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Effect of adding natural and synthetic antioxidants to broiler drinking water as antistressor on productivity, antioxidant statues and hematological traits under heat stress

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ABSTRACT

This study was conducted to explore the benefit of adding synthetic and natural antioxidants nutritional additives in drinking water to increase broiler resistance to heat stress in the hot summer climate on productive performance, and physiological status. A total of 600 chicks of broiler (Ross-308) were distributed into ten treatments, each treatment with three replicates of 20 chicks. The treatments of study are: T0: (Positive control: drinking water without adding-DW)- without heat stress, treatments exposed to heat stress: [T1: (Negative control: drinking water without adding-DW, but exposed to heat stress), T2: (100 mg BHT/1 L DW) (BHT – butylhydroxytoluene: synthetic antioxidant), T3: (100 mg vit E/1 L DW) (synthetic antioxidant), T4: (200 mg saffron/1 L DW) (natural antioxidant, T5: (200 mg curcumin/1 L DW), T6: (100 mg saffron+ 50 mg BHT/1 L DW), T7: (100 mg saffron+50 mg vit E/1 L DW), T8: (100 mg curcumin+ 50 mg BHT/1 L DW), T9: (100 mg curcumin+ 50 mg vit E/1 L DW)]. Data of the study analyses and display that the synthetic and natural antioxidants nutritional additives had significantly decreases in the temperature of body parts surface (head, back, under wings, cloaca), general body temperature, mortality%, Malondialdehyde (MDA), heat shock proteins (HSP-40 and HSP -70), Corticosterone hormone concentration in the blood serum and heterophiles: lymphocytes (H/L) ratio. However, body weight, body weight gain, Production index (PI), feasibility (economic profit), water intake (WI), Glutathione Peroxidase (GSH-Px), super oxide dismutase (SOD), total antioxidant capacity (TAC), Newcastle (ND), Infectious bursal (IBD) diseases and Infection bronchitis virus (IB), so feed conversion ratio (FCR) had significantly improved in the all treatments of water additive in the treatments of positive control T0 compared with the negative control T1. Whereas, feed intake had non-significant differences among all treatments of the study. In all traits of the study natural antioxidant additives: saffron and curcumin additives seen superiorly.

تأثير إضافة مضادات الأكسدة الطبيعية والاصطناعية في مياه شرب فروج اللحم كمضاد أجهادي على صفات الإنتاج و حالة الأكسدة وصفات الدم تحت الأجهاد الحراري

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الخلاصة

أجريت هذه الدراسة لمعرفة فعالية إضافة مضادات الأكسدة الطبيعية والاصطناعية في ماء شرب فروج اللحم لزيادة مقاومته للإجهاد الحراري في جو الصيف الحار على الأداء الإنتاجي والحالة الفسيولوجية. وزعت 600 فرخة فروج لحم (Ross-308) بعمر يوم واحدعلى 10 معاملات، بواقع 3 مكررات و كل مكرر احتوت على 20 فرخة. و كانت معاملات الدراسة كالآتي: T0 السيطرة الموجبة - شرب الماء بدون إضافة و بدون إجهاد حراري، المعاملات المعرضة للإجهاد الحراري: T1 :السيطرة السلبية: شرب الماء بدون إضافة، T2: 100ملغم BHT مضاد أكسدة اصطناعي /لتر ماء) T3 100 ملغم لتر ماء مضاد أكسدة صناعي فيتامين E ، T4 200 ملغم زعفران / 1 لتر ماء (مضاد أكسدة طبيعي) :T5 200ملغم/ لتر ماء كركمين مضاد أكسدة طبيعي)، T6 100ملغم زعفران + (50 ملغمBHT / لتر ماء) :T7 100ملغم زعفران + 50 ملغم فيتامين هـ / 1 لتر ماء) :T8 100ملغم كركمين + 50 ملغم BHT /لتر ماء) :T9 100ملغم كركمين + 50 ملغم فيتامين هـ/ لترماء. أوضحت نتائج الدراسة أن المواد المضافة الغذائية الطبيعية والاصطناعية خفضت بشكل ملحوظ درجة حرارة سطح أجزاء الجسم (الرأس ، الظهر ، تحت الأجنحة ، فتحة المخرج) ، درجة حرارة الجسم العامة ، نسبة الهلاكات، مالونديالديهايدMDA ، بروتينات الصدمة الحرارية HSP-40 و HSP-70، تركيز هرمون الكورتيكوستيرون في مصل الدم والهيتروفيل: الخلايا الليمفاوية. سجلت النتائج ايضا ارتفاع معنوي في وزن الجسم ،الزيادة الوزنية للجسم ، مؤشر الإنتاج PI، الجدوى أو الربح الاقتصادي، استهلاك الماء، الجلوتاثيون بيروكسيدي GSH-Px ، أكسيد الفائق ديسميوتز SOD والقدرة الإجمالية لمضادات الأكسدة TAC، ونيوكاسل ND، وأمراض الجراب المعدية IBD وفيروس التهاب الشعب الهوائية IB، كذلك تحسن في كفاءة التحويل الغذائي FCR بشكل ملحوظ في جميع معاملات الإضافات الغذائية الطبيعية والأصطناعية الى ماء الشرب و معاملة السيطرة الموجبة T0 مقارنة بمعاملة السيطرة السالبة T1 في حين أن عدم وجود فروق معنوية في استهلاك العلف بين معاملات الدراسة. نستنتج من ذلك أن جميع صفات الدراسة ت تفوق معاملات إضافة الزعفران و الكركمين الى الماء.

الكلمات المفتاحية: الزعفران ، الكركمين ، فيتامين E ،، BHT، الصفات الإنتاجية ، الصفات الفسلجية.

INTRODUCTION

Heat stress is one of the most challenging environmental stresses affecting broiler chicken diseases and stress dynamics are liable for heavy mortality nowadays especially in Iraq, high production targets and heat stress initiate free radical injuries and gastrointestinal oxidative, subsequently causing severe yearly economic losses. Heat stress, a major source of oxidative stress, stimulates mitochondrial oxidative stress and cell dysfunction, leading to cell damage and apoptosis (Shehata et al, 2020). Heat stress increases the probability of death, especially of larger broilers into which several weeks of input costs have been invested (May *et al.*, 1997; Simmons *et al.*, 1997). It also affects physiological parameters including chemistry and blood gas parameters in animals and the responses vary depending on the time and degree of thermal challenge (Boddicker *et al.*, 2014), and affects the immune response in poultry, immunosuppression has been observed in both broilers and layers during exposure to a heat stress environment (Padgett and Glaser, 2003).

Antioxidants are the best choice to delay the onset of oxidation by preventing the formation of free radicals. Free radicals oxidize fats, and antioxidants stop the reaction by donating the hydrogen atoms (Sies, 1997). The objective of the use antioxidants is to enhance the oxidative stability for a reasonable length of time. Antioxidants are used to ensure the safety and nutritional values of poultry meals used in the animal industry (Dozier *et al*,. 2003). The synthetic antioxidant of the study composed vitamin E and butylated hydroxytoluene (BHT), the supplementation of vitamin E and ascorbic acid into broiler diet improve the growth performance, feed efficiency, nutrient digestibility, immune response and antioxidant balance (Khan et al. 2011).

The natural antioxidants composed of saffron and curcumin. Saffron, the dried stigmas of *Crocus sativus* L., is an aromatic spice, also used as an analgesic in traditional medicinal preparations and has recently been shown to have distinct antioxidant properties (Rios *et al.*, 1996. The main biologically active constituents of saffron are safranal and carotenoid pigmen crocin, a family of red-color and water-soluble carotenoids, which are all glycosides of crocetin (Tarantilis and Polissiou, 1997). The adding saffron petals in broiler diet had increased body weight, body weight gain, feed conversion ratio, immune response against Newcastle disease virus (NDV) and decreased the abdominal fat, serum cholesterol and heterophil: lymphocyte (H/ L) ratio (Hosseini-Vashan and Piray, 2021). Curcumin a natural polyphenol, is the principle active ingredient of turmeric. It has been a popular spice (Chattopadhyay *et al*., 2004), it has many biological activities: anti-inflammatory, antioxidant (Gandhi *et al.*, 2011), antimicrobial (Araujo and Leon, 2001), anticoagulant, antidiabetic and antiulcer (Lokova *et al.*, 2001), improve the nutrients digestibility, metabolism, and prevent biliary disorders (Chattopadhyay *et al.*, 2004). The objective of the study was planned to evaluate the synthetic and natural antioxidant sources as antistressor on body temperature, growth performance, antioxidant statue and immunological traits of broilers.

MATERIALS AND METHOD

The experimental procedures involving the use of synthetic and natural antioxidants in broiler drinking water under heat stress, this study was carried out in the Poultry Hall in the farm of Grdarasha/ Animal Resources Dept./ College of Agricultural Engineering Sciences/ University of Salahaddin- Erbil/ Iraq.

A total of 600 newly hatched broiler chicks (Ross -308) with similar body weights (43 ± 2 g) were purchased from a local commercial hatchery and reared for 38 days from 15-9 to 22-10/ 2022. They were randomly assigned to Ten treatments of 60 chicks in each treatment, each with three replicate floor pens from 1 to 38 days of age, the treatments of the study are: T0: (Positive control: drinking water without adding-DW)- without heat stress, treatments exposed to heat stress: T1: (Negative control: drinking water without adding-DW, but exposed to heat stress), T2: (100 mg BHT/1 L DW) (BHT – butylhydroxytoluene: synthetic antioxidant), T3: (100 mg vit E/1 L DW) (synthetic antioxidant), T4: (200 mg saffron/1 L DW) (natural antioxidant, T5: (200 mg curcumin/1 L DW), T6: (100 mg saffron+50 mg BHT/1 L DW), T7: (100 mg saffron+50 mg vit E/1 L DW), T8: (100 mg curcumin+50 mg BHT/1 L DW), T9: (100 mg curcumin+50 mg vit E/1 L DW)].

All broiler chickens were reared under standard management conditions positive control treatment was kept in a temperature-controlled room at 21±2 C° (thermoneutral group), other nine treatments were raised under heat stress (28-30) C° temperature room for 24 h/day. The average relative humidity of the rooms was 55%. Feed in pellet form and freshwater were provided *ad libitum* the feed was prepared by a private feed company that contains 3072, 3122 kcal/kg metabolizable energy, 22.5, 20 %) crude protein (starter (1-19 d) and grower (20-38 d) diets respectively as recommended in NRC (1994), and continuous light (22 h light: 2 h dark) was applied throughout the experimental period. Vaccination program was consisting of Newcastle at 7 and 21 days and IB at 14 days of rearing, according to Ross-308 guide (Broiler management handbook 2018).

The chemical analysis of total antioxidant, phenolic and flavonoid compounds in Saffron and curcumin were determined by High performance liquid HPLC chromatography as in Table 1.

Table 1. Results of chemical analysis of total (antioxidant, phenolic and flavonoid) compounds in Saffron and curcumin.

	Saffron flower	**Curcumin**
Antioxidant activity (mg / 100 g)	87.33	61.48
Total phenolic (mg /100 g)	56.73	115.00
Flavonoids (µg CE/g)	37.05	40.90

Samples and data collection:

At the end of 38 d body performance were measured (body weight, body weight gain, feed intake and feed conversion ratio in the study, Body temperature (C°) of head, back, under wing, cloaca and general body temp. were taken by portable digital infrared thermometer according to the procedure by Kettlewell et al. (1997). Blood samples were centrifuged, and collected plasma samples were stored at -20 °C until analysis. At the end of the study (38 d), Glutathione peroxidase (GPx) and superoxide dismutase (SOD) enzyme activities were measured in serum kits, from Randox Laboratories Ltd, UK. Also, antibodies titer response against NDV, IBD and IB were measured by ELISA using Kits from BioCompare.

Statistical analysis

All data were analyzed using CRD (Completely Randomized Design) by the SAS institute program (SAS, 2014). Duncan's multiple range tests were used to compare differences among the followed a completely randomized design at the level of probability 0.01 and 0.05 (Duncan, 1955).

RESULTS AND DISCUSSION

The result in Table 2. shows the using of synthetic butylhydroxytoluene (BHT) T2 and Vitamin E (T3) and natural (saffron T4 and curcumin T5) antioxidants and their mix (T6, T7, T8 and T9) in broiler drinking water as antistressor had significantly ($P \leq 0.05$) decreased the temperature of body parts surface (head, back, under wings and cloaca) and general body temperature at age (1-38) d (C°) in the all treatments of adding of water additive and the positive control T0 (without adding additives or heat stress) compared with the negative control T1 (without adding additives, that exposed to heat stress). The phenolic, antioxidant contents of saffron and curcumin as point out in Table 1 have a positive role to decrease body temperature of broiler, also vit E and BHT in the treatment added in drinking water. Sugiharto *et al*. (2017) suggested that the strong antioxidant (anti-stress activity) and hepato-protective capacities of curcumin were attributed to the alleviating effect of curcumin (turmeric) on the increased stress hormone levels due to heat stress.

Table 2. Efficacy of synthetic and natural antioxidants in broiler drinking water as antistressor on the temperature of body parts surface and general body temp at age (1-38) under heat stress.

Treatments	The means of body parts surface temperature at age (1-38) d (C°)				General body Temp. (C°)
	Head	Back	Under wings	Cloaca	
T0	40.35 ^{b}	35.80 ^{b}	42.25 ^{b}	40.35 ^{b}	39.59 ^{b}
T1	41.95 ^{a}	40.35 ^{a}	44.65 ^{a}	42.80 ^{a}	42.45 ^{a}
T2	40.20 ^{b}	35.75 ^{b}	42.20 ^{b}	40.20 ^{b}	39.59 ^{b}
T3	40.05 ^{b}	35.60 ^{b}	41.95 ^{b}	40.00 ^{b}	39.39 ^{b}
T4	40.05 ^{b}	35.55 ^{b}	41.95 ^{b}	39.90 ^{b}	39.37 ^{b}
T5	40.00 ^{b}	35.55 ^{b}	41.85 ^{b}	40.00 ^{b}	39.36 ^{b}
T6	40.05 ^{b}	35.45 ^{b}	42.05 ^{b}	40.00 ^{b}	39.39 ^{b}
T7	40.00 ^{b}	35.50 ^{b}	41.95 ^{b}	39.80 ^{b}	39.30 ^{b}
T8	39.95 ^{b}	35.45 ^{b}	41.85 ^{b}	39.75 ^{b}	39.25 ^{b}
T9	39.85 ^{b}	35.35 ^{b}	41.85 ^{b}	39.80 ^{b}	39.22 ^{b}
SE	1.78	1.55	1.49	1.60	1.52
S.L	*	*	*	*	*

T0: (Positive control: drinking water without adding-DW)- without heat stress, treatments exposed to heat stress: [**T1**: (Negative control: drinking water without adding-DW, but exposed to heat stress), **T2**: (100 mg BHT/1 L DW) (BHT – butylhydroxytoluene: synthetic antioxidant), **T3:** (100 mg vit E/1 L DW) (synthetic antioxidant), **T4**: (200 mg saffron/1 L DW) (natural antioxidant, **T5**: (200 mg curcumin/1 L DW), **T6**: (100 mg saffron+50 mg BHT/1 L DW), **T7**: (100 mg saffron+50 mg vit E/1 L DW), **T8**: (100 mg curcumin+50 mg BHT/1 L DW), **T9**: (100 mg curcumin +50 mg vit E/1 L DW)]. ^{a,b,c} Means within columns with different superscripts differ significantly at *(P≤ 0.05).

The result in Table 3. displays the adding of synthetic and natural antioxidants to broiler drinking water as antistressor had significantly (P≤0.01) increased the body weight, body weight gain, Production index (PI) and feasibility (economic profit) in the treatments T3, T4. T5, T6, T7, T8 and T9 compared with the T0, T1, and T2. Also, water intake (WI) had significantly (P≤0.01) increased in the treatments that exposed to heat stress (the negative control T1 and all additive treatments T2, T3, T4. T5, T6, T7, T8 and T9) compared with the positive control T0 which had not exposed to heat stress T0. So, feed conversion ratio (FCR) had significantly (P≤0.01) improved in the treatments T4, T5, T6, T7, T8 and T9 compared with the T0, T1, T2, T3. However, feed intake had no significant differences among all treatments of the study. While the percentage of mortality had significantly (P≤ 0.01) decreased in the treatments T2, T3, T4, T5, T6, T7, T8 and T9 compared with the positive T0 and negative T1 controls, In the most characteristics the treatments T9 then T7 had superiority fulfill. Heat stress is attributed to impaired productive performance and health in broiler chickens. As a part of the nutritional interventions, turmeric in alleviating the negative effect of heat stress on broilers in terms of production, physiological conditions, immune responses, and antioxidant system (Sugiharto, 2020). It is therefore possible that turmeric may increase the deposit of body protein instead of body fat (Samarasinghe et al 2003). Sadeghi and Moghaddam (2018) documented that turmeric powder could restore the activities of triiodothyronine and thyroxine in heat-stressed broilers. This may consequently correct the compromised growth rate in broilers due to heat stress. Candra and Putri (2020) recently reported that turmeric powder (500 mg per kg body weight of broiler) did not have any influence on the growth rate of broilers maintained in thermal stress conditions. In the case of growth rate, feeding turmeric may not improve the weight gain.

Table 3. Efficacy of synthetic and natural antioxidants in broiler drinking water as antistressor on body performance and Feasibility at age 38 d under heat stress.

Treat ments	Body performance and Feasibility at age 38 days								
	IBW (g)	BW (g)	BWG (g)	FI (g)	FCR	WI (ml)	Mortality %	(PI)	F $/ bird
T0		2605 ^{bc}	2564 ^{bc}	4410.1 ^{a}	1.72 ^{ab}	9689 ^{c}	4.00 ^{ab}	382.6 ^{b}	1.45 ^{bc}
T1		2541 ^{c}	2500 ^{c}	4675.0 ^{a}	1.87 ^{a}	11883 ^{a}	6.66 ^{a}	333.6 ^{c}	1.20 ^{c}
T2		2651 ^{bc}	2610 ^{bc}	4437.0 ^{a}	1.70 ^{ab}	11126 ^{ab}	2.66 ^{b}	399.5 ^{b}	1.58 ^{bc}
T3		2713 ^{b}	2672 ^{b}	4462.2 ^{a}	1.67 ^{b}	11185 ^{ab}	2.66 ^{b}	416.1 ^{b}	1.75 ^{b}
T4	41.0	2700 ^{b}	2659 ^{b}	4281.0 ^{a}	1.61 ^{bc}	10732 ^{b}	1.33 ^{bc}	435.5 ^{ab}	2.00 ^{b}
T5		2777 ^{ab}	2736 ^{ab}	4377.6 ^{a}	1.60 ^{bc}	10985 ^{ab}	1.33 ^{bc}	450.7 ^{ab}	1.98 ^{b}
T6		2732 ^{ab}	2691 ^{b}	4359.4 ^{a}	1.62 ^{bc}	10938 ^{ab}	2.66 ^{b}	432.0 ^{ab}	2.50 ^{ab}
T7		2800 ^{a}	2759 ^{a}	4414.4 ^{a}	1.60 ^{bc}	11065 ^{ab}	1.33 ^{bc}	454.4 ^{ab}	2.63 ^{ab}
T8		2790 ^{ab}	2749 ^{ab}	4425.9 ^{a}	1.61 ^{bc}	11083 ^{ab}	1.33 ^{bc}	450.0 ^{ab}	2.59 ^{ab}
T9		2830 ^{a}	2789 ^{a}	4406.6 ^{a}	1.58 ^{c}	11033 ^{ab}	0.00 ^{c}	471.4 ^{a}	2.70 ^{a}
SE		215.6	188.7	292.0	0.411	339.0	0.338	13.57	0.29
S.L		**	**	N.S	**	**	**	**	**

T0: (Positive control: drinking water without adding-DW)- without heat stress, treatments exposed to heat stress: [**T1**: (Negative control: drinking water without adding-DW, but exposed to heat stress), **T2**: (100 mg BHT/1 L DW) (BHT – butylhydroxytoluene: synthetic antioxidant), **T3:** (100 mg vit E/1 L DW) (synthetic antioxidant), **T4**: (200 mg saffron/1 L DW) (natural antioxidant, **T5**: (200 mg curcumin/1 L DW), **T6**: (100 mg saffron+50 mg BHT/1 L DW), **T7**: (100 mg saffron+50 mg vit E/1 L DW), **T8**: (100 mg curcumin+50 mg BHT/1 L DW), **T9**: (100 mg curcumin +50 mg vit E/1 L DW)]. **IBW**: Initial body weight, **BW**: Body weight, **BWG**: Body weight gain, **FI**: feed intake. **FCR**: feed conversion ratio. **WI**: water intake, **M**: mortality. **PI**: Production Index. **F**: Feasibility. N.S: had no significant differences. [a,b,c] Means within columns with different superscripts differ significantly at ** (P≤ 0.01),

Table 4. point out the efficacy of synthetic and natural antioxidants in broiler drinking water had significantly (P≤ 0.01) decreased heat shock proteins (HSP-40 and -70), Corticosterone hormone concentration in the blood serum and heterophiles: lymphocytes (H/L) ratio of broilers in the all treatments of water additives and positive control T0 compared with the negative control T1.

Table 4. Efficacy of synthetic and natural antioxidants in broiler drinking water as antistressor on heat shock proteins and Corticosterone hormone concentrations in blood serum, and H/L ratio at age 38 d under heat stress.

Treatments	Heat shock proteins HSP 40 (ng/ml)	Heat shock proteins HSP 70 (ng/ml)	Cs hormone (ng/ml)	H/L ratio
T0	55.9 ^{b}	143.5 ^{b}	16.3 ^{b}	0.32 ^{b}
T1	97.6 ^{a}	255.4 ^{a}	23.7 ^{a}	0.62 ^{a}
T2	51.8 ^{bc}	148.9 ^{b}	14.9 ^{bc}	0.43 ^{b}
T3	46.9 ^{bc}	153.7 ^{b}	14.1 ^{bc}	0.37 ^{b}
T4	43.2 ^{c}	123.8 ^{c}	13.5 ^{bc}	0.34 ^{b}
T5	44.0 ^{bc}	121.0 ^{c}	13.7 ^{bc}	0.35 ^{b}
T6	43.1 ^{c}	119.2 ^{c}	13.5 ^{bc}	0.31 ^{bc}
T7	42.5 ^{c}	105.3 ^{cd}	13.4 ^{c}	0.30 ^{bc}
T8	42.8 ^{c}	110.8 ^{c}	13.4 ^{c}	0.31 ^{bc}
T9	42.3 ^{c}	103.5 ^{d}	13.1 ^{c}	0.28 ^{c}
SE	1.42	5.73	0.863	0.037
S.L	**	**	**	**

T0: (Positive control: drinking water without adding-DW)- without heat stress, treatments exposed to heat stress: [**T1**: (Negative control: drinking water without adding-DW, but exposed to heat stress), **T2**: (100 mg BHT/1 L DW) (BHT – butylhydroxytoluene: synthetic antioxidant), **T3:** (100 mg vit E/1 L DW) (synthetic antioxidant), **T4**: (200 mg saffron/1 L DW) (natural antioxidant, **T5**: (200 mg curcumin/1 L DW), **T6**: (100 mg saffron+50 mg BHT/1 L DW), **T7**: (100 mg saffron+50 mg vit E/1 L DW), **T8**: (100 mg curcumin+50 mg BHT/1 L DW), **T9**: (100 mg curcumin +50 mg vit E/1 L DW)]. **Cs**: corticosterone, **H/L ratio** (heterophil/ lymphocyte). ^{a,b,c} Means within columns with different superscripts differ significantly at ** (P≤ 0.01),

Table 5, display the results of Malondialdehyde (MDA) had significantly (P≤0.05) decreased in the T0 and T2, T3, T4. T5, T6, T7, T8 and T9 compared to the T1. Otherwise, Glutathione Peroxidase (GSH-Px), super oxide dismutase (SOD) and total antioxidant capacity (TAC) had significantly (P≤0.01) increased in the treatments T0 and T2, T3, T4. T5, T6, T7, T8 and T9 compared with The T1. Also, in the same table the antibodies titer by ELISA test against Newcastle (ND), Infectious bursal (IBD) diseases and Infection bronchitis virus (IBV) had significantly (P≤0.01) higher titer in the T0, T2, T3, T4. T5, T6, T7, T8 and T9 compared to the negative control T1. Saffron and its constituents, crocin, safranal, and crocetin, can strengthen the immune system via regulating the antioxidant system, cytokine and immunoglobulin secretion, histamine release, lymphocyte activation, phagocytosis, and cellular coreceptor expression (Boskabady and Farkhondeh 2016). The PCA confirmed the positive association between bursa percentage and humoral immune responses and antioxidant status. Samarghandian et al. (2017). Also, Saffron petals possess good antioxidant activity, which is mainly due to the presence of carotenoid and flavonoid compounds, notably glycosides of crocin and kaempferol (Zeka et al. 2015). Heat stress (HS) enhances catabolism of muscle proteins and mobilization of amino acids to facilitate gluconeogenesis and thus eventually results in elevated plasma uric acid levels. (Khodadadi et al. 2016). Mohaqiq et al. (2020) found that aqueous extract of saffron stigma and petal (SPE) enhanced the total antioxidant capacity, while reduced the lipid peroxidation. Additionally, Alipour et al. (2019) reported that oral administration of SPE enhanced the activity of GPx. Its cutaneous injection caused an increase in the SOD activity and a decrease in the plasma MDA concentration. Swathi et al (2016) reported that turmeric powder (0.4% of diets) increased cell-mediated immune response against phyto-haemagglutinin and humoral immune response against Newcastle disease vaccine (NDV) in broilers exposed to heat stress. The increased corticosterone and decreased antioxidant enzyme activities have been associated with the immune depression in broilers maintained under high thermal conditions (Sugiharto et al 2017). Turmeric was previously reported to prevent the increased corticosterone level in the bloodstream (Swathi et al 2016) while increasing the antioxidant enzyme activities in heat-stressed broilers (El-Maaty et al 2014; Swathi et al 2016). The antioxidant content in Saffron and curcumin (Table 1) given the crucial role of lymphoid organs in producing and maturing immune cells, the capacity of turmeric in maintaining the development of these organs is therefore essential to keep the chicks healthy during thermal stress conditions.

Table 5. Efficacy of synthetic and natural antioxidants in broiler drinking water as antistressor on antioxidant enzymes activities concentrations and antibodies titer against ND, IBD and IBV by ELISA in blood serum at age 38 d under heat stress.

Treatments	Antioxidant enzymes activities				ELISA test of antibodies titer against diseases (ng/ml)		
	MDA (nmol/ml)	GSH-Px (U/mL)	SOD (U/mL)	TAC (U/mL)	ND	IBD	IBV
T0	8.48 [b]	138.3 [b]	118.5 [c]	20.1 [b]	2855 [c]	1610 [c]	1937 [c]
T1	10.65 [a]	108.4 [c]	94.2 [d]	16.29 [c]	1505 [d]	1173 [d]	1055 [d]
T2	7.33 [ab]	149.1 [b]	128.9 [b]	23.3 [b]	3420 [bc]	2375 [bc]	3188 [b]
T3	7.25 [ab]	153.5 [b]	133.3 [ab]	25.7 [ab]	3800 [b]	2863 [b]	3450 [ab]
T4	6.93 [b]	159.2 [ab]	134.8 [ab]	25.1 [ab]	3993 [ab]	3094 [ab]	3011 [b]
T5	6.77 [b]	167.0 [ab]	133.0 [ab]	24.8 [ab]	4430 [ab]	3075 [ab]	3755 [ab]
T6	6.81 [b]	160.3 [ab]	129.7 [b]	26.3 [ab]	4520 [a]	3183 [ab]	3700 [ab]
T7	6.55 [b]	163.7 [ab]	138.4 [ab]	27.0 [ab]	4592 [a]	3215 [a]	3802 [a]
T8	6.67 [b]	164.4 [ab]	137.6 [ab]	25.8 [ab]	4670 [a]	3319 [a]	3910 [a]
T9	6.58 [b]	167.0 [a]	138.2 [a]	27.2 [a]	4625 [a]	3278 [a]	3850 [a]
SE	0.377	10.35	8.19	1.50	216	179	164
S.L	*	**	**	**	**	**	**

T0: (Positive control: drinking water without adding-DW)- without heat stress, treatments exposed to heat stress: [**T1**: (Negative control: drinking water without adding-DW, but exposed to heat stress), **T2**: (100 mg BHT/1 L DW) (BHT – butylhydroxytoluene: synthetic antioxidant), **T3:** (100 mg vit E/1 L DW) (synthetic antioxidant), **T4**: (200 mg saffron/1 L DW) (natural antioxidant, **T5**: (200 mg curcumin/1 L DW), **T6**: (100 mg saffron+50 mg BHT/1 L DW), **T7**: (100 mg saffron+50 mg vit E/1 L DW), **T8**: (100 mg curcumin+50 mg BHT/1 L DW), **T9**: (100 mg curcumin +50 mg vit E/1 L DW)]. **MDA**: Malondialdehyde, **SOD**: Super oxide dismutase (U/g tissue), **TAC**: total antioxidant capacity, **GSH-Px**: Glutathione Peroxidase (U/g tissue), **ND**: Newcastle Diseases, **IBD:** Infectious bursal diseases- Gamboro **IBV:** Infection bronchitis virus, [a,b,c] Means within columns with different superscripts differ significantly at *($P \leq 0.05$) &** ($P \leq 0.01$),

CONCLUSIONS

The impact of heat stress is a major challenge causing economical losses to the poultry industry. Heat stress exerts a damaging effect on physiological responses such as immunity, oxidative stress, and intestinal and muscular functions. In this review, we highlight the potential of adding natural antioxidant (saffron and curcumin) then synthetic antioxidant (vit. E and BHT) in broiler drinking water had significantly affected on body performance, economic profit, immune statues and decreasing of body temperature, heat-shock proteins, corticosterone hormone concentrations in blood to antagonize the adverse effects of heat stress, and how we can modulate their functions with nutritional interventions.

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