

The effect of dietary *Sanguinaria canadensis* extract and/or Mannan-Oligosaccharide supplementation on body weight and serum total antioxidant activity in broilers under heat stress

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Abstract: *Sanguinaria canadensis* L. is an herbalceous perennial that contains benzophenanthridine alkaloids, including sanguinarine and dihydrosanguinarine. Mannan-oligosaccharide (MOS) is derived from the cell wall of the yeast *Saccharomyces cerevisiae*. The aim of the study was to investigate the effects of the supplementation of *Sanguinaria canadensis* extract (SCE) and/or MOS on body weight and serum total antioxidant activity in broilers under heat stress (HS[+]) and normal (HS[-]) conditions. A total of 72 one-day-old Ross 308 broiler were randomly assigned to 8 pens in two environmentally controlled rooms (4 pens per room). The dietary treatments were: (1) basal diet (control), (2) basal diet plus 1 g/kg of SCE, (3) basal diet plus 1 g/kg of MOS, (4) basal diet plus 1 g/kg of SCE and 1 g/kg of MOS. At 15 days of age, the chickens in one of the two rooms were exposed to HS (34±2°C) for 6 h, while the chickens in another room were continuously kept under normal conditions, serving as control treatment (22±2°C). During the study, body weights were significantly different and these differences were depended on diet and heat. HS[+] groups had lower body weights, however, the supplementation of SCE and MOS improved this situation positively. During the study, it was also determined that there was an interaction between diet and heat. Differences for serum antioxidant activity between HS[-] and HS[+] groups were significant for CUPRAC analysis results and insignificant for ABTS analysis results.

Keywords: Broiler; Heat stress; Mannan oligosaccharide; *Saccharomyces cerevisiae*; *Sanguinaria canadensis*

Broyler diyetlerine *Sanguinaria canadensis* ekstraktı ve/veya Mannan-Oligosakkarit ilavesinin sıcaklık stresi altında canlı ağırlık ve serum total antioksidan aktivitesi üzerine etkisi

Özet: *Sanguinaria canadensis* L., sanguinarine ve dihydrosanguinarine dahil olmak üzere benzophenanthridine alkaloidleri içeren çok yıllık bir bitkidir. Mannan-oligosakkaritler (MOS) *Saccharomyces cerevisiae* mayasının hücre duvarından elde edilmektedir. Bu çalışma, *Sanguinaria canadensis* ekstraktı (SCE) ve/veya MOS ilavesinin sıcaklık stresi (HS[+]) ve normal (HS[-]) koşullar altındaki broylerlerde canlı ağırlık ve serum total antioksidan aktivitesi üzerindeki etkilerini araştırmak amacıyla yapılmıştır. Toplam 72 adet bir günlük broyler civcivleri (Ross 308), çevre kontrollü iki odada 8 kümese (oda başına 4 küme) rastgele ayrılmıştır. Gruplara (1) bazal diyet (kontrol), (2) bazal diyet + 1 g/kg SCE, (3) bazal diyet + 1 g/kg MOS, (4) bazal diyet + 1 g/kg SCE + 1 g/kg MOS şeklinde 42 gün boyunca besleme uygulanmıştır. Hayvanlar 15 günlük olunca, iki odadan birindeki gruplar 6 saat boyunca sıcaklık stresine (34±2°C) maruz bırakılırken, diğer odadaki gruplar kontrol muamelesi olarak sürekli normal (22±2°C) koşullarda tutulmuştur. Çalışma boyunca, vücut ağırlıkları önemli ölçüde farklılık göstermiş ve bu farklılıklar diyet ve sıcaklığa bağlı olmuştur. Sıcaklık stresine maruz kalan gruplar daha düşük vücut ağırlığına sahipken, SCE ve MOS takviyesi bu durumu olumlu yönde iyileştirmiştir. Çalışma sırasında diyet ve sıcaklık arasında bir etkileşim olduğu da tespit edilmiştir. HS[-] ve HS[+] gruplar arasındaki serum antioksidan aktivite farklılıkları CUPRAC analiz sonuçları için önemli, ABTS analiz sonuçları için ise önemsiz bulunmuştur.

Anahtar kelimeler: Broiler; Sıcaklık stresi; Mannan oligosaccharide; *Saccharomyces cerevisiae*; *Sanguinaria canadensis*

Introduction

Stress is considered as a reflex response that inevitably occurs in organisms exposed to adverse environmental conditions and can affect many systems, leading to negative effects such as decreased

immunity, live weight gain, feed consumption, and even death (Puvadolpirod and Thaxton 2000; Etim et al. 2013). Heat stress is the most important stressor in poultry and one of the most important causes of economic losses in poultry production. The re-

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commended ambient temperature for broiler chickens from the 4th week of age is between 20–24°C and when this temperature exceeds 35°C, it leads to an increase in mortality and morbidity of broilers (Arjona et al. 1988). Although it is thought that the decrease in performance parameters such as body weight, feed conversion, meat and egg yield due to heat stress in poultry is directly related to the decrease in feed intake, many findings from studies on this subject have shown that heat stress can also cause oxidative stress leading to the formation of reactive oxygen species (ROS) that disrupt cell structure in poultry and antioxidant system disorders that negatively affect nutrient absorption and metabolism (Sur et al. 2023). Indeed, various stress factors on living organisms, including heat and cold stress, lead to the overproduction of ROS and the formation of free radicals that exceed antioxidant capacity causes damage to proteins, lipids and DNA (Babior 2000; Belhadj Slimen et al. 2014). Although free radicals are normally neutralized by the body's antioxidant defense system, it is not successful in eliminating the excessive free radical load caused by high environmental stressors and abnormal conditions, and therefore the necessity for the addition of natural or synthetic antioxidants to poultry diets arises (Hosseini-Vashan et al. 2015).

Different feeding strategies to reduce the negative effects of heat stress on livestock include reducing heat production (e.g., increasing dietary fat), compensating for low nutrient content in the diet (e.g., increasing the amount of concentrate), and reducing metabolic changes due to heat stress (e.g., using different feed additives) (Babinszky et al. 2011). Within the scope of different feed additives; especially after the banning of antibiotics by the European Union in 2006, the interest in alternative feed additives to antibiotics such as plant and plant extracts, botanical mixtures, organic acids, probiotics, prebiotics, symbiotics, essential oils, enzymes as growth promoters has increased (Huyghebaert et al. 2011).

Sanguinaria canadensis, also known as bloodroot, is a member of the *Papaveraceae* family, a family of lactiferous latex-producing plants (Croaker et al. 2016). *S. canadensis* biologically contains eight isoquinoline alkaloids, including six quaternary benzophenanthridine alkaloids as sanguinarine, chelerythrine, sanguilutine, chelilutine, sanguirubine, chelirubine and two protopine alkaloids as protopine and allocryptopine (Bambagiotti-Alberti et al. 1991). Among phytobiotics, isoquinoline alkaloids constitute the most interesting group due to their antimicrobial, anti-inflammatory and immunomo-

dulatory effects (Kishore et al. 2009) and within this group, benzophenanthridine alkaloids are believed to be the main bioactive components of *Sanguinaria* spp. (Harkrader et al. 1990) and almost all studies report that sanguinarine is the most bioactive component of this alkaloid group (Senchina et al. 2009). It has also been reported that sanguinarine has antimicrobial, anti-inflammatory and antioxidant properties (Adhami et al. 2004).

Mannanligosaccharide (MOS) is known as a new active antigen substance derived from the cell wall of *Saccharomyces cerevisiae*, a yeast cell (Xue et al. 2022). The main reasons for the addition of MOS to diets as an alternative to antibiotics are that it prevents the adhesion of pathogenic bacteria to intestinal cells and increases the immunological effect by stimulating the immune system (Genç et al. 2011). In addition, it has also been reported that MOS improves body antioxidant capacity in various animal species such as laying hens (Bozkurt et al. 2012), sheep (Zheng et al. 2018), rabbits (Attia et al. 2015) by acting as a free radical scavenger.

The aim of this study was to investigate the effects of single and combined supplementation of *S. canadensis* extract (SCE) and MOS on body weight and total antioxidant activity in broilers exposed to heat stress, which is an important stressor for poultry.

Materials and Methods

Animals, diets and experimental design

A total of 72 one-day old broiler chicks (Ross 308) were used in this study. The animals were randomly allocated equally into two temperature-controlled rooms with 4 pens each (8 birds per pen). The basal diets used for starter (d 1–21) and grower (d 22–42) phases in the experiment were formulated as isocaloric (ME: 12.34 and 12.74 MJ/kg for starter and grower diets, respectively.) and isonitrogenous (CP: 21% and 18.2% for starter and grower diets, respectively.) to contain all nutrients at appropriate levels according to NRC (1994). During the experiment, the control group in both rooms was fed only with the basal diet, while the experimental groups were fed with basal diets supplemented (1 g/kg diet) with MOS, a prebiotic obtained from the cell wall of the *Saccharomyces cerevisiae* yeast cell, and/or *Sanguinaria canadensis* extract (SCE). After a 15-day adaptation period, the groups in the room without heat stress (HS[-]) were kept under normal thermal conditions of 22±2°C during the experiment, while the groups in the room under heat stress (HS[+]) were exposed to 34±2°C for 6 hours a day. Chicks

were provided *ad libitum* access to feed and water during the experimental period (42 days).

Collection of data and analytical profiles

On day 21 and 42, the animals were fasted for 12 hours and weighed individually, and their body weights (BW) were recorded. Samples collected from the diets given to each group were analyzed for ME and CP according to the standard procedures reported by the Association of Official Analytical Chemists (AOAC 2000).

Blood samples were collected from the brachial vein via vacuum tubes on day 42 from all animals in the experiment before the morning feeding. Serums were separated by centrifugation for 15 min at 3000 g at 4°C and stored (-86°C) for further analysis. CUPRAC (cupric reducing antioxidant capacity) assay according to the procedures reported by Apak et al. (2005) and ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) assay according to the method reported by Re et al. (1999) were applied to determine total antioxidant activity in serum samples. Analyte concentrations were measured by ICP-OES (Agilent 700 ICP-OES). Assays were calib-

rated with trolox and results are expressed in trolox equivalents (mM).

Statistical analysis

All data were subjected to ANOVA using the general linear models (GLM) procedure of SPSS (1999). Statistical differences among means ($p < 0.05$) were identified using Tukey's multiple range test.

Results

The effects of the experimental period on BWs of broilers on d 21 and 42 are presented in Table 1. In HS[-] groups, SCE supplementation to the diet caused a significant increase in BW on d 21 compared to the control and MOS groups ($P < 0.05$), while MOS supplementation did not cause a significant difference compared to the control group. The addition of both additives together caused a significant increase in live weight compared to the control and MOS group ($P < 0.05$). When the groups in the same room were compared in terms of body weight on day 42, no significant difference was found between the SCE, MOS and control groups, but the combination of SCE and MOS resulted in significantly higher final BW than both the control and MOS groups ($P < 0.05$).

Table 1. Body weights of broilers (n=9)

Groups	HS Exposure	Supplementation (g/kg diet)		Body weight (g)	
		SCE	MOS	d 21	d 42
1	[-]	-	-	889.78 ^{cd}	2650.23 ^b
2	[-]	1	-	1009.34 ^{ab}	2780.89 ^{ab}
3	[-]	-	1	898.89 ^{cd}	2658.45 ^b
4	[-]	1	1	1036.67 ^a	2994.20 ^a
5	[+]	-	-	772.00 ^e	2158.88 ^c
6	[+]	1	-	959.32 ^{bc}	2821.34 ^{ab}
7	[+]	-	1	870.89 ^d	2618.89 ^b
8	[+]	1	1	906.40 ^{cd}	2762.67 ^{ab}
SEM				6.85	23.12
Diet					
Control				830.89	2404.56
SCE				984.33	2801.11
MOS				884.89	2638.67
SCE + MOS				971.56	2878.44
Heat					
[-]				958.67	2770.94
[+]				877.17	2590.40
Variation source (p<)					
Diet				0.0001	0.0001
Heat				0.0001	0.0001
Diet × Heat				0.024	0.001

HS: Heat stress; **SCE:** *Sanguinaria canadensis* extract; **MOS:** Manan-oligosaccharide; **SEM:** Standard error mean; ^{a, b, c, d, e} Means within a column with different superscript letters indicate significant differences ($p < 0.05$).

In HS[+] groups, BWs on d 21 were significantly higher in the experimental groups than those of control ($P<0.05$). It was also recorded that SCE supplementation had a higher effect than MOS in terms of BWs on the same day ($P<0.05$). On d 42, when BWs of the groups in the same room were compared, the addition of SCE and MOS to the diet individually and combined did not cause a significant difference between these experimental groups, but all experimental groups had significantly higher BW compared to the control group ($P<0.05$).

When the groups in HS[+] and HS[-] rooms were compared, temperature stress caused a significant decrease in body weight in the unsupplemented group ($P<0.05$). The addition of SCE and MOS to the diets individually and combined significantly improved the decreased BW in the HS[+] groups ($P<0.05$) and brought it closer to BW value of the control group in the HS[-] room. In addition, when the experimental groups in the HS[+] and HS[-] rooms were compared, except for the group fed diet supplemented with the combination of the relevant additives in both rooms, it was noted that there was no significant difference in BW between the counterparts of the experimental

groups in the HS[-] room on d 21. On day 42, it was also observed that there was no significant difference in final BW between all experimental groups in both rooms. As a result, it was determined that significant differences in BWs during the experiment were related to diet and heat ($P<0.001$). Heat stress led to lower BW, but the addition of SCE and/or MOS to the diets improved this situation positively. It was also found that there was an interaction between diet and heat during the experiment ($P=0.024$ and $P=0.001$, respectively).

The results of CUPRAC and ABTS analysis performed to determine antioxidant activity in serum samples obtained from the control and experimental groups at the end of the trial (d 42) are presented in Table 2. According to the CUPRAC analysis results, no statistically significant difference was found in serum antioxidant activity at the end of the trial among all groups in HS[-] room. The same situation was also found among the groups in HS[+] room. When HS[+] and HS[-] rooms were compared, HS treatment had a negative effect on serum antioxidant activity and it was significantly lower than the groups housed at normal temperature ($P<0.05$).

Table 2. Serum antioxidant activities on d 42 according to CUPRAC and ABTS assay (n=9)

Groups	HS Exposure	Supplementation (g/kg diet)		Assay (mM TR-equivalent)	
		SCE	MOS	CUPRAC	ABTS
1	[-]	-	-	0.59 ^a	1.83
2	[-]	1	-	0.53 ^a	1.92
3	[-]	-	1	0.54 ^a	1.80
4	[-]	1	1	0.59 ^a	1.82
5	[+]	-	-	0.28 ^b	1.94
6	[+]	1	-	0.33 ^b	1.88
7	[+]	-	1	0.32 ^b	1.93
8	[+]	1	1	0.31 ^b	2.05
SEM				0.013	0.027
Diet					
Control				0.44	1.88
SCE				0.42	1.90
MOS				0.43	1.86
SCE + MOS				0.46	1.93
Heat					
[-]				0.56	1.84
[+]				0.32	1.95
Variation source (p<)					
Diet				0.793	0.827
Heat				0.0001	0.046
Diet × Heat				0.388	0.377

HS: Heat stress; **SCE:** *Sanguinaria canadensis* extract; **MOS:** Manan-oligosaccharide; **SEM:** Standard error mean; ^{a, b} Means within a column with different superscript letters indicate significant differences ($p<0.05$).

According to the ABTS analysis results, whether or not the groups were subjected to HS and whether or not the related feed additives were used in the diets did not have a significant effect on serum antioxidant activities and no significant differences were found between all groups in terms of the related parameter. In addition, there was no significant interaction between diet and heat in terms of serum antioxidant activity according to both analysis methods ($P=0.39$ and $P=0.38$, respectively).

Discussion and Conclusion

Due to increasing concerns about the transfer of residues to consumers through the food chain and the growth of resistant bacteria that threaten both public and animal health, studies on the use of herbal extracts and purified ingredients obtained from plants as alternative additives have increased, especially following the ban on the use of antibiotic feed additives as growth promoters by the European Union in 2006 (Kostandinović and Lević 2018). In addition to herbal extracts, various nutraceuticals such as botanical mixtures, organic acids, prebiotics, probiotics, symbiotics, exogenous enzymes, essential oils are also considered as alternative feed additives to antibiotics (Huyghebaert et al. 2011; Yadav and Jha 2019). In this context, this study was conducted to evaluate individual and combined dietary supplementation of *Sanguinaria canadensis* plant extract, which is rich in quaternary benzo phenanthridine alkaloids (Wu et al. 2020), and MOS derived from the cell wall of *Saccharomyces cerevisiae*, a yeast cell, in terms of body weight and serum antioxidant activities in broilers housed under normal thermal and heat stress conditions as two different environmental temperatures. In this study, the addition of SCE alone to the diet of broilers housed at normal temperature caused a significant increase in BW on d 21 compared to the control group, but did not cause a significant difference on d 42. This positive result obtained in terms of BW on d 21 was comparable with the results of the same period in the studies conducted on broilers by Vieira et al. (2008), Liu et al. (2020), Hasan et al. (2020) and Abudabos et al. (2020). In contrast, a 28-day trial in broilers with a phytobiotic containing 1.5% sanguinarine (sangrovit WS[®]) showed no significant difference in final BW between groups (Khatun et al. 2023). In the present study, the addition of the relevant extract to the diet created a statistically insignificant difference in terms of BW on d 42 compared to the control group, which was similar to the results of no significant difference between the control and experimental

groups in terms of BW values on d 42 reported by Vieira et al. (2008), on d 35 reported by Zdunczyk et al. (2010) and Aljumaah et al. (2020), and on d 56 reported by Liu et al. (2020). MOS is a natural substance derived from the cell wall of the yeast cell *S. cerevisiae* and contains a carbohydrate-like mannan component (Zbeda 2021). In this study, the addition of MOS alone to the diet at a rate of 1 g/kg in broilers housed in a normal temperature environment did not cause a significant difference in BWs on d 21 and 42 compared to the control group. Contrary to this finding, in a study in which MOS was added to the diet at a rate of 50 g/100 kg in broilers, Taye et al. (2021) reported that there was an improvement in weekly BW values compared to the control group, and this result was attributed to the view that the carbohydrate in the structure of MOS can reduce pathogenic bacterial colonization in the gastrointestinal tract by binding to bacterial fimbriae, thus improving broiler performance. However, in the broiler diets, there was no significant difference compared to the control group in the BW values on d 21 and 50 in the study in which 0.15%, 0.1% and 0.05% MOS was added by Eseceli et al. (2010) for the starter, grower and finisher periods, respectively, and in the BW values on d 28 and 42 in the study in which 0.5, 1.0 and 1.5 g/kg MOS was added by Zhou et al. (2021), which are parallel to the results of the present study. The combined addition of SCE and MOS to the diets of broilers housed in the HS[-] room resulted in a significant increase in BWs on d 21 and 42 compared to the control group. The fact that the combined use of the relevant additives was more effective than adding them separately to the diet, especially on day 42, strengthens the possibility of a possible synergistic effect. There are also evidences that the combination of phytobiotics with probiotics or prebiotics has a synergistic effect on performance. Indeed, the combination of rosmarinic acid as a phytobiotic and multi-strain *Lactobacillus* as a probiotic in dairy calves (Stefańska et al. 2021), the mixture of carob pulp, chicory and fenugreek as a phytobiotic-prebiotic mixture in fattening pigs (Juhász et al. 2023), the combination of hop extract, oregano essential oil and MOS in broilers (Bozkurt et al. 2009) resulted in a significant improvement in BW compared to the control group, which is remarkable in terms of synergistic effect. In other studies where phytobiotics were used together with other additives such as probiotics and prebiotics, including the study conducted by Ren et al. (2019) in broilers to determine the effects of the combination of a commercial phytobiotic, containing carvacrol, cinnamaldehyde and eugenol as active ingredients,

together with host-specific *Lactobacillus* strains as probiotics, the common view is that these combinations have an improving effect on the intestinal microbiota and animal health by reducing the survival of potential pathogenic microorganisms in the intestine. With this context, it would not be unreasonable to consider the possibility that such a mechanism of action may play a role behind the improving effect of the combined dietary supplementation of SCE and MOS on BW in broilers in the present study.

Heat stress is the most important environmental stressor for the poultry industry worldwide. Factors such as thick feather layer, inadequate sweat glands and high metabolic characteristics, make poultry more sensitive and vulnerable to heat stress (Song et al. 2018). In this study, it was observed that the BW values of the HS[+] control group on d 21 and 42 were significantly lower than the HS[-] control group, as expected. It is known that HS has negative effects on parameters such as feed intake, body weight gain, carcass weight and immunity in poultry (Sahin and Kucuk 2003; Niu et al. 2009; Ghazi et al. 2012). It is also believed that the decrease in appetite and feed consumption leading to less body weight gain in animals exposed to HS is a defense mechanism to reduce heat production in the animal (Sohail et al. 2012). It has also been reported that exposure to high ambient temperature increases the production and release of corticosteroids in poultry, leads to lower levels of important growth hormones such as triiodothyronine and thyroxine, and causes the release of various cytokines and glucocorticoids that induce protein catabolism (Sahin and Kucuk 2003; Zhou et al. 2016; Yildirim 2016). In the HS[+] room, although HS caused a significant decrease in BW in the control group, dietary SCE supplementation resulted in a significant increase in BW on d 21 and 42, resulting in an improvement of 24.3% and 30.7%, respectively. Even though it seems to be no significant difference between the control group in the HS[-] room and SCE supplemented group in HS[+] room, it is also noteworthy that the addition of SCE caused an increase in BW values of approximately 8% and 6.5% for the same days, respectively. In a trial conducted by Wang et al. (2022) on broilers with the extract of *Macleaya cordata* plant, which has similar alkaloids to *S. canadensis*, the addition of 1000 mg/kg of the relevant extract to the diet of the HS group significantly increased the final BW value compared to the HS control group, and also, the addition of this extract reduced the deterioration in the intestinal flora caused by HS by reducing the relative density of *Bacteroidota* and *Bacteroides* in

the intestinal flora, and thus reducing the decrease in growth performance caused by HS. In studies with 100 ppm by Kikusato et al. (2021) and 60 and 100 mg/kg isoquinoline alkaloids by Khongthong et al. (2023) in broilers under HS, it was reported that the addition of these extracts to the diet led to a significant increase in final BW. In these studies, which are consistent with the positive effects of SCE on BW in broilers under HS in the present study, the ameliorative effects of the relevant extracts on BW were attributed to the modulating effect on caecal flora composition (Wang et al. 2022), the reducing effect on oxidative damage, protein catabolism, intestinal barrier function, intestinal inflammation and the correcting effect on cortisone release (Kikusato et al. 2021), and the improving intestinal integrity by reducing inflammation in the intestines and the suppressing effect of anorexigenic regulation by modulating the gut-brain axis (Khongthong et al. 2023). This situation also raises the possibility that there are similar mechanisms underlying the improving effect of SCE on BW in broilers under HS in the present study.

According to Gibson and Roberfroid (1995), prebiotics are nondigestible food substances that exert beneficial effects by selectively stimulating the growth and activity of one or a limited number of beneficial bacteria in the host intestine. In this study, the addition of 1 g/kg MOS to the diet of broilers under HS significantly improved the decrease in BW values caused by HS on d 21 and 42 and even succeeded in approaching the BW values of the control group housed under normal temperature. When compared to the group supplemented with SCE under HS, it was observed that although MOS supplementation fell behind the group supplemented with SCE on d 21 in terms of BW, it closed this gap on d 42. In this study, the result, which showed that the addition of MOS to broiler diets under HS had a significantly higher BW value compared to the control group in the same environment, was in accordance with the results obtained in studies in which 0.2 g/L MOS was added to drinking water by Hasan et al. (2014), 0.5 ml/L commercial prebiotic was added to drinking water by Sayed et al. (2023), and 0.5% MOS was added to the diet by Sohail et al. (2012) in broilers under HS. The positive effects of MOS on BW under HS may be a result of its ability to reduce the proliferation of potentially pathogenic bacteria in the intestinal environment due to stress, to increase the population of beneficial bacteria, to support intestinal functions, and in addition to these, to reduce the release of cortisone, which increases under

stress, to normal levels. As a matter of fact, it has been reported that dietary MOS supplementation significantly decreased serum corticosterone levels in broilers under HS (Sohail et al. 2012; Hosseini et al. 2016; Cheng et al. 2018; Cheng et al. 2019). In the present study, it was found that the group supplemented with the combination of SCE and MOS housed in the HS[+] room had significantly higher BW than the control group, as in the same group housed in the HS[-] room. Considering the previously mentioned positive effects of adding SCE and MOS individually to the diet on BW under HS, the fact that using them together produced similar results shows that there is no negative interaction between themselves in terms of BW, and it is also possible that they created a cumulative effect in terms of the possibilities leading to the positive result on BW.

Free radicals and antioxidants, which still attract the attention of researchers as a subject of study, are in balance under normal conditions in living organisms, and when this balance changes in favor of free radicals, it can lead negative effects caused by oxidative stress (Gheisar and Kim 2018). The antioxidant activity of additives such as plant extracts, essential oils, prebiotics and probiotics is a subject of great interest due to their free radical scavenging abilities that may play a role in the prevention of free radical-induced diseases (Miguel 2010; Gheisar and Kim 2018; Chaves et al. 2020; Musazadeh et al. 2023). Total antioxidant status is an important criterion used to evaluate the entire antioxidative status in the body (Erel 2004). In this context, measurement of total antioxidant capacity is an application that provides important data in determining antioxidant effects. In particular, measuring total antioxidant capacity or activity in biological samples such as plasma, which contain various antioxidant compounds, provides valuable clues about the antioxidant status (Ghiselli et al. 2000). There are various analysis methods such as ORAC (oxygen radical absorbance capacity), FRAP (ferric reducing antioxidant power), CUPRAC (cupric reducing antioxidant capacity), ABTS (2,20 - azinobis - (3 - ethylbenzothiazoline - 6 - sulfonic acid)), DPPH (2,20 - diphenyl - 1 - picrylhydrazyl), Folin for analyzing antioxidant capacity (Apak et al. 2005). In this study, CUPRAC and ABTS analysis methods were used to determine serum antioxidant activity. When the serum antioxidant activity values obtained according to the CUPRAC method were examined, the first striking finding was that the antioxidant capacity in the HS[+] groups was significantly lower than in the HS[-] groups. On a room basis, the statistically insignificant differences

in serum antioxidant activities between the control and experimental groups of each room showed that the differences were entirely due to heat stress, regardless of dietary additives. This may be due to the change in the antioxidant defense system due to increasing environmental temperature. Indeed, it has been reported that broilers exposed to heat stress produce excessive levels of reactive oxygen species (ROS) leading to oxidative stress due to decreased mitochondrial respiratory chain activity and that heat stress disrupts the balance between synthesis and catabolism in this production (Lin et al. 2006; Yang et al. 2010). It was observed that there was a decrease in the antioxidant defense system due to the decrease in SOD, catalase and glutathione peroxidase concentrations in broilers housed under heat stress (Sahin et al. 2010), and there was a significant decrease in the activity of antioxidant enzymes paraoxonase and arylesterase (Sohail et al. 2011). In the present study, when CUPRAC and ABTS analysis methods were compared in terms of HS, it was observed that the CUPRAC method provided more effective results than the ABTS method, while the ABTS method did not exhibit effective sensitivity results against the changes in serum antioxidant activity due to HS. Cecchini and Fazio (2020), who determined that there was no significant difference in antioxidant capacity analyzed by ABTS method in blood samples collected from hens artificially stressed with dexamethasone compared to the healthy group, while this difference was significantly higher in the FRAP test, reported that the lower sensitivity of the ABTS test in the detection of imbalance in antioxidant status compared to the FRAP test may be due to the different technology on which the two tests are based. In the present study, the lower sensitivity of the ABTS method compared to the CUPRAC method in measuring the change in antioxidant activity due to HS may be due to a similar reason. In addition, it is also thought that some unique advantages of the CUPRAC method may play a role in its higher sensitivity than the ABTS method. For example, better reagent stability than radical reagents such as ABTS, a neutral pH of 7.0, which is close to biological systems, and compatibility with hydrophilic and lipophilic solvents are some of the advantages of the CUPRAC assay (George et al. 2022).

In the present study, the addition of SCE to the diet did not cause a significant difference in serum total antioxidant activity between the control and experimental groups housed in each of the HS[-] and HS[+] rooms. Although there are no data on total antioxidant capacity directly related to SCE

supplementation in broilers, similar results were obtained from some studies in which partially similar active ingredients were used on different animal species. The use of dihydrosanguinarine in rats by Vrublova et al. (2008), Sangrovit, a mixture of quaternary benzo[c]phenanthridine alkaloids obtained from *M. cordata* in rats by Zdarilova et al. (2008), and Sangrovit® Extra, a mixture of isoquinoline alkaloids obtained from *M. cordata* in pigs by Le et al. (2020) did not cause any significant difference in terms of total antioxidant capacity. Additionally, Bavarasadi et al. (2017) reported that sanguinarine had no directly remarkable antioxidant properties in laying hens, and in a study by Karakçı et al. (2022) on laying quails, it was reported that a mixture of magnolia and sanguinarine as aromatic plant extracts did not create a significant difference in plasma total antioxidant status.

In this study, the addition of MOS to the diet did not cause significant differences in serum antioxidant activity between the control and experimental groups housed in each of the HS[-] and HS[+] rooms. This result was in agreement with the recent study conducted by Abd El Latif (2023) in broilers, in which no significant difference was detected in serum total antioxidant capacity with the addition of MOS to the diet compared to the control group. Also, in a trial conducted by Yang et al. (2022), the addition of xylo-oligosaccharides, fructo-oligosaccharides and iso-maltooligosaccharides to broiler diets had no significant difference in total antioxidant capacity in liver, breast muscle and thigh muscle samples. In an experiment conducted by Zbeda (2021) in rabbits as a different animal species, it is also a similar finding that the addition of MOS to the diet did not cause any difference in terms of serum total antioxidant capacity values.

In conclusion, in this study, it was determined that body weights were significantly different and these differences were depended on diet and heat stress. Heat stress caused lower body weights in broilers, however, the supplementation of *Sanguinaria canadensis* extract and mannan-oligosaccharide improved this situation positively. In terms of serum antioxidant activity, differences between thermoneutral and heat stressed room were significant for CUPRAC and insignificant for ABTS analysis results. The supplementation of *Sanguinaria canadensis* extract and/or mannan-oligosaccharide had no significant effect on serum antioxidant activity in both analysis method.

Ethics committee for the use of experimental animals and other ethical committee decisions and permissions: Local Ethics Committee for Animal Experiments, Istanbul University approved the ethical compliance of the study (2010/151).

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