Performance

The effects of supplemental zinc and PHE on the performance parameters of broiler chickens are shown in Table 2. Average body weight of broiler chickens did not significantly alter in zinc and PHE treated groups when compared to the control group at 10 days of age ($P > 0.05$). However, at 24 and 44 days of age, there was a tendency toward an increase in the average body weight of birds fed diets supplemented with zinc and PHE combinations and BHT under summer conditions ($P = 0.059$; $P = 0.061$). No significant alteration in the DFI or FCR was observed when experimental groups

were compared with the control group. The treatment effect did not influence mortality ($P > 0.05$, data not shown).

3.2 Serum biochemical and antioxidant parameters

As shown in Table 3, none of the dietary treatments resulted in a significant alteration in the serum TG concentrations at 24 and 44 days of age. Although there was a significant ($P < 0.05$) decrease in the ASAT activity in the birds fed 100 mg kg^{-1} BHT and a combination of 100 mg kg^{-1} zinc and 600 mg kg^{-1} PHE at 24 days of age. However, at 44 days of age there was no significant reducing effect of BHT, while 600 mg kg^{-1} of PHE and its combination with 100 mg kg^{-1} zinc resulted in a significant decrease in the serum ASAT activity ($P < 0.05$). At 44 days of age, LDH activity of heat-exposed broiler chickens received 100 mg kg^{-1} BHT, 600 mg kg^{-1} PHE or 100 mg kg^{-1} zinc plus 600 mg kg^{-1} PHE significantly ($P < 0.05$) decreased than those fed control diet.

The effects of supplemental zinc and PHE on the serum antioxidant enzyme activity and MDA concentration in broiler chickens are shown in Table 4. MDA content of serum was reduced in broiler chickens fed 100 mg kg^{-1} BHT or 100 mg kg^{-1} zinc plus 600 mg kg^{-1} PHE under summer conditions. Moreover, serum MDA concentrations in the birds fed 600 mg kg^{-1} PHE in combination with 100 mg kg^{-1} zinc showed a significant reduction ($P < 0.05$). The results showed that there was a tendency toward reduction in the activity of SOD in the broilers fed diets containing BHT or ZnPHE600 during high ambient temperature exposure ($P = 0.050$).

3.3 Response to SRBC and Newcastle vaccination

The effect of dietary treatments of the serum antibody titer against SRBC and Newcastle disease is shown in Table 4. When compared with the control group, antibody titer against SRBC and Newcastle virus did not improve when birds received diets supplemented with 300 or 100 mg kg^{-1} supplemental dietary zinc. However, there was a significant increase in the SRBC and broiler chickens fed 600 mg PHE per kg of diet at 34 days of age ($P < 0.05$).

4 Discussion

Positive effects of purslane on the performance of broiler chickens during the growing period have been studied by Zaho et al. (2013) and Ghorbani et al. (2013). However, they reported the effect of purslane powder or PHE in the thermoneutral housing systems. Supplementation of broiler diets with purslane extract improved feed consumption and daily body weight gain in broiler chickens (Zhao et al., 2013; Ghorbani et al., 2014). Lee et al. (2003) suggested that improved digestibility and availability of feedstuff nutrients

Table 2. Effects of dietary Zn and PHE on performance of broiler chickens from 1 to 44 days of age. ^{a-c} indicates that results with the same superscript in each row are not significantly different.

Item	Treatments							p value	SEM
	Control	BHT	Zn	PHE300	PHE600	ZPHE300	ZPHE600		
BW (g)									
day 10	228.50 ^{abc}	258.67 ^{ab}	211.17 ^c	240.84 ^{abc}	260.59 ^a	227.00 ^{bc}	243.50 ^{abc}	0.023	4.61
day 24	715.44	745.73	735.31	726.76	768.59	740.90	774.36	0.059	5.92
day 44	1985.19	2146.71	2081.06	2121.35	1966.06	2126.17	2216.60	0.061	24.89
DFI (g d⁻¹ bird⁻¹)									
days 01–10	21.59 ^{abc}	21.91 ^{ab}	19.20 ^c	23.48 ^a	23.18 ^a	20.00 ^{bc}	21.79 ^{ab}	0.012	0.38
days 11–24	62.13	68.05	68.35	64.68	69.16	67.49	69.24	0.307	0.90
days 25–44	152.76	166.54	157.68	146.78	164.98	145.54	164.98	0.560	3.31
days 01–44	92.74	100.84	96.39	91.30	96.76	91.35	100.09	0.511	1.55
FCR (g g⁻¹)									
days 01–10	1.17	1.04	1.15	1.20	1.07	1.10	1.11	0.303	0.02
days 11–24	1.78	1.96	1.82	1.93	1.84	1.91	1.77	0.597	0.03
days 25–44	2.28	2.28	2.21	2.01	2.47	2.00	2.18	0.232	0.05
days 01–44	2.00	2.03	1.98	1.85	2.11	1.84	1.94	0.377	0.03

Abbreviations: BHT, butylated hydroxytoluene; Zn, zinc; PHE300, purslane hydroextract 300 mg kg⁻¹ of diet; PHE600, Purslane hydroextract 600 mg kg⁻¹ of diet; ZPHE300, zinc 100 mg kg⁻¹ diet + purslane hydroextract 300 mg kg⁻¹ of diet; ZPHE600, zinc 100 mg kg⁻¹ diet + purslane hydroextract 600 mg kg⁻¹ of diet; BW, body weight; DFI, daily feed intake; FCR, feed conversion ratio; SEM, standard error of means; values are means ± SEM.

Table 3. Effects of dietary Zn and PHE on the serum level of TG, ASAT and LDH activity in broiler chickens. ^{a-c} means that results with the same superscript in each row are not significantly different.

Item	TG (mg dL ⁻¹)		ASAT (UL ⁻¹)		LDH (UL ⁻¹)	
	Days 24	44	24	44	24	44
Control	91.70	124.92	182.42 ^{ab}	303.76 ^a	1938.65 ^a	1268.24
BHT	281.61	97.47	113.46 ^c	250.70 ^{ab}	1220.96 ^c	1227.63
Zn	95.86	81.28	182.21 ^{ab}	266.53 ^{ab}	1581.58 ^{abc}	1070.67
P300	84.70	112.48	154.03 ^{abc}	264.22 ^{ab}	1531.32 ^{abc}	1156.90
P600	140.79	97.25	145.32 ^{bc}	242.79 ^{bc}	1423.04 ^{bc}	1286.34
ZP300	111.03	89.63	196.58 ^a	279.80 ^{ab}	1781.16 ^{ab}	1313.66
ZP600	109.79	119.46	122.96 ^c	193.09 ^c	1334.87 ^c	1030.06
SEM	5.13	4.77	7.31	8.41	59.84	36.61
p value	0.327	0.084	0.026	0.042	0.043	0.333

Abbreviations: BHT, butylated hydroxytoluene; Zn, zinc; PHE300, purslane hydroextract 300 mg kg⁻¹ of diet; PHE600, purslane hydroextract 600 mg kg⁻¹ of diet; ZPHE300, zinc 100 mg kg⁻¹ diet + purslane hydroextract 300 mg kg⁻¹; ZPHE600, zinc 100 mg kg⁻¹ diet + purslane hydroextract 600 mg kg⁻¹ of diet; TG, triglyceride; ASAT, aspartate aminotransferase; LDH, lactate dehydrogenase; SEM, standard error of means; values are means ± SEM.

could be due to greater digestive enzyme production and/or activity by supplementing broiler diets with purslane.

Heat challenge leads to an increase in free radical production and oxidative damage which subsequently increases LDH and ASAT, reflecting tissue damage (Mikami et al., 2004; Sandercock et al., 2006). In the current study, reduced activity of serum ASAT at 24 and 44 days in birds fed a combination of 100 mg kg⁻¹ zinc and 600 mg kg⁻¹ PHE sug-

gests a possible alleviating effect of liver oxidative injuries in broiler chickens during high ambient temperature.

Oxidative damages reflect the imbalance between pro-oxidant and antioxidant systems. In intact rats, it is demonstrated that high hyperthermic conditions increased formation of ROS in the portal circulation (Hall et al., 1994; Lin et al., 2006). In broiler chickens, high ambient temperature increased plasma MDA which indicated an oxidant/antioxidant equilibrium disturbance leading to oxidative stress (Lin et al.,

Table 4. Effects of dietary Zn and PHE on the serum concentration of MDA, TAC and T-SOD activity and antibody titers against SRBC and Newcastle virus in broiler chickens.

Item	MDA (nmol mL ⁻¹)	TAC (mmol ⁻¹)	T-SOD (U mL ⁻¹)	SRBC	Newcastle titer
Control	2.02 ^a	0.79 ^c	142.71	3.37 ^c	3.16 ^c
BHT	1.38 ^b	1.16 ^a	106.51	2.62 ^c	3.14 ^c
Zn	2.02 ^a	0.95 ^{abc}	138.20	2.62 ^c	3.08 ^c
P300	2.01 ^a	0.90 ^{bc}	129.77	3.50 ^c	3.70 ^{bc}
P600	1.78 ^{ab}	1.13 ^a	118.21	5.50 ^a	5.53 ^a
ZP300	1.76 ^{ab}	1.03 ^{ab}	130.16	3.50 ^c	4.12 ^{abc}
ZP600	1.48 ^b	1.16 ^a	109.76	4.37 ^b	4.92 ^{ab}
SEM	0.06	0.03	3.76	0.17	0.23
<i>p</i> value	0.031	0.004	0.050	< 0.0001	0.010

Abbreviations: BHT, butylated hydroxytoluene; Zn, zinc; PHE300, purslane hydroextract 300 mg kg⁻¹ of diet; PHE600, purslane hydroextract 600 mg kg⁻¹ of diet; ZPHE300, zinc 100 mg kg⁻¹ diet + purslane hydroextract 300 mg kg⁻¹ of diet; ZPHE600, zinc 100 mg kg⁻¹ diet + purslane hydroextract 600 mg kg⁻¹ of diet; MDA, malondialdehyde; TAC, total antioxidant capacity; CAT, catalase; SRBC, sheep red blood cells; HA, hemagglutination assay; SEM, standard error of means; values are means ± SEM. ^{a-c} means that results with the same superscript in each row are not significantly different.

2000, 2006; Yang et al., 2010). In our present study, supplemented basal diet with 100 mg kg⁻¹ BHT or 100 mg kg⁻¹ zinc plus 600 mg kg⁻¹ PHE significantly decreased the content of serum MDA in broiler chickens reared under summer conditions. As a consequence of high ambient temperature, increased ROS production led to increased lipid peroxidation that was indicated by the concentration of MDA. Therefore, the content of MDA in serum can be used as an indirect index of lipid peroxidation and overproduction of ROS (Yang et al., 2010). This effect could be due to increased antioxidant capacity of the birds. As the results showed, the level of serum TAC increased in the BHT and ZnP600 groups. Purslane is known as an important source of biologically active components with high antioxidant activity (Youssef and Mokhtar, 2014). Although the PHE we used in the present study showed a high total phenolic content and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity, none of the groups that were fed supplemented diets with 300 or 600 mg kg⁻¹ of PHE showed a reduction in the serum MDA. It is strongly possible that higher levels of PHE are needed to improve broiler chickens antioxidant capacity during high ambient temperature condition. Interestingly, there was a reduction in serum MDA concentration in the birds fed 600 mg kg⁻¹ PHE in combination with 100 mg kg⁻¹ Zn. It has been well discussed that Zn is able to exert antioxidant effects by inducing the expression of metallothioneins, as potent electrophilic scavengers and cytoprotective agents and activation of antioxidant proteins and enzymes, such as glutathione and catalase (Jarosez et al., 2017). Hence it is likely that a combination of 100 mg kg⁻¹ zinc and 600 mg kg⁻¹ PHE resulted in a synergistic effect and reduced MDA production.

One of the important enzymes that can protect animals from oxidative injury is SOD, which clears superoxide an-

ions. It is reported that a small increase in ROS can induce the expression of SOD (McArdle and Jackson, 2000). Consequently, SOD activity and MDA concentration are commonly combined when investigating the effect of antioxidant feed additives. In our present study, the activity of SOD tended to be decreased in the broilers fed diets supplemented with BHT or ZnP600 during high ambient temperature exposure. BHT is known as a strong antioxidant and therefore in the present study it is strongly possible that due to its ability to neutralize ROS, dietary BHT supplementation led to lower activity of T-SOD in the serum of broiler chickens. Although there is no previous report on the possible effect of BHT (synthetic antioxidant) on SOD activity, previous studies reported that vitamin E (natural antioxidant) supplementation decreases T-SOD activity (Mantha et al., 1993; Muller and Pallauf, 2003; Song et al., 2017).

It is well known that antibody responses decreased in the birds reared under high ambient temperature conditions (Donker et al., 1990; Bartlett and Smith 2003; Mahmoud and Edens, 2003). Under stress conditions, increased inflammatory cytokine production (Ogle et al., 1997) stimulates corticotrophin-releasing factor from the hypothalamus ending with release of corticosterone and finally inhibits antibody production (Gross, 1992). In the present study antibody titers against Newcastle virus increased in broiler chickens fed 600 mg PHE per kg of diet at 34 days of age. Some previous studies showed that dietary vitamins E and C, as natural antioxidants, significantly increased antibody response to SRBC in the broiler (Singh et al., 2008) and layers (Panda et al., 2008) under high ambient temperature. Antibody response against SRBC, as an indicator for routine evaluation of humoral immunity, involves both B and T lymphocytes and are used in immunological studies (Hörak et al., 2003). Oxidative stress directly induces immune system damage

(Biller-Takahashi et al., 2015; Marri and Richner, 2015). Hence it is likely that high ambient temperature initiated oxidative stress resulting in lower antibody titer against SRBC and Newcastle virus; however, supplemented broiler diets with 600 mg kg⁻¹ of PHE enhanced serum antibody. Positive effect of antioxidants on the humoral immune system response has been previously reported; however, physiological mechanisms need to be further elucidated.

5 Conclusions

1. Inclusion of 600 mg kg⁻¹ of supplemental PHE and also a combination of PHE and zinc (100 mg kg⁻¹ basal diet) partially improved the body weight of broiler chickens reared under summer high ambient temperature conditions.
2. Dietary supplementation of 600 mg PHE per kilogram of diet could be advisable to be used in broiler chickens during summer conditions to improve their antioxidant capacity and immune response against Newcastle virus.

Data availability. The original data of the paper are available upon request from the corresponding author.

Author contributions. RK performed this experiment and provided data. AS designed this experiment, analyzed the data and wrote the paper; GS designed the experiment, edited the paper and gave advice.

Competing interests. The authors declare that they have no conflict of interest.

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