The effects of the mixture of betaine, vitamin C, St John's wort (*Hypericum perforatum L.*), lavender, and *Melissa officinalis* on performance and some physiological parameters in broiler chickens exposed to heat stress

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ABSTRACT The unprecedented rate of global warming requires more immaculate strategies to fight the heat stress and its detrimental effects on poultry sector. Nutritional strategies, particularly herbal extracts, play a crucial importance in this scenario. Therefore, this study was conducted to evaluate the effects of a novel selected mixture (hereafter refers to as Stress-Bio-Max, **SBM**) comprising St John's wort, lavender, *Melissa officinalis* extracts, betaine, and Vit C on heat-stressed broilers. A total of 300 male broiler chicks were allocated to 4 treatments: T1, Control (no SBM in drinking water); T2, 0.25 mL/L of SBM in drinking water; T3, 0.5 and T4, 1 mL/L of SBM, which applied since d 32. The birds underwent heat stress (40 ± 1) daily from d 35 to 42 of the experiment (2 pm−8 pm). The results show the superior weight gain of T2 and T3 from d 7 to 42 compared to other groups, 72.5 and 72.7 g/d, respectively ($P < 0.05$). Corticosterone level was higher on d 35 in T1 (4.674 ng/mL, $P < 0.05$) and lower in T2 and T3 (2.64 and 2.952 ng/mL, respectively, $P < 0.05$); T2 and T3 also caused the lowest concentrations of corticosterone on d 40 (5.198 and 6.458 ng/mL, respectively, $P < 0.05$). The superior triiodothyronine levels belonged to T2 (0.935 ng/mL, $P < 0.05$) on d 35 and T2 and T3 on d 40 (0.699 and 0.582 ng/mL, respectively, $P < 0.05$). T2, T3, and T4 caused a rise in glutathione peroxidase (**GPX**) and superoxide dismutase (**SOD**) activities ($P < 0.05$). A significant higher amount of total antioxidant capacity (**TAC**) belonged to the groups treating with SBM ($P < 0.05$). The SBM in T2 and T3 resulted in the lowest levels of malondialdehyde (**MDA**), aspartate transaminase (**AST**), alanine aminotransferase (**ALT**), and alkaline phosphatase (**ALP**). Results from this study indicate that SBM may alleviate the negative impacts associated with heat stress in broiler chickens.

Key words: corticosterone, triiodothyronine, broiler, heat stress, herbal extract mixture

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INTRODUCTION

Although there have been many significant developments in management, nutrition, and controlling environment to alleviate the pernicious effects of heat stress, it is still one of the most crucial stressors challenging poultry industry worldwide. The adverse effects of heat stress on wellbeing and production of poultry would be aggravated when accompanied by high relative humidity in hot climates. Moreover, lack of sweat glands, having feathers, and having higher body temperature than that of mammals contribute even more to the heat stress challenge in tropical areas (Park and Kim, 2017). Heat stress has also been demonstrated to deteriorate the meat quality in broilers through inducing and accelerating oxidative stress in muscle mitochondria, thereby heightening protein carbonyl production. It would also yield a less juicy and tender meat, lower meat pH, higher drip and cooking loss, L value, and shear force (Song and King, 2015).

On the other hand, much attention has been attracted to extracts, essential oils, and herbs in recent years which has root in traditionally use for overall improvement in sensory characteristics of foods and their shelf-life. In poultry industry such substances have been used for many purposes, from battling against oxidative and

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heat stress and improving overall performance (Habibi et al., 2014; Ghazi et al., 2015; Behboodi et al., 2017; Baradaran et al., 2019; Barbarestani et al., 2020; Cai et al., 2020), carcass quality (Brzóska et al., 2010) to boosting immune (Davoodi et al., 2014; Sedaghat and Karimi Torshizi, 2017) and reproductive function (Sedaghat et al., 2016), to name a few. St John's wort (*Hypericum perforatum L.*) is one of such examples comprising a high proportion of bioactive agents such as flavonoids, hyperforin, and hypericin with considerable antioxidant and antimicrobial properties, showing promising effects in broiler chickens (Landy et al., 2012). Lavender (*Lavandula angustifolia*) is another well-known aromatic herb demonstrating beneficial antioxidant and antimicrobial effects (Salarmoini et al., 2019). It has also been shown to be a suitable alternative to antibiotic growth promoters and a feed additive in broiler diet which can have immune-stimulatory effect as well (Salarmoini et al., 2019). Its antioxidative effect has also been revealed in broiler chickens (Salarmoini et al., 2019; Barbarestani et al., 2020).

The antioxidant effect of *Melissa officinalis*, also known as lemon balm, has extensively been demonstrated (Dastmalchi et al., 2008; De Sousa et al., 2004; Petrovic et al., 2012). Using lemon balm in heat-stressed broiler diets is shown to lead to a better performance (Amiri et al., 2015) as well as a superior immune function and performance in laying hens (Nobakht et al., 2012). Petrovic et al. (2012) found that the antioxidant status of broilers was superior when the clove flower buds powder was supplemented to the diet along with solving lemon balm extract in drinking water, even though it was not reflected in a significant growth performance.

Betaine is known to be a methyl groups donor, similar to choline and methionine, which functions in numerous metabolic processes such as energy metabolism, synthesis of proteins, carnitine, and creatine (Park and Kim, 2017). Betaine has been found to markedly improve growth performance of animals and alleviate detrimental effects of heat stress (Sosnówka-Czajka and Skomorucha, 2013; Chand et al., 2017). The antioxidant capacity of betaine in heat-stressed broiler chickens has already been revealed (Akhavan-Salamat and Ghasemi, 2016). Several studies have found it to result in a better performance by supplementation (Sakomura et al., 2013; He et al., 2015; Akhavan-Salamat and Ghasemi, 2016) and concluded that betaine could improve feed intake, BWG, and FCR in broilers by exerting morphological changes in intestinal epithelia during osmotic disturbance caused by heat stress, thereby leading to an improved feed efficiency and growth rate.

Vit C is a natural antioxidant capable of breaking down the chain of lipid peroxidation in cell membranes. The synthesis of Vit C is not sufficient under heat stress, leading to an increased requirement for the birds, thereby raising the need for supplementation to the diet or drinking water (Sahin and Sahin, 2002; Ghazi et al., 2015). Vit C could alleviate the noxious effects of stress by virtue of reducing corticosteroids synthesis and secretion (Sahin and Sahin, 2002).

With the unprecedented and extensive detrimental effects of global warming, it is crucial more than ever to combat heat stress in broiler industry by utilizing nutritional strategies and exploiting herbal extracts not only alone but in combination with other effective substances such as betaine and Vit C. The purpose of this study, therefore, was to evaluate the function of SBM supplied with drinking water on broilers under heat stress and elucidate the extent or proper dose in which it can counterbalance the pernicious effects of heat stress.

MATERIALS AND METHODS

Birds, Housing, and Management

A total of 300 one-day-old, Ross 308, male broiler chicks, provided by a local commercial hatchery, were randomly allocated into 4 groups comprising 5 replicates per group (15 birds per pen). Each pen was 200 × 150 cm^2 and equipped with a bell-shaped drinker and feeder. Concrete flooring covered with wood shavings was used to raise the birds. All birds were fed (as mash) with a commercial diet (soy-corn base diets) and water ad libitum. The ingredients, composition, and nutritional value of the starter (1 to 21 d of age) and grower (21 to 42 d of age) are provided in Table 1. Four treatments were used in drinking water: T1, Control (no SBM in drinking water); T2, 0.25 mL/L of SBM; T3, 0.5 and T4, 1 mL/L of SBM. The treatments were applied since d 32 and the whole experiment lasted 42 d. All experimental treatments underwent heat stress (40 ± 1°C) daily from d 35 to 42 of the experiment (from 2 pm to 8

Table 1. Ingredients and composition of basal diets in starter and grower phases of the experiment.

Items (% unless stated otherwise)	Starter (1−21 d)	Grower (21−42 d)
Corn	56.50	60.56
Soybean meal (44% CP)	37.27	32.33
Soy oil	2.38	3.69
Dicalcium phosphate[1]	1.44	1.09
$CaCO_3$ (38%)	1.28	1.38
Sodium chloride	0.43	0.33
DL-Methionine	0.15	0.07
Mineral supplement[2]	0.25	0.25
Vitamin supplement[3]	0.25	0.25
Calculated analyses		
ME (Kcal/kg)	2950	3100
CP	21.20	19.38
Met + Cys	1.31	1.06
Lys	1.15	1.03
Thr	0.81	0.73
Ca	0.92	0.87
Available P	0.41	0.34

[1]Contained 23% Ca and 20% P.

[2,3]Supplied the following per kilogram of diet: retinyl acetate, 9,000 IU; cholecalciferol, 2,000 IU; dl-α-tocopheryl acetate, 12.5 IU; menadione sodium bisulfite, 1.76 mg; biotin, 0.12 mg; thiamine, 1.2 mg; riboflavin, 3.2 mg; calcium d-pantothenate, 6.4 mg; pyridoxine, 1.97 mg; nicotinic acid, 28 mg; cyanocobalamine, 0.01 mg; choline chloride, 320 mg; folic acid, 0.38 mg; MnSO4•H2O, 60 mg; FeSO4•7H2O, 80 mg; ZnO, 51.74 mg; CuSO4•5H2O, 8 mg; iodized NaCl, 0.8 mg; Na2SeO3, 0.2 mg.

pm) to resemble the climate of the region and the time frame when the heat stress is exacerbated. Other times, chickens were maintained at the recommended comfort temperature according to Ross 308 company, the ambient temperature of 32°C and RH of 60 to 70% at d 1 with a gradual decrease of 2 degrees per week until the constant temperature of 20°C and RH of 50% prior to d 32. A typical day during heat stress included a temperature of 20 to 25 before heat stress exposure with the RH of 50 to 60%. Heaters would be turned on at the time of heat exposure and set to provide the constant desired temperature (approximately 40°C). The time to reach the desired temperature was approximately 20 min and the time after heat exposure to return to the normal temperature of 20 to 25°C was approximately 30 min. The following lighting program was adhered: 23L:1D (35 LX) for the first 7 d, 20L:4D with the light intensity of 15 LX (1.5 FC) over the course of 8 to 28 d, and 23L:1D with the light intensity of 5 LX (0.5 FC) from d 29 to 42. The provided lighting was through incandescent lamps with electronic controller and dimmer systems to control the photoperiod and light intensity in the house.

The study protocol was conducted in accordance with the Animal Care and Use Review Committee guidelines of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

SBM Preparation and Composition

The SBM being investigated was composed of betaine, Vit C, St John's wort (*Hypericum perforatum L.*), lavender, and *Melissa officinalis* extracts which were amalgamated so that the composition of the final solution had a fixed concentration with elements described in Table 2. Commercially available betaine and Vit C in addition to each commercial extract was purchased (Kimiagaran Raze Tabiat co, Mashhad, Iran) and applied in preparation. Compositions of SBM were determined by gas chromatography according to Dunford and Vazquez (2005), with Hypericin, Linalool, Rosmarinic acid, and Chlorogenic acid being the principal active components.

Table 2. The composition and the attributes of the final solution of SBM, the mixture of betaine, Vit C, St John's wort (*Hypericum perforatum L.*) extract, lavender extract, and *Melissa officinalis* extract.

Item	Content[1]
Hypericin (mg/g)	19.568
Linalool (mg/g)	14.421
Rosmarinic acid (mg/g)	15.412
Chlorogenic acid (mg/g)	12.214
Flavonoids (mg/g)	2.231
Antioxidant (%)	91.61
Dry matter (%)	8
Total ash (mg/g)	1.3
Color	Brown
pH	4.6
Alcohol (%)	0

[1]The values represent the amount of each component in dry matter sample of SBM.

Growth Performance

Body weight and feed intake of broilers were recorded on a pen basis at regular intervals: the day of hatch, 7, 14, 21, 28, 35, and 42 d. The recorded data was used to calculate BWG, FI, and FCR in each phase and for the whole experimental period. The broiler chicks were monitored several times a day to remove any mortalities and adjust feed intake.

Determination of Blood Indices, Triiodothyronine, and Corticosterone

On d 35 and 40, the blood samples of 10 birds were randomly collected from each treatment (2 birds/pen) for triiodothyronine and corticosterone measurements after heat exposure. The blood samples of 2 birds per pen were also collected at d 42, before slaughter, to quantify the levels of some blood biochemical indices and liver enzymes activity.

The serum concentrations of triglycerides, cholesterol, high-density lipoproteins (**HDL**), and malondialdehyde (**MDA**) were determined by spectrophotometric methods following the instructions of manufacturer (Pars Azmoon, Tehran, Iran). The values of very low-density lipoprotein (**VLDL**) were determined by dividing triglyceride values to unit 5. Subtracting total concentrations of HDL and VLDL from total cholesterol was performed in order to determine LDL (Barbarestani et al., 2020).

The values of total antioxidant capacity (**TAC**), glutathione peroxidase (**GPX**), superoxide dismutase (**SOD**), alanine aminotransferase (**ALT**), aspartate transaminase (**AST**), and alkaline phosphatase (**ALP**) were measured using a microplate reader based on the manufacturer's instructions (Pars Azmoon Co., Tehran, Iran; Awareness Technology Inc., State Fax 3200, Palm City, FL). Analysis of plasma concentrations of triiodothyronine was performed by using enzyme-linked immunosorbent assay (Pishtaz Teb, Tehran, Iran). Corticosterone concentration was also determined using a commercial high sensitivity ELISA kit (IDS Ltd., Bolton, UK).

Carcass Yield

At the end of the experiment, 2 birds per pen were randomly selected for carcass yield. The live body weight of each bird at slaughter was recorded; then, after the evisceration, the hot carcass weight, and also after the chilling for 4 h at 1°C in ice water the carcass weight was again recorded. Next, the carcass, breast, thigh, and abdominal fat weights were recorded for carcass yield calculations.

Statistical Analysis

The experiment was conducted in a completely randomized design. All data were analyzed via one-way ANOVA using General Linear Model procedure of Statistical Analysis System (SAS, 2014) with treatment means deemed significantly different at $P < 0.05$. The means were compared by the Duncan's multiple range test.

RESULTS

Performance

No significant difference was found in FCR among experimental groups throughout the research and no significant differences have been observed in average daily feed intake (**ADFI**) and average daily weight gain (**ADWG**) among groups prior to heat stress and SBM application (data not shown for brevity). Figure 1 shows the significant effect of SBM on overall ADFI ($P < 0.05$) in which T2 and T3 groups consumed more feed than those in T1 and T4 groups. The same significant effect was observed in overall ADWG ($P < 0.05$), as shown in Figure 1B.

Carcass Yield

The yield of the whole carcass was not influenced by the applied SBM treatments ($P > 0.05$; data not shown for brevity) but the effects were significant for the yield of thigh and breast meat, as well as abdominal fat ($P < 0.05$) (Figure 2). Using SBM in all levels yielded a better performance in thigh (Figure 2A), but T2 resulted in a better yield for breast meat numerically, even though it was not statistically different from T1, T3, and T4 (Figure 2B). Results show that the amount of abdominal fat as a percentage of body weight decreased by using SBM ($P < 0.05$) in which T2 and T3 groups could cause less abdominal fat compared with the other 2 groups (Figure 2C) and the highest value was observed in the control group ($P < 0.05$).

Blood Parameters

Plasma triglyceride and cholesterol levels of broiler chickens under heat stress are presented in Figure 3. The SBM has been dramatically effective in lowering plasma triglyceride and cholesterol levels, in which all the groups treated with SBM showed lower cholesterol concentrations ($P < 0.05$) when compared with the control group. T3 and T2 were found to be the most effective of all in lowering triglyceride and cholesterol, respectively.

Figure 4 illustrates the effect of SBM used in this study on the amounts of VLDL, LDL, and HDL. The VLDL and LDL concentrations were significantly lower in T2, T3, and T4 groups than that of T1 group ($P < 0.05$). As regards HDL, all the groups receiving SBM, T2, T3, and T4, revealed the higher levels of HDL.

Corticosterone and Triiodothyronine

The results of the effects of SBM on triiodothyronine at d 35 and 40 of the broiler chickens under heat stress are summarized in Figure 5. At d 35 of the experiment, T2 caused the highest level of triiodothyronine in plasma compared to the other groups ($P < 0.05$) and the trend was almost the same at d 40 with having sharper results in which T3 also showed a significant rise in triiodothyronine along with T2 ($P < 0.05$). The control group, T1, and T4 had no significant difference in the concentration of

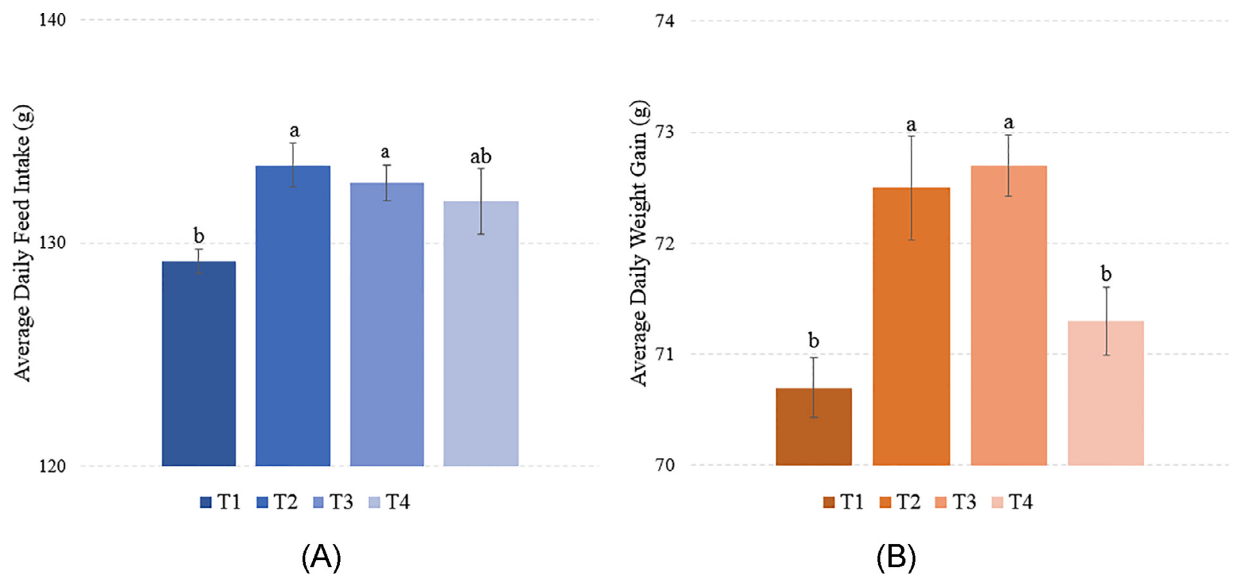

Figure 1. Effect of different levels of SBM, the mixture of St John's wort, lavender, and *Melissa officinalis* extracts, betaine, and Vit C, on overall average daily feed intake (A) and average daily weight gain (B) of broilers under heat stress; Treatments T1, T2, T3, and T4 stand for control (no SBM in drinking water), 0.25, 0.5, and 1 mL/L of SBM, respectively. The data is given as means ± SEM. Means with different letters are significantly different ($P < 0.05$). Abbreviation: SBM, Stress-Bio-Max.

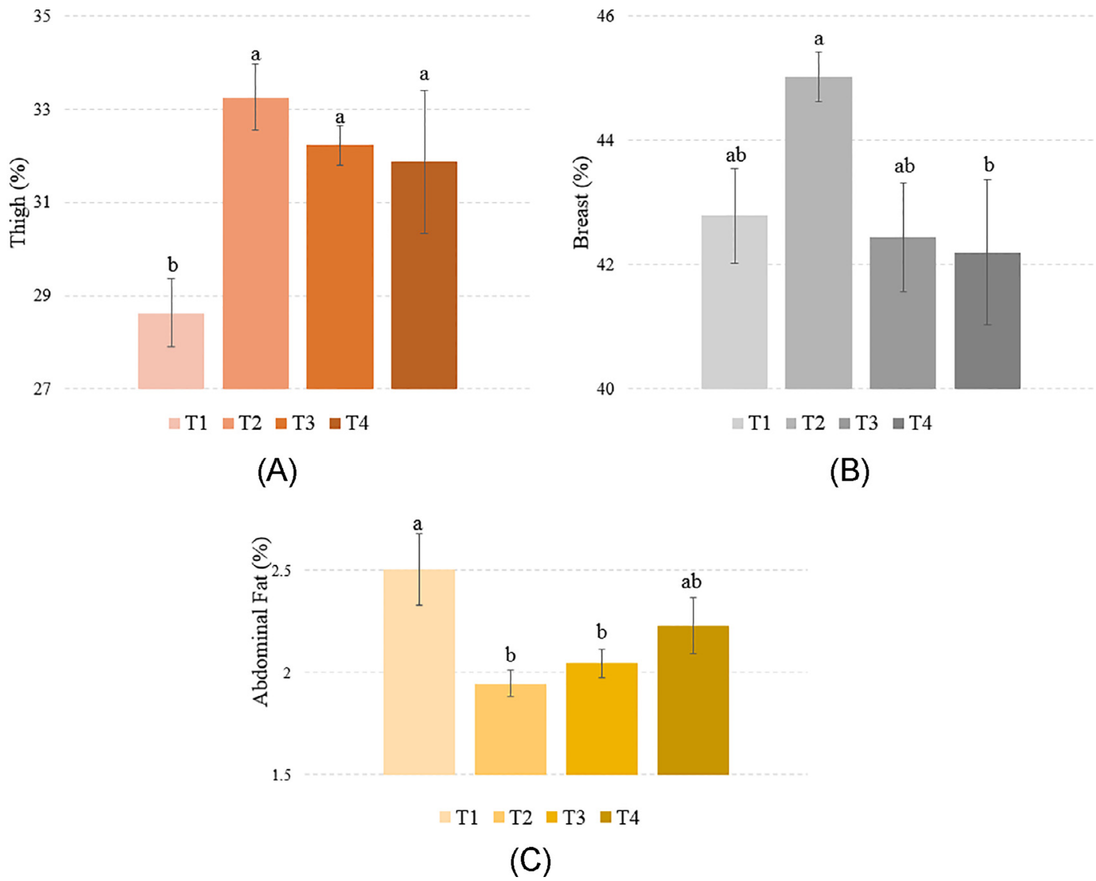

Figure 2. Effect of different levels of SBM, the mixture of St John's wort, lavender, and *Melissa officinalis* extracts, betaine, and Vit C, on thigh (A) and breast (B) meat yield, and abdominal fat percentage (C) of broilers under heat stress; Treatments T1, T2, T3, and T4 stand for control (no SBM in drinking water), 0.25, 0.5, and 1 mL/L of SBM, respectively. The data is given as means ± SEM. Means with different letters are significantly different ($P < 0.05$). Abbreviation: SBM, Stress-Bio-Max.

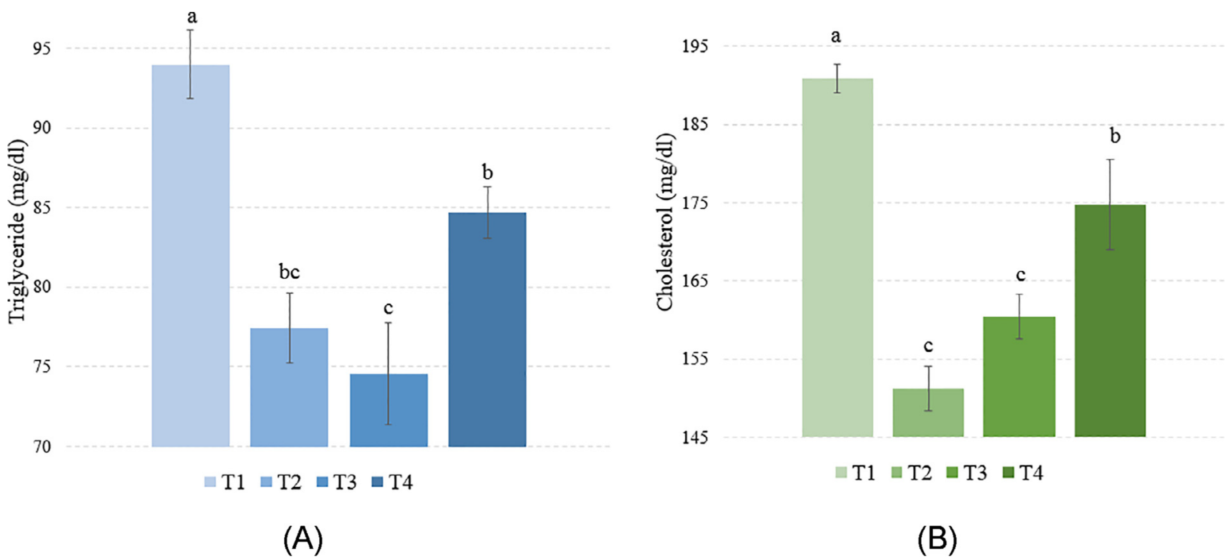

Figure 3. Effect of different levels of SBM, the mixture of St John's wort, lavender, and *Melissa officinalis* extracts, betaine, and Vit C, on plasma triglyceride (A) and cholesterol (B) levels of broilers under heat stress; Treatments T1, T2, T3, and T4 stand for control (no SBM in drinking water), 0.25, 0.5, and 1 mL/L of SBM, respectively. The data is given as means ± SEM. Means with different letters are significantly different ($P < 0.05$).

triiodothyronine at either d 35 (Figure 5A) or 40 (Figure 5B) of the experiment ($P > 0.05$).

As for the plasma concentration of corticosterone, there was a significant difference ($P < 0.05$) between the groups receiving SBM and the control group at both 35 and 40 d of the experiment (Figure 6). In fact, T2 showed to have the lowest level of corticosterone among the other groups at both d 35 (Figure 6A) and 40 (Figure 6B) in broilers under heat stress; the control group had the highest concentration of corticosterone in these 2 stages of measurement (Figures 6A and 6B).

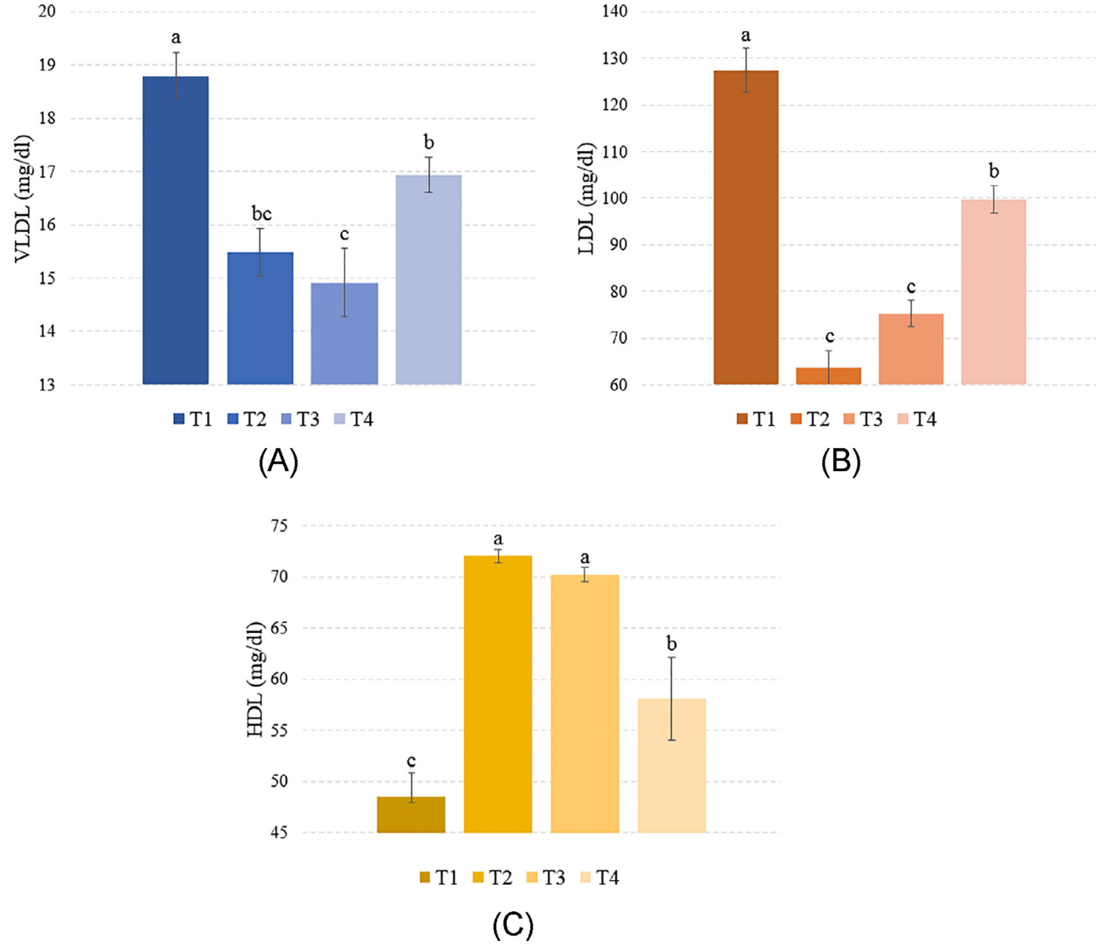

Figure 4. Effect of different levels of SBM, the mixture of St John's wort, lavender, and *Melissa officinalis* extracts, betaine, and Vit C, on plasma VLDL (A), LDL (B), and HDL (C) levels of broilers under heat stress; Treatments T1, T2, T3, and T4 stand for control (no SBM in drinking water), 0.25, 0.5, and 1 mL/L of SBM, respectively. The data is given as means ± SEM. Means with different letters are significantly different ($P < 0.05$). Abbreviations: HDL, high-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low-density lipoprotein; SBM, Stress-Bio-Max.

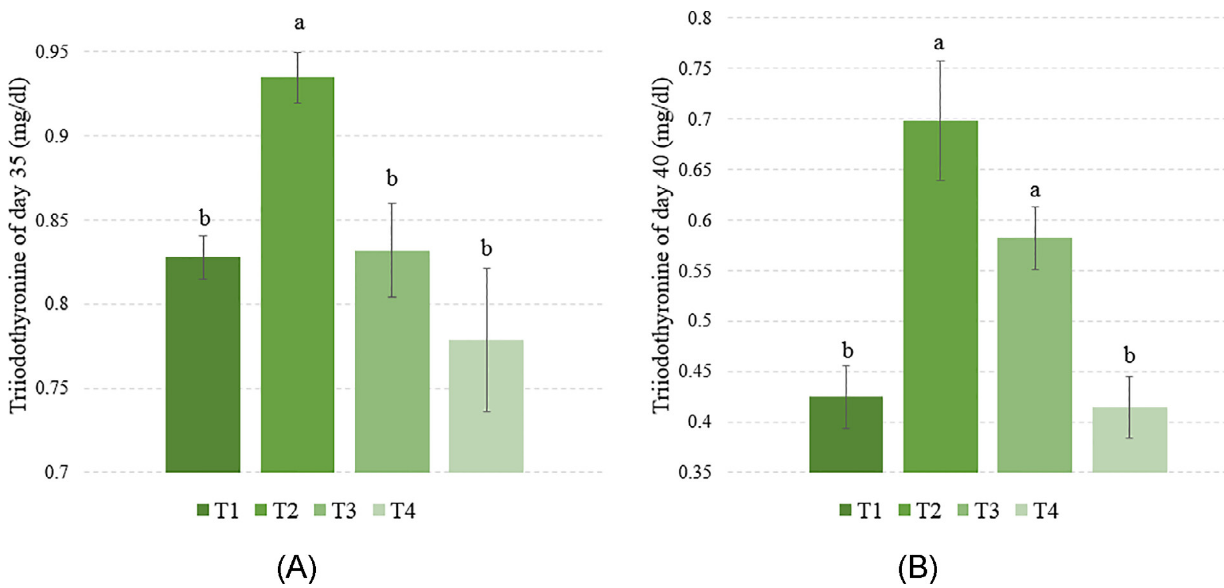

Figure 5. Effect of different levels of SBM, the mixture of St John's wort, lavender, and *Melissa officinalis* extracts, betaine, and Vit C, on plasma triiodothyronine at d 35 (A) and 40 (B) of broilers under heat stress; Treatments T1, T2, T3, and T4 stand for control (no SBM in drinking water), 0.25, 0.5, and 1 mL/L of SBM, respectively. The data is given as means ± SEM. Means with different letters are significantly different ($P < 0.05$). Abbreviation: SBM, Stress-Bio-Max.

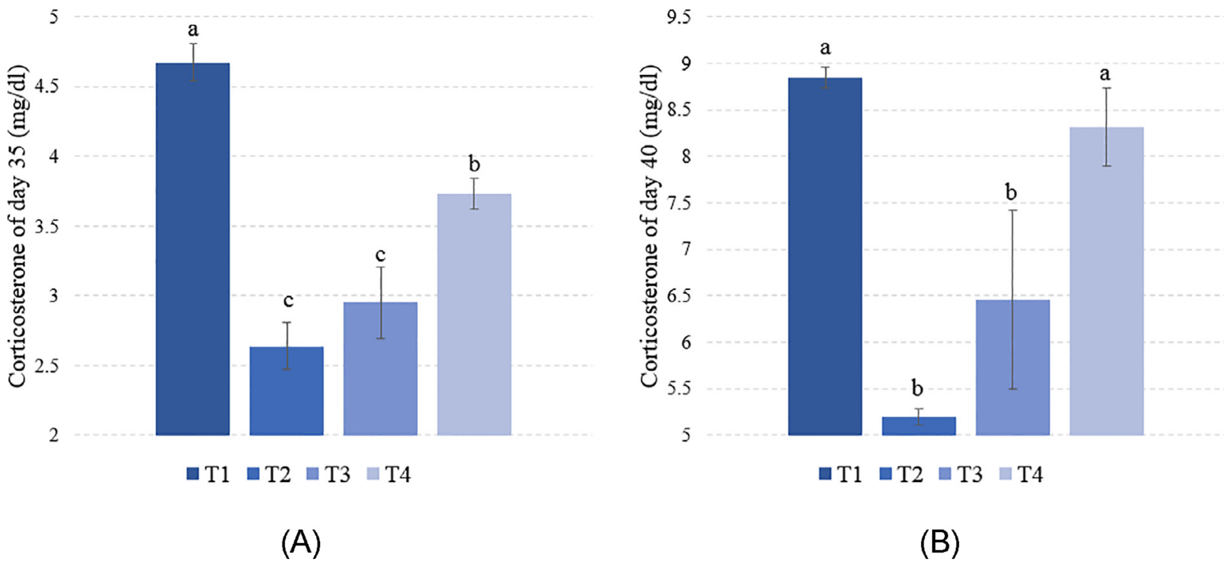

Figure 6. Effect of different levels of SBM, the mixture of St John's wort, lavender, and *Melissa officinalis* extracts, betaine, and Vit C, on plasma corticosterone concentration of broilers under heat stress at d 35 (A) and 40 (B) of rearing; Treatments T1, T2, T3, and T4 stand for control (no SBM in drinking water), 0.25, 0.5, and 1 mL/L of SBM, respectively. The data is given as means ± SEM. Means with different letters are significantly different ($P < 0.05$). Abbreviation: SBM, Stress-Bio-Max.

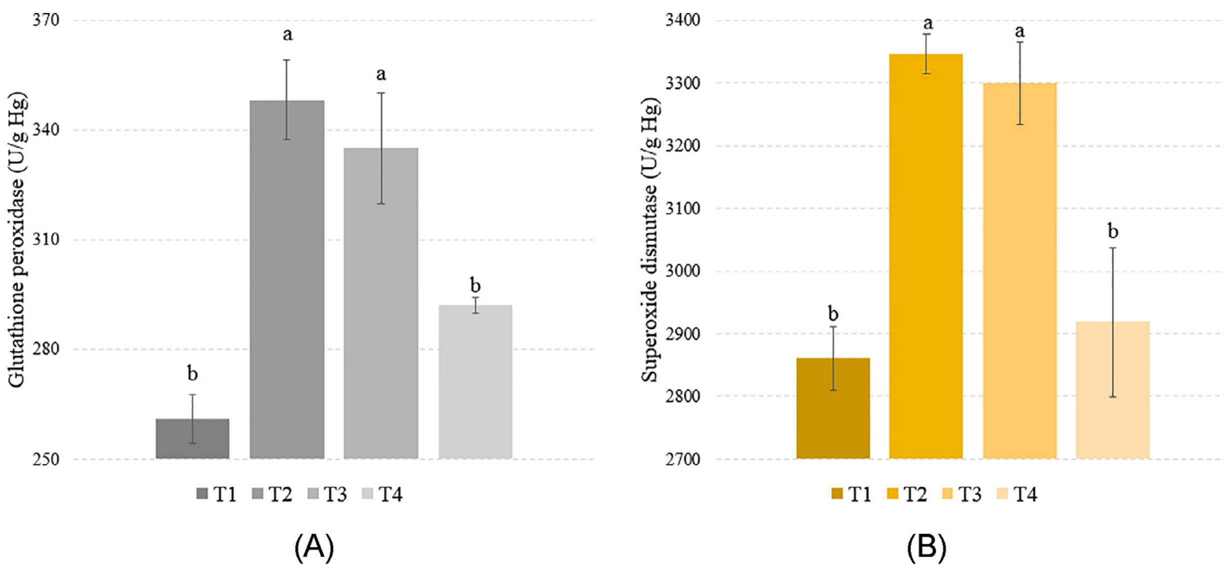

Figure 7. Effect of different levels of SBM, the mixture of St John's wort, lavender, and *Melissa officinalis* extracts, betaine, and Vit C, on plasma glutathione peroxidase (A) and superoxide dismutase (B) concentrations of broilers under heat stress; Treatments T1, T2, T3, and T4 stand for control (no SBM in drinking water), 0.25, 0.5, and 1 mL/L of SBM, respectively. The data is given as means ± SEM. Means with different letters are significantly different ($P < 0.05$). Abbreviation: SBM, Stress-Bio-Max.

GPX, SOD, TAC, and MDA

The results concerning the effects of SBM on 2 main liver enzymes activity such as GPX and SOD in heat-stressed broiler chickens are provided in Figure 7. As it is shown in the figure, SBM treatments, T2, T3, and T4 caused a rise in GPX ($P < 0.05$; Figure 7A) and SOD activities ($P < 0.05$; Figure 7B).

Figure 8 shows the effects of SBM on TAC (a) and MDA (b) of plasma in broiler chickens exposed to heat stress. As in the section a of the figure, a significant higher amount of TAC belongs to T2 ($P < 0.05$) and subsequently to T3 and T4, despite having no statistically significant difference ($P > 0.05$) compared with the control group. The MDA levels of the birds in T2 and T3 groups happened to be the lowest value among the experimental groups ($P < 0.05$) and no significant difference was seen between T1 and T4 (Figure 8B).

ALT, ALP, and AST

The results from the influence of SBM on 3 key liver enzymes activity, ALT, ALP, and AST of the broiler chickens under heat stress are summarized in Figure 9. The SBM in T2 and T3 caused a reduction in the activity of ALT, ALP, and AST significantly ($P < 0.05$), and no significant differences were observed between T1 and T4 in AST and ALT activities.

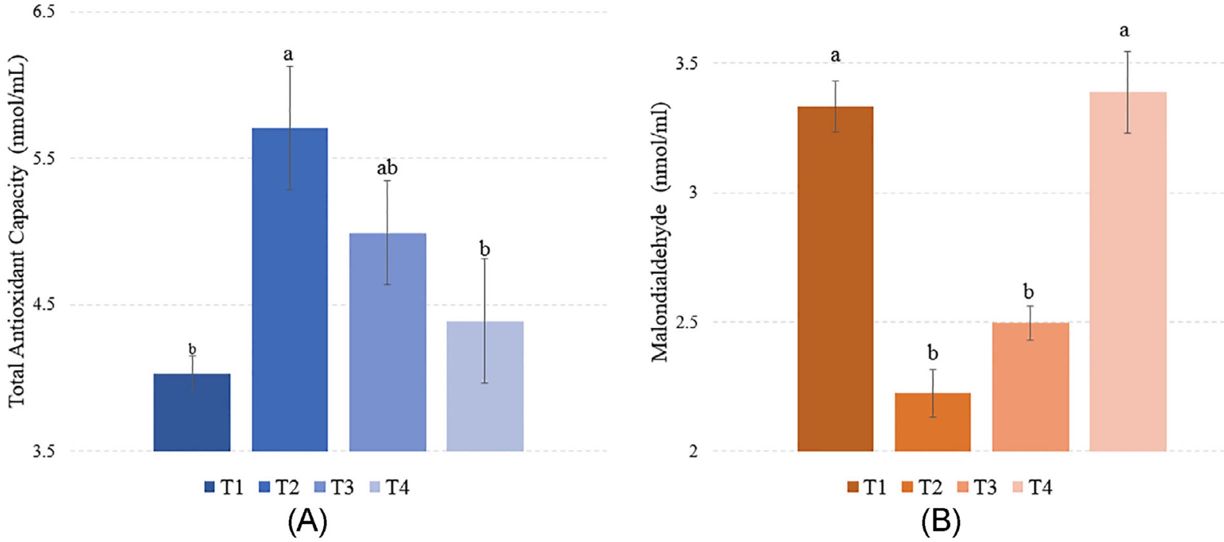

Figure 8. Effect of different levels of SBM, the mixture of St John's wort, lavender, and *Melissa officinalis* extracts, betaine, and Vit C, on plasma total antioxidant capacity (A) and malondialdehyde (B) of broilers under heat stress; Treatments T1, T2, T3, and T4 stand for control (no SBM in drinking water), 0.25, 0.5, and 1 mL/L of SBM, respectively. The data is given as means ± SEM. Means with different letters are significantly different ($P < 0.05$). Abbreviation: SBM, Stress-Bio-Max.

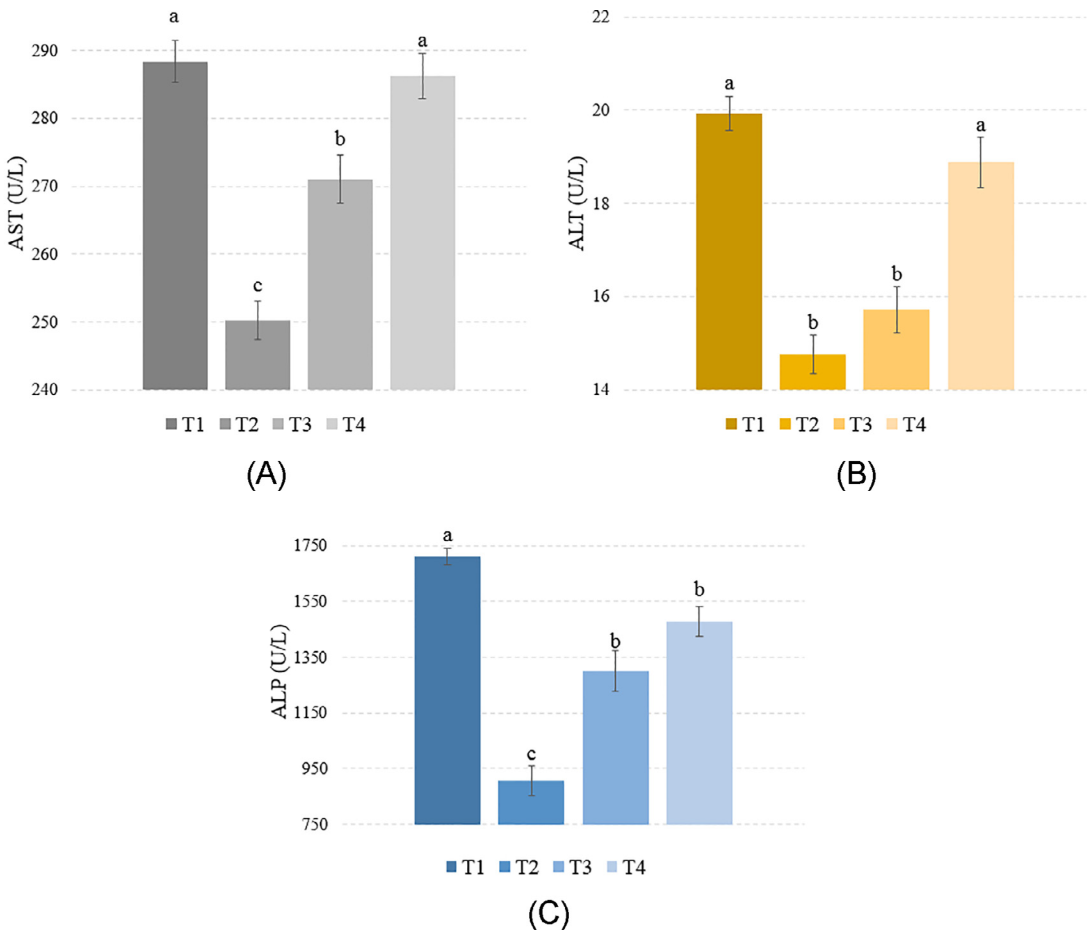

Figure 9. Effect of different levels of SBM, the mixture of St John's wort, lavender, and *Melissa officinalis* extracts, betaine, and Vit C on alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) of broilers under heat stress; Treatments T1, T2, T3, and T4 stand for control (no SBM in drinking water), 0.25, 0.5, and 1 mL/L of SBM, respectively. The data is given as means ± SEM. Means with different letters are significantly different (P < 0.05). Abbreviation: SBM, Stress-Bio-Max.

DISCUSSION

Although it is well known that essential oils and extracts are capable of augmenting feed efficiency, body weight, and antioxidant capacity, very few studies, if any, have been conducted to illustrate the effects of SBM under extreme environmental challenges.

It has been demonstrated that essential oils could increase nutrient availability in gastrointestinal tract leading to a better performance (Murugesan et al., 2015). A great deal of SBM was made of St John's wort extract, with having hypericin and flavonoids as its major components among others (Barnes et al., 2001), which has been revealed in a study not be able to improve performance of broilers but alter HDL and total cholesterol of serum (Landy et al., 2012). This result is partially in agreement with the current study, manifesting a positive impact on triglyceride, cholesterol, HDL, VLDL, and LDL concentrations. However, it has also been reported that St John's wort had no positive effects on growth performance of broilers (Landy et al., 2012; Hosseini et al., 2015). Moreover, Hosseini et al. (2015) revealed a lower amount of abdominal fat in broiler chickens by using St John's wort which is in concordance with the results obtained in the present study showing a significant decrease in abdominal fat (Figure 2) by SBM. They also showed an enhanced liver enzyme activity which is also in line with the current results of liver enzyme assay. It has been revealed that St John's wort has a positive impact on body weight, cholesterol levels, broiler survival, and improving welfare by exerting a potent physiological response to stress (Sosnówka-Czajka and Skomorucha, 2013). The studies that found a better body weight posit that the logic behind this improvement is a better flora of the digestive tract pursuant to antibacterial effect of volatile oils, which in turn gives rise to an improved digestion by an increase in the production of digestive enzymes (Hippenstiel et al., 2011). On the other hand, there are also a few studies reporting no effect of herb mixtures on body weight, feed intake, and feed conversion ratio (Brzóska et al., 2010; Sosnówka-Czajka and Skomorucha, 2013) which could be attributed to providing the extract for a brief period of time, for example 5 h/d during d 21 to 35 of rearing while in the current study SBM administered by drinking water continuously 24 h/d for 10 d. This alone, does not necessarily substantiate the discrepancies since other confounding factors such as single vs. combined usage could play a critical role in this scenario.

There are a multitude of studies reporting either a positive or no effect of extracts on carcass traits. For example, there are reports about St John's wort, as one component of SBM, resulting in a better carcass yield (Davoodi et al., 2014) or no effect altogether (Landy et al., 2012); or lavender, another component of SBM, showing to have no effects on carcass yield and relative weights of breast, thigh, and other parts of the carcass (Salajegheh et al., 2018; Salarmoini et al., 2019). This inconsistency may be due to the different approaches of application, the level of usage, or other environmental and management factors such as hygiene, the duration of heat exposure or the extent of heat stress. In the current study, we observed that SBM could act effectively and produced an improved thigh and breast yield, especially T2 group ($P < 0.05$). Indeed, it is well documented that the superior effect of extracts is due to a positive impact on FI and performance parameters, pursuant to antioxidant agents which could improve nutrient utilization and decrease the noxious effect on protein breakdown along with a drop in corticosterone synthesis during heat stress (Hayashi et al., 1994; Sahin et al., 2003; Seven et al., 2008). These are all in agreement with the results of ADFI and ADWG and corticosterone in the current study. In other words, the results are compatible with and support each other, manifesting the effective impact on the overall condition of the broilers under heat stress. Moreover, it has been demonstrated that using a mixture of properly chosen herbs would yield better results on account of synergistic effect than using individually the same herbs (Hippenstiel et al., 2011). Hence, it can explain the proper mixture of chosen substances for SBM in the current study due to better results of ADFI and ADWG in broilers under heat stress, despite no significant difference in FCR.

Thyroid hormones play an important role in thermoregulation of avian species. A suppression in thyroid axis is believed to be associated with heat stress (Oke et al., 2017). In this scenario, the conversion of T4 to T3, which is biologically more active, is decreased due to the suppression of thyroid-stimulating-hormone secretion during heat stress, leading to a lower level of triiodothyronine in broilers (Lin et al., 2006; Oke et al., 2017). Therefore, as one effective strategy, every attempt must be made to improve this harsh condition by increasing the concentration of this hormone. Some researchers have reported a rise in this hormone by applying essential oils and extracts such as olive leaf oil (Oke et al., 2017). In the present study, we observed that using SBM proved to be effective in raising the level of triiodothyronine especially at 0.25 mL/L (T2 group). This is also clear from the MDA results of the present study from which we observed the lowest level of MDA in T2 group. In fact, it has been revealed that elevated thyroid level is not responsible for oxidative stress induction (Lin et al., 2006). Therefore, 0.25 mL/L of SBM proved to be the most effective dose to protect the cells against oxidative stress and augment the capacity for scavenging free radicals. Furthermore, a study conducted in heat-stressed broilers showed a decreased level of MDA by inclusion of betaine in diet (Akhavan-Salamat and Ghasemi, 2016). Therefore, it can be concluded that betaine, which is incorporated in SBM, has exerted its effect, along with other components, to reduce the concentration of MDA to enhance the antioxidant capacity of the SBM during heat stress in broiler chickens.

Hypothalamic-pituitary-adrenal axis is a well-known pathway controlling the reactions to stress. An elevated corticosterone level is believed to be a sign of overactivation of this axis (Sohail et al., 2012), inducing oxidative stress (Lin et al., 2006). The overexcitation of this axis is also considered to be detrimental and compromises the body immune function (Sohail et al., 2012). In the current experiment, we observed a drop in circulating corticosterone concentrations in groups receiving SBM which is in agreement with that of the study reporting a decrease in corticosterone of chickens receiving St John's wort extract (Sosnówka-Czajka and Skomorucha, 2013).

A significant drop in serum triglyceride, cholesterol, VLDL, and LDL was observed in groups receiving SBM. A rise was also seen in serum HDL levels of the aforementioned groups. These findings are all in agreement with those studies reporting positive impacts of the extracts and essential oils on blood constituents. For instance, Barbarestani et al. (2020) found that supplementing 600 mg/kg lavender essential oil to the diet reduced serum cholesterol and LDL concentration. They also found an increase in intestinal lactic acid bacteria, and concluded that lowering cholesterol and increasing lactic acid bacteria are the result of inhibiting 3-hydroxy-3-methyl glutaryl-CoA (known as HMG-CoA) reductase activity in the liver. In fact, this inhibition has been shown to come from linalool that lowers the expression of HMG-CoA reductase protein, which is a marker for hepatic cholesterol synthesis. This hypocholesterolemic effect of linalool, one of major components in essential oils as well as SBM of the current study, has already been revealed (Cho et al., 2011; Eissa et al., 2017). Many studies also confirm the positive effects of herbal extracts on blood constituents. In this regard, Davoodi et al. (2014) revealed that adding St John's wort to the diets of broiler chickens increased feed intake, body weight, serum HDL level, and antibody titers, and decreased abdominal fat, serum triglyceride, cholesterol and LDL levels. Similarly, Sosnówka-Czajka and Skomorucha (2013) have found a decrease in serum cholesterol by using St John's wort.

Oxidative stress is one of the physiological changes in heat-stressed chickens (Lin et al., 2006). Liver enzymes activities are the salient features of oxidative status. In fact, SOD and GPX are believed to be the most potent enzymes in protecting the cells and are known to act against ROS products and scavenge free oxygen radicals (Barbarestani et al., 2020). In the present study, SBM was revealed to exert a significant impact on the liver enzymes activities, raising the levels of GPX, SOD, and TAC, and decreasing the levels of AST, ALT, and ALP. These results demonstrate a better function of the liver and the body to protect the cells and tissues against free radicals and damages to body tissues, which has already been reported as a mechanism for protection against oxidative stress (Sohail et al., 2011). A study conducted using betaine in broilers under heat stress found a significant increase in GPX and TAC which is also in line with our results (Akhavan-Salamat and Ghasemi, 2016), considering that betaine was a main component of SBM in the present study. Moreover, as one major bioactive compound of SBM which is found in lavender, linalool has been demonstrated to have a potent antioxidant activity. Considering chlorogenic acid and flavonoids included in SBM, phenolic compounds have also been shown to be responsible for antioxidant activities by donating hydrogen and chelating metal (Barbarestani et al., 2020). This whole scenario will not only suppress the production of free radicals but also foster the antioxidant systems to exert a more robust protective function, suggesting a strong protecting measure employed by SBM.

In addition to herbal extracts, betaine and Vit C have also been two main parts of SBM. Many studies have already proved the beneficial effects of their application in heat stress condition (Sahin and Sahin, 2002; Sahin et al., 2003; Chand et al., 2017; Park and Kim, 2017). The effect of betaine in heat-stressed birds has extensively been revealed and also reviewed by Saeed et al. (2017) in which its role from lipid metabolism and yielding quality meat to gut development, immune response, and intracellular osmoregulatory in poultry is discussed in depth (Saeed et al., 2017). Similarly, hypericin, which is the main compound of St John's wort extract and 19.568% of SBM, has been shown to target the heat shock protein 90 chaperone (Blank et al., 2003) playing an important role in protecting the cells against oxidative stress. Moreover, 15.412% of SBM was made of rosmarinic acid. This compound is believed to prevent apoptosis of intestinal epithelial cells and shield the intestinal barrier. The mechanism by which rosmarinic acid exerts its influence is activating Nrf2 pathway and increasing the levels of antioxidant enzyme activity leading to a better performance during heat stress (Cai et al., 2019, 2020).

In conclusion, our results suggest that SBM introduced in this experiment could exert a robust influence in protecting the birds against harmful effects of heat stress, manifesting a synergistic effect of its components on broiler chickens exposed to heat stress, thereby improving overall growth performance, carcass yield, and the levels of some blood constituents. Therefore, it is highly recommended that broiler industry considers it as a potential nutritional practice to ameliorate the detrimental effects of heat stress. Clearly, these results have both productive and health implications and therefore warrant further investigations.

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DISCLOSURES

The authors declare no conflicts of interest. They declare that they have no personal and/or financial relationships with any other corporation or individual that can inappropriately influence our work. There are also no personal or professional interests of any nature or kind in any service, product and/or company that could be interpreted as influencing the content of this paper.

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