Effect of vitamin C and acetylsalicylic acid supplementation on some hematological value, heat shock protein 70 concentration and growth hormone level in broiler exposed to heat stress

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Abstract

The goal of the current investigation is to study the effect of vitamin C and acetylsalicylic acid in minimizing the effects of heat stress in terms of hematological values, growth hormone, HSP70 and glutathione. Broiler were randomly distributed into four groups: 1st group was served as the control, the 2nd group subjected to heat stress 40±2 ºC up to 4 hours/day, the 3rd group was subjected to heat stress and vitamin C 360 mg/L via drinking water and the 4th group was exposed to heat stress and acetylsalicylic acid 0.03% via drinking water. The result showed that exposure to heat stress decline in RBCs count, Hb concentration, PCV and percentage of lymphocyte, furthermore elevation in of MCV, heterophils, H/L ratio, and Hsp70 concentration. Administration of vitamin C caused a significant rise RBCs, percentage of lymphocyte and reduces in MCV, MCH value, percentage of heterophils and H/L ratio compared with the heat stress group. Administrations of acetylsalicylic acid were significantly increased the RBCs, and PCV and decrease in MCV, MCH, MCHC values, and H/L ratio compare with the heat stress group. The results did not show a significant change between the tested groups in the levels of glutathione and growth hormone. The study concluded that Vitamin C and acetylsalicylic acid administration as feed additive ameliorating the opposing effect caused by heat stress in the broiler; thus, its administration recommends when there is heat stress exposure.

***Keywords*:** Growth hormone, HSP70, Vitamin C, glutathione, Acetylsalicylic acid

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تأثير إضافة فيتامين ج وحامض الساليسيلك في بعض القيم الدموية، وتركيز بروتين الصدمة الحرارية ٧٠ ومستوى هرمون النمو في فروج اللحم المعرضة للإجهاد الحراري

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الخلاصة

هدفت الدراسة الحالية لمعرفة تأثير فيتامين ج وحامض الساليسيلك في تقليل الإجهاد الحراري من خلال تأثيره على بعض مكونات الدم ومستوى هرمون النمو وتركيز بروتين الصدمة الحرارية ٧٠ والكلوتاثايون. وزعت أفراخ الدجاج عشوائيا الى أربع مجاميع. المجموعة الأولى هي مجموعة السيطرة، والمجموعة الثانية هي مجموعة التعريض الحراري حيث عرضت للإجهاد الحراري ٤٠±٢ ºم لمدة ٤ ساعات يوميا، والمجموعتين الثالثة والرابعة هي المجاميع المعرضة حراريا والمعاملة بفيتامين ج ٣٦٠ ملغم / لتر، وحامض الساليسيلك ٠.٠٣% بماء الشرب. أظهرت النتائج أن التعرض للإجهاد الحراري خفض من عدد خلايا الدم الحمر وتركيز الهيموكلوبين والنسبة المئوية لحجم الخلايا المرصوصة والخلايا اللمفية إحصائيا فضلا عن زيادة إحصائية في معدل حجم الكرية والخلايا البيضاء المغايرة ونسبة المغايرة/ الخلايا اللمفية وبروتين الصدمة الحرارية ٧٠. في حين إضافة فيتامين ج زيادة معنوية في عدد خلايا الدم الحمر والخلايا اللمفية وقلل من

معدل حجم الكرية ومعدل تركيز الهيموكلوبين ونسبة الخلايا المغايرة ونسبة الخلايا المغايرة/ الخلايا اللمفية مقارنة مع مجموعة الإجهاد الحراري. أدى إعطاء حامض الساليسيلك الى حدوث زيادة معنوية في عدد خلايا الدم الحمر وحجم الخلايا المرصوصة وقلة في معدل حجم الكرية ومعدل تركيز هيموكلوبين الكرية ومعدل هيموكلوبين الكرية ونسبة المغايرة / اللمفية بالمقارنة مع المجموعة المعرضة للإجهاد الحراري. لم تظهر النتائج تغير معنوي ما بين المجاميع في مستوى الكلوتاثايون وهرمون النمو. يستنتج أن إعطاء فيتامين ج وحامض الساليسيلك كإضافات غذائية استطاع معالجة أو التقليل من التأثيرات التي أحدثها الإجهاد الحراري في فروج اللحم لذا نوصي بإعطائها.

Introduction

One of the most significant environmental factors affecting the physiological functions of broiler is the high temperature, when the temperature exceeds optimum temperature conditions, adversely affects the growth rate, feed conversion and live weight gain (1). Stress factors in birds involve many elements of variation in atmospheric temperature, low manipulation, removal of water and feed, elevated relative moisture and ambient temperature (2). Cells increase the synthesis of heat shock proteins when environmental conditions change, and cells respond to heat stress by decreasing the production quantity of all cellular proteins and increasing the development of heat shock proteins (3). It was recorded that cells which absence heat shock protein genes, so devoid of the protein expression, show little tolerance to stress compared to cells expressing heat shock protein (4). Moreover, it was determined that induced thermal tolerance degree was related to the expression of heat shock protein (4). Hsp70 expression demonstrates an important function in folding cellular proteins. It is highly stimulated by heat stress and cell protection from stress toxic impacts when environmental circumstances alter cells react to changes and elevate the threshold of thermal shock or stressor proteins (3). Another indicator of heat stress is the calculation of the heterophil/lymphocyte proportion is a sensitive measure of heat stress in broilers, which in the first and last days of heat stress increased significantly (5).

In sequence to decrease the impact of heat stress in the broiler, adding some additional feeds (6). Acetylsalicylic acid is an antipyretic substance (7), act to prevent prostaglandin synthesis; and therefore, it alters the hypothalamic thermostat capacity (8). The addition of acetylsalicylic acid in chicken diets has also been shown to improve the rate of growth and physiological character during hot environment (9). Alagawang *et al.* (10) noted that acetylsalicylic acid improving nutrient digestion and absorption, growth performance, as well as it plays an important role in decreasing triglyceride and cholesterol in the circulation and enhancement of immune functions and antioxidant enzymes in birds.

As a nutritional supplementation, vitamin C is not needed in the poultry because it can be synthesized; thus, the use of ascorbic acid in poultry production is to prevent environmental oxidative stress impacts (11). The formation of vitamins is inadequate or reduced in stressful circumstances such as high environmental temperatures (12).

One of the extremely fundamental impacts antagonistically influencing poultry physiology is the heat stress, and broilers reared in elevated ambient temperatures induce changes in the physiological processes of the body, so the purpose of the current experiment was to determine the effect of adding vitamin C and acetylsalicylic acid treatment in minimizing the adverse effects of heat stress on broilers.

Materials and methods

Bird and experimental design

This experiment was conducted at the animal house/college of the Veterinary Medicine, University of Mosul. Eighty broilers 14th days old were distributed randomly into four groups, and four replicates each of 5 chicks. During the experiment, the chicks were housed in (1mx2m) floor pens provided with constant light 17 hours/day and clean drinking water ad libitum throughout the rearing periods (7th weeks). The dietary composition of the diet, according to (13).

The chicks were allocated into four groups during the last 14th days. 1st group respected as control group placed into a controlled room and was kept in 25±27ºC ambient heat during the whole day from the 14th day to the end of the experiment. 2nd group birds were subjected to heat stress 40±2˚C up to 4 hrs /day (9:00-1:00). The 3rd group was exposed to extreme heat stress 40±2ºC up to 4 hrs /day (9:00-1:00) and were dietary supplemented with vitamin C (360 mg / L) via drinking water (14). The last 4th group birds were exposed to heat stress 40±2ºC lasted 4 hrs. /day (9:00-1:00) .and provided with acetylsalicylic acid 0.03% via drinking water (15).

Blood samples collection was performed at the end of the experiment 7th week and obtained from the wing veins. Blood with anticoagulant (EDTA) was used for hematology analysis. Red blood cell count was performed according to the technique of Natt and Herrick, Packed cell volume (PCV %), Hemoglobin concentration (Hb g/dl) of the blood samples were estimated. Blood smears were prepared from fresh blood-stained using Wright's stain for examine the differential leucocyte count (DLC) and heterophils /lymphocyte ratio calculations. Blood sera stored at -20 ºC for biochemical analysis including growth hormone analysis

using GH Chicken ELISA kit and heat shock protein 70 using HSP70 Chicken ELISA kit and glutathione level Ellman's procedure (16). At the end of the experiment, the broilers were slaughtered and visceral organs were collected including liver, heart, spleen, and bursa of fabric and their weights were recorded as relative weight /100 g body weight. The cloaca temperature was measured.

Statistical analysis

Statistical analysis among the groups was performed using one-way variance analysis and the significant within groups by Duncan test. At P<0.05 (17) were regarded as statistically significant. All statistical analyzes with the SPSS 10.00 pack were carried out.

Results

The influence of vitamin C and acetylsalicylic acid on some hematological values in broiler subjected to high temperature 40±2ºC 4 hrs/day (Table 1). Broiler exposed to heat stress caused a significant decline in RBCs count, Hb and PCV as compared with the control group at P values 0.000, 0.03, 0.000, respectively. Supplementation of vitamin C to the stress broilers caused a significant increase in RBCs count but did not change Hb concentration and PCV% compared with heat stress group, while administration of acetylsalicylic acid to the stress broilers cause significant rise in the RBCs and PCV% (P=0.000), while there was no significant alteration (P> 0.05) in Hb concentration as related with heat stress group and each of Hb and PCV value was similar to control group values.

Table 1: Effect of vitamin C and acetylsalicylic acid on some blood variables in broiler exposed to heat stress

Groups	Mean ± Standard Error		
	RBCs 10^6 cell/cm²	Hb gm/ dl	PCV %
First group	2.54±0.15 b	8.56±0.29 a	26.00±0.62 ab
Second group	1.91±0.13 c	7.60±0.19 b	23.76±0.25 c
Third group	3.02±0.21 b	8.22±0.98 ab	25.00±0.26 bc
Fourth group	3.25±0.25 a	7.96±0.25 ab	26.62±0.49 a
P value	0.000	0.03	0.000

Values with different letters in the column are significantly different at P<0.05.

The results in table 2 showed that the heat stress exposure causes a significant elevation in MCV value (P=0.003) but the MCH and MCHC did not influenced significantly (P=0.056) as compared with the control value. Administration of vitamin C significantly declined the MCV and MCH values (P=0.001), while MCHC did not changed as compared with the heat stress group but the values similar to control value (P=0.056). On the other hand, supplementation of acetylsalicylic acid to the stress, broilers caused a significant decrease in MCV, MCH, and MCHC concerning heat stress group.

Table 2: Effect of vitamin C and acetylsalicylic acid on blood indices in broiler exposed to heat stress

Groups	Mean ± Standard Error		
	MCV fl	MCH pg	MCHC gm/100 ml
First group	105.26±7.48 b	33.80±2.65 ab	32.89±2.65 a
Second group	128.94±7.83 a	40.72±3.45 a	32.61±3.45 a
Third group	89.30±7.12 b	28.22±2.14 bc	32.93±2.14 a
Fourth group	84.98±6.21 b	25.25±1.74 c	29.91±1.74 b
P value	0.003	0.001	0.056

Values with different letters in the column are significantly different at P<0.05.

The results in table 3 appeared that the broilers exposed to the heat stress caused significant decline in the lymphocyte and a significant elevation in the Heterophils and basophils (P=0.000), with no significant change in monocyte and eosinophil when compared with the unexposed group. Administration of vitamin C caused a significant rise in the lymphocyte and significant decreased in the Heterophils (P=0.000), with no significant change in the monocyte, basophil and eosinophil counts as compared with heat stress group (P=0.109). Supplementation of the stressed broiler with acetylsalicylic acid did not affect the percentage of the DLC concerned with the heat stress group (P>0.05).

As shown in table 4, no significant alterations between groups in the weights of heart, spleen, bursa of fabresia at P values (0.278, 0.597, and 0.100) respectively. The results of the current experiment showed that exposure to heat stress significantly decreases in the liver weight as compared with the control group (P=0.000). The addition of vitamin C and acetylsalicylic acid did not affect liver weights as compared with the heat stress and control groups (P> 0.05). The results indicate a significant decrease in body weight in the heat stress group, vitamin C group and the acetylsalicylic group as compared with the control group (P=0.002).

The data in table 5 demonstrated that broilers exposed to heat stress were increased significantly the heterophils

/lymphocyte ratio as compared with the control group (P=0.000). Supplementation of stressed broilers with vitamin C produces a significant diminish in the H/L ratio as associated with the heat stress group (p=0.000) but did not affect significantly as compared with the control group (P> 0.05). Supplementation of acetylsalicylic acid caused a significant decrease in H/L ratio as compared with the heat stress group but there was a significant increase in H/L when compared with the control value (P=0.000).

Broilers subjected to heat stress at 40±2˚C for 4 hr./day lead to significant rise of Hsp70 compared the control values (P=0.026). Supplementation of stress broilers with vitamin C and acetylsalicylic acid diminished the Hsp70 level significantly as compared with the heat stress group (P= 0.026).

Table 5 revealed that the broilers exposed to heat stress did not affect significantly on the growth hormone level, and the values were similar to that observed in the control group (P=0.680). Furthermore, administration, each of vitamin C and acetylsalicylic acid to stress broilers, did not significantly influence on the growth hormone as compared with the heat stress group (P=0.680). The results of the current study showed no changes significant in the level of glutathione among the experimented groups (P=0.259).

Table 3: Effect of vitamin C and acetylsalicylic acid on differential leucocytes count in broiler exposed to heat stress

	Mean ± Standard Error				
Groups	Lymphocyte (%)	Heterophils (%)	Monocyte (%)	Basophil (%)	Eosinophil (%)
First group	63.80 ± 1.89^{a}	28.10±1.75^{b}	2.20±.49^{a}	3.0±0.51^{b}	2.9±0.4^{a}
Second group	53.00 ± 1.43^{b}	42.80 ± 1.35^{a}	1.60 ± 0.4^{ab}	0.7 ±0.30^{a}	1.90 ±0.23^{a}
Third group	64 30 ±1.54^{a}	31.6 ± 1.59^{b}	1.30 ±0.26^{ab}	0.9±0.27^{a}	1.9 ±0.37^{a}
Fourth group	56.90 ± 2.77^{b}	39.40±2.74^{a}	0.70 ±0.21^{a}	1.10±0.34^{a}	1.90 ±0.31^{a}
P value	0.000	0.000	0.041	0.000	0.109

Values with different letters in the column are significantly different at P<0.05.

Table 4: Effect of vitamin C and acetylsalicylic acid on organ weights in broiler exposed to heat stress

	Mean ± Standard Error				
Groups	Heart (gm/100gm BW)	Liver (gm/100gm BW)	Spleen (gm/100gm BW)	Bursa of Fabresia (gm/100gm BW)	Total body (weight gm)
First group	0.449±0.02^{a}	2.71±0.11^{a}	0.10±0.011^{a}	0.14±0.017^{ab}	2756±117^{a}
Second group	0.523±0.02^{a}	2.03±0.07^{b}	0.10±0.004^{a}	0.15±0.013^{a}	2347±86^{b}
Third group	0.450±0.20^{a}	2.01±0.12^{b}	0.10±0.009^{a}	0.13±0.014^{ab}	2327±19^{b}
Fourth group	0.490±0.40^{a}	1.95±0.05^{ab}	0.08±0.010^{a}	0.10±0.008^{b}	2278±55^{b}
P value	0.278	0.0000	0.597	0.100	0.002

Values with different letters in the column are significantly different at P<0.05.

Table 5: Effect of vitamin C and acetylsalicylic acid on stress index Hsp70, growth hormone and glutathione in broilers exposed to heat stress

	Mean ± Standard Error			
Groups	H/L ratio	Hsp70 (ng/ml)	Growth hormone (ng/ml)	Glutathione (µmol/L)
First group	0.434±0.04^{c}	0.1040±0.003^{b}	0.618±0.05^{a}	0.384±0.08^{a}
Second group	0.818±0.44^{a}	0.2148±0.056^{a}	0.552±0.04^{a}	0.380±0.26^{a}
Third group	0.499±0.38^{bc}	0.1059±0.003^{b}	0.568± 0.01^{a}	0.369±0.07^{a}
Fourth group	0.629±0.88^{b}	0.1095±0.004^{b}	0.578±0.03^{a}	0.339±0.16^{a}
P value	0.000	0.026	0.680	0.259

Values with different letters in the column are significantly different at P<0.05.

Discussion

Endothermic animals (including mammals and birds) are able to control their body temperature by the regulation of their basal metabolic rate (thermoregulation). The significance of endothermy is enhanced enzymatic activities and a continuous body temperature that allows animals to be active at different external temperatures (18). Body

thermoregulation plays an important role in tissue protection against oxidative stress which enhanced by elevation of body temperature, oxidative stress took place as a result of activation of ROS production in mitochondrial matrix through respiratory chain process (19). In cells, the respiratory chain is responsible for reactive oxygen species (ROS) production, mainly by complexes I and III. When the electrochemical gradient increases, the activity of the respiratory chain diminishes, raising the half-life of ubisemiquinone (20). Thus, the ROS production is dependent on the mitochondrial membrane potential and alteration in body temperature will lead to alteration in response capacity of the cells to produce ROS. Heat stress plays an important role in tissue oxidation due to enhance the phosphorylation of ADP into ATP takes place within the mitochondrial inner membrane respiratory chain via reoxidize the reduced cofactors (NADH, FADH2) produced by the various cellular metabolic processes (18).

The present study revealed that heat stress exposure induced a significant reduction in RBCs, Hb concentration, and PCV accompanied by an elevation in MCV. These results, are similar to the previous findings, detailed that exposure to oxidative stress on the 6th week of adult rooster caused a significant decrease in Hb and PCV values as compared with the control group (21), other researchers documented that oxidative stress causes a significant increase in MCV (22). The etiology of that result is may be due to that the RBCs are highly sensitive to oxidative injury because of its high level of oxygen contents. Moreover, the hemoglobin is a potent initiator of the oxidative stress process development in the RBCs (23). So, the RBCs are considered as the main cells that influenced by oxidative stress (24). Hemoglobin reduction due to heat stress might be due to the influence oxygen binding protein as an antioxidant mechanism and gradually converted to methemoglobin (metHb), so MetHb is unable to bind or transport oxygen (25,26). Under normal circumstances, the concentration of metHb in RBCs is preserved at less than 1% of the whole hemoglobin (26). Hemoglobin oxidation also initiates the development of disulfide cross-links between globin chains and deforming the hemoglobin structure that leads to Heinz bodies' formation (26). This accumulation of membrane-bound denatured protein (Heinz bodies) may be eliminated through reticuloendothelial macrophages system, but more significant cellular changes results in RBC hemolysis (25).

Vitamin C addition in drinking water to stressed broiler lead to a significant rise in RBCs count associated with decreased MCV. Whereas giving of acetylsalicylic acid improve RBC and PCV, associated with reduction MCV, MCH, and MCHC. Our results oppose with (27) who documented no significant influence on red blood cell, packed cell volume and hemoglobin were noticed when acetylsalicylic acid additive to heat stress layers. The current findings disagree with Tras *et al.* (28), who found that the hematological values of vitamin C, acetylsalicylic acid treated groups, had no effect on the serum superoxide dismutase liver, glutathione peroxidase, and catalase-like antioxidant enzymes because of the nature of the non-steroidal anti-inflammatory reagents as an antioxidant agent. Furthermore, Usman *et al.* (29) found that there was no changes in RBC, HB, PCV, MCV, MCHC values in the quails under the heat stress at 38 ºC supplemented to the vitamin C. Ultimately similar to (30) who report in his work that supplementation of vitamin C during summer season improves the hematological values. Vitamin C may be attributed to its capability to preserve the integrity of the erythrocyte membrane (31) and to its antioxidant capacity by scavenging the free radicals from the cellular environment leading to high malondialdehyde formation (31). Current results found that administration of vitamin C simultaneously with heat stress had no effect on hematological values. The etiology of this effect, is that the vitamin C plays a major role as antioxidant cellular compounds which has the ability to neutralize free radicals when poultry are undergoing stress (14).

Data of current study of the broiler exposure to the heat stress caused significant decline in the lymphocyte accompanied with a significant elevation in Heterophils as a result of the heterophils/lymphocyte ratio greater than normal, whereas giving of vitamin C in drinking water caused significant increase in percentage of lymphocyte and decreased in percentage of Heterophils which cause H/L ratio decreased. However, the administration of acetylsalicylic acid with drinking water did not affect the percentage of the DLC but decrease in H/L ratio. Leucocyte response has been linked to heat stress due to its relation to circulating ACTH hormone which effect on corticosterone levels through decreasing its serum level leading to decreasing white blood cell count (32). The high temperature in hot weather is enough to cause increased body temperature as well as changes circulating leucocyte components in chickens, in addition, to that, pervious results observed that vitamin C avoided the releasing of leucocytes into peripheral bloodstream from their body reservoir, presumably because of its inhibition function in the secretion of corticosteroids during stress (31). Another previous work reported that after 2 hours of heat stress exposure, chickens showed higher heterophils correlated with declining lymphocytes (33). Consequently, the percentage of heterophils /lymphocytes increased owing to heat stress and is considered as an indicator of physiological stress in harmony with earlier research (34). The H/L ratio was expressed in birds as a stress index (35). Adenkola *et al.* (31) reported that vitamin C supplementation reduces the negative effects of heat stress in birds. Therefore, it is advisable to add vitamin C during the hot-dry season to reduce heat stress effect. Normally, vitamin C, a synthesis in the fowl's kidneys, is concerned

with expelling surplus free radicals from the body, yet are either drained or collected under pressure condition; in this way presenting cells to their ruinous impacts (36). Another work by (37) have suggested various methods by using electrolytes and vitamins to improve the harmful impacts of heat stress on birds.

In our study, the administration of acetylsalicylic acid declines the H/L ratio and this finding agrees with (27). This decrease could be due to the action of acetylsalicylic acid on reducing the release of serum corticosterone in the summer season (38). The result of the present study revealed a significant elevation in Hsp70 in heat stressed broiler. Our findings are in agreement with other researchers who found a significant rise in Hsp70 level relative to the control group in laying hens when exposed to mild heat stress (39). This outcome was relating to other investigation which uncovers that administration of male rabbits to the cadmium chloride as oxidative stress in tap water daily for 6^{th} weeks cause significant increase in serum Hsp70 concentration in compassion with control group (40). The cause of the rise in serum Hsp70 level may be due to an increase in the expression of Hsp70 genes in broiler's liver during the altered ambient temperature (14), and the heat shock protein 70 gene is very susceptible to changes in the environment (41). The results of the research show that enhanced expression of Hsp70 in chicken tissue is one of the most significant protective reactions to prevention the detrimental changes in protein structure and functions owing to multiple stresses (42).

The result of the present study demonstrated that exposure to heat stress reduces the body weight and liver weight, this result agrees with (43) who reported that six weeks of oxidative stress induced by hydrogen peroxide supplementation with drinking water caused a significant reduction in the body weight of adult white Leghorn male chicken. Lin *et al.* (44), the researcher found that elevated body temperatures could lead to metabolic changes involving oxidative stress induction and the liver is more susceptible to oxidative stress in broilers during acute exposure to heat stress. Oxidative stress should be considered as part of broiler stress response to heat stress exposure (44). Under stressful environmental circumstances, enhanced concentrations of ROS happen as the bird's body tries to keep its heat homeostasis. As a result, the body begins to produce heat shock proteins in an attempt to safeguard itself from ROS deleterious cellular effects (45). Heat stress reduced feed consumption and activation of the hypothalamus pituitary hypophysial axis and decreased feed consumption is consequent to detrimental impacts of heat stress leading to reduced body weight, feed effectiveness, egg production and quality (46). Current research has shown no significant change in serum glutathione concentration between groups, which is not agreement with Hassan *et al.* (47) who found that serum glutathione concentration increased in Awassi ewes during the spring season compared to those lactating their lambs in hot dry season. This finding also disagree with prior research that found the administration of vitamin C to adult rooster with hydrogen perioxide resulted in a significant increase in liver glutathione levels relative to hydrogen peroxide group (43). In the present research, the cause of uninfluenced growth hormone and glutathione concentration maybe due to the length of the experiment or the dose of vitamin C and acetylsalicylic acid were not sufficient to trigger modifications in growth hormone and glutathione.

Conclusion

From this research, it is found that vitamin C and acetylsalicylic acid lowered the adverse effect of heat stress so suggested to offer vitamin C and acetylsalicylic acid during the warm season.

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Conflict of interest

The researchers note that there is really no conflict of interest.

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