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Synergistic effect of *Spirulina platensis* and selenium nanoparticles on growth performance, serum metabolites, immune responses, and antioxidant capacity of heat-stressed broiler chickens

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Abstract

This study examined the effects of dietary *Spirulina platensis* (SP) at levels of 0, 5, and 10 g.kg^{-1} and selenium nanoparticles (SeNPs) at 0, 0.1, and 0.2 mg.kg^{-1}, individually and in combination, on heat-stressed broiler chickens for 5 weeks. Four hundred fifty one-day-old Ross-308 chicks were allocated to 9 dietary groups with 5 replicates (10 chicks each). The control diet was consisted of corn-soybean-based basal diet. The obtained results displayed a significant increase in final body weight ($p = 0.005$) and weight gain during the periods from 22 to 35 days ($p = 0.002$) and 1 to 35 days ($p = 0.005$) in birds fed supplemented diets compared to those fed control diet, with the highest being in birds fed with both 10 g SP and 0.1 mg SeNPs. Feed conversion ratio was also improved in birds fed supplemented compared to control group. Dietary supplements significantly improved carcass dressing ($p < 0.001$), carcass yield ($p = 0.001$) percentages, and blood lipid profile. Blood triiodothyronine was higher ($p = 0.005$) with all treated diets except that contain 5 g SP compared to the control, with the highest being in birds fed diet contains 5 g SP + 0.2 mg SeNPs. Immunoglobulin subclasses IgG, IgM, and IgA were higher in birds fed supplemented diets compared to the control group. Antibody titers to Newcastle disease, avian influenza, and infectious bursal disease were numerically increased with dietary supplementation compared to the control group. Dietary treatments increased ($p < 0.001$) glutathione peroxidase and superoxide dismutase (SOD) levels, except diet contains 5 g SP for SOD level and decreased ($p < 0.001$) malondialdehyde level. It is concluded that dietary inclusion of SP and SeNPs, particularly their combination at levels 5 g SP plus 0.2 mg SeNPs kg^{-1} and 10 g SP plus 0.1 mg SeNPs kg^{-1}, improved growth performance, carcass yield, immunity, and antioxidant capacity of heat-stressed broilers.

Keywords Heat stress · Broilers · Spirulina · Selenium nanoparticles · Growth · Antioxidant

Introduction

The intensive commercial production of poultry has created numerous challenges and stressors on birds, including environmental stress, high stocking densities, nutritional imbalances, and disease outbreak [1, 2]. Hatched broiler chicks are susceptible to various infectious diseases, oxidative stress, and high ambient temperature, which lead to increased mortality and economic losses [3, 4]. Exposure to heat stress encourages the outbreak of contagious diseases and threats the growth, nutrients uptake, digestibility, absorption, and physiological functions of the host [5–7]. Moreover, prolonged exposure to heat stress suppresses the innate immune response and induces immune disorders via altering the organs' immune functions [8, 9] and circulating antibody levels [10]. Heat stress also induces oxidative stress by damaging the membrane of immune cells, leading to apoptosis [11] and increased intestinal barrier permeability and consequently translocation of toxic agents into the body [12, 13].

Consequently, it is of importance to implement mitigation strategies for environmental stressors and climate change that

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can alleviate their undesirable effects on growth performance, immune functions, and antioxidant capacity of broiler chickens, particularly under heat stress conditions. Several mitigation strategies have previously been used including feed additives such as trace elements [14, 15], vitamins [16], probiotics [7, 17–21], and herbal products [3, 22–24].

The microalgae *Spirulina platensis* (SP), a filamentous cyanobacterium, has various health benefits, therapeutic properties, and several other biological activities. The pharmaceutical, medicinal properties of SP may be attributed to its nutritional value (Table 2) particularly its high content of proteins, and essential amino and fatty acids. Besides the nutritional value of spirulina, several medicinal properties and therapeutic potential also have been reported [25]. Spirulina contains a wide range of bioactive compounds, including phycocyanin, polysaccharides, carotenoids, chlorophyll, phenolic compounds, and desired vitamins and minerals [26, 27]. A large and growing body of literature has demonstrated the immunostimulatory, hepatoprotective, anti-inflammatory, antimicrobial, antiviral, and antioxidative activities of spirulina via enhancing disease resistance, stimulating antibodies and cytokines production, effectively scavenging free radicals, and inhibiting lipid peroxidation, thereby improving poultry production and achieving high profitability [27–31].

Selenium plays a vital role in enhancing antioxidant defenses, immune system, and stress prevention. Besides, selenium nanoparticles (SeNPs) displayed several benefits (e.g., low cytotoxicity, increased surface area and interaction with biological targets, and high drug loading capacity) [32]. Accordingly, SeNPs appears to be the priority form in poultry nutrition. It has been reported that SeNPs as natural bioactive molecules can effectively contribute to maintaining cellular functions against oxidative stress and lipid peroxidation, enhancing host immunity and indirectly promoting growth performance [15, 33].

Despite the demonstrated beneficial effects of SP or SeNPs on host physiological functions, the literature on feeding broiler chickens on SP or SeNPs or their combination, particularly under heat stress conditions, are very limited and needed. Therefore, the objective of this work was to explore the beneficial effects of dietary supplementation with SP, SeNPs, and their combinations on growth performance, carcass characteristics, blood biochemistry, immune responses, and antioxidants capacity of heat-stressed broiler chickens.

Materials and methods

The experiment on broiler chickens was carried out according to the Animal Care and Research Ethics Committee's guidelines at the Biological Application Department, Nuclear Research Center, Egypt, as well as of the Egyptian Desert Research Center.

Selenium nanoparticles and *Spirulina platensis* sources

The SeNPs and SP were obtained from Department of Microbiology, Faculty of Agriculture, Zagazig University.

Birds and experimental design

The trial was conducted on 450 post-hatch Ross-308 broiler chicks, ≈ 40 g body weight. They exposed to temperature of 34 ± 2 °C using electric heaters for 24 h during the first 14 days. Later, birds were exposed for three consecutive days a week to 34 ± 2 °C for 12 h (from 9:00 to 18:00) and then to 25 ± 2 °C during the remaining experimental period. They were randomly allocated to 9 groups with 5 replicates of 10 chicks each. Birds were fed on two corn-soybean-based basal diets for 35 d divided into 2 stages: stage 1 (starter, days 1 to 21), stage 2 (grower, days 21 to 35). The experimental groups were arranged as follows: (1) control basal diet with no supplementation; (2) control diet containing 0.1 mg SeNPs kg^{-1}; (3) control basal diet containing 0.2 mg SeNPs kg^{-1}; (4) control basal diet containing 5 g SP kg^{-1}; (5) control basal diet containing 5 g SP plus 0.1 mg SeNPs kg^{-1}; (6) control basal diet containing 5 g SP plus 0.2 mg SeNPs kg^{-1}; (7) control basal diet containing 10 g SP kg^{-1}; (8) control basal diet containing 10 g SP plus 0.1 mg SeNPs kg^{-1}; and (9) control basal diet containing 10 g SP plus 0.2 mg SeNPs kg^{-1}.

All of the chicks were grown in metal cages with water and food provided ad libitum. They were subjected to the same managerial, environmentally controlled, clean, and hygienic conditions. Throughout the first week, the chicks were exposed to 24 h of light per day and then reduced to 22 h per day. The chicks, at day 7, were injected subcutaneously in the back of the neck inactivated Newcastle disease (NDV) plus inactivated avian influenza (H9N1) vaccines while they were vaccinated against infectious bursal disease (IBD) at day 14. The basal diet was formulated to meet the recommendations of Aviagen [34] for broiler chicks. Diet composition for starter and grower basal diets are presented in Table 1.

Nutrients and fatty acid analysis of *Spirulina platensis*

SP samples were analyzed for chemical composition and fatty acids contents (Table 2). Crude protein (CP) (2000 #984.13), crude fiber (CF) (2000 #973.18), and ether extract (EE) (2003 #2003.05) contents in SP were analyzed according to the AOAC International procedures [35, 36]. Riboflavin was determined using ultra-high-performance liquid chromatographic (UHPLC) according to Edelmann et al. [37]. β-Carotene was assessed using HPLC-based quantification method according to Leema et al. [38]. Fatty acids were determined by gas chromatography coupled with mass spectrometry (GC-MS) (Thermo Fisher, Waltham, MA, USA) after its conversion to fatty acid methyl esters according to [39]. Relative

Table 1 Diet composition for starter and grower basal diets

Ingredient	Starter (1–21 days)	Grower (22–35 days)
Corn	55.00	59.00
Soybean meal (44%)	38.7	33.4
Vegetable oil	2.38	3.90
Limestone	1.17	1.06
Dicalcium phosphate	2.00	1.82
Premix[1]	0.30	0.30
NaCl (salt)	0.25	0.25
L-lysine	0.04	0.10
DL-methionine	0.16	0.17
Calculated composition		
ME (kcal.kg^{-1})	2913	3060
Crude protein	22.06	20.15
Calcium	1.04	0.94
Non-phytate phosphorus	0.52	0.48
Methionine	0.52	0.50
Lysine	1.30	1.29
TSAA	0.87	0.82

[1] *Premix* (1%) provided per kg of diet. vitamin A, 14,000 IU; vitamin D3, 300 IU; vitamin E, 50 mg; vitamin K, 4 mg; vitamin B6, 3 mg; vitamin B12, 6 mg; niacin, 60 mg; pantothenic acid, 20 mg; folic acid, 0.20 mg; choline, 150 mg; Ca, 48 mg; P, 3.18 mg; Mn, 100 mg; Fe, 50 mg; Zn, 80 mg; Cu,10 mg; Co, 0.25 mg; iodine, 1.5 mg

ratios of fatty acids were calculated as percentage of the total fatty acids.

Growth performance

Live body weight (LBW) and feed intake (FI) were recorded weekly. Body weight gain (BWG) was calculated weekly, and feed conversion ratio (FCR) was calculated based on feed intake divided by BWG. On day 35, three representative birds were randomly selected from each replicate for slaughter test. Before slaughter, these birds were fasted for 12 h and then individually weighed, slaughtered, de-feathered, opened, and eviscerated. The hot carcass, dressing, edible parts (liver, heart, and gizzard), non-edible parts (thymus, bursa of Fabricius, and spleen), and abdominal fat were weighed and calculated as relative weight as a percentage to live body weight.

Table 2 Proximate composition and fatty acids contents of *Spirulina platensis*

Proximate composition, g.kg DM^{-1}	*Spirulina platensis*
Crude protein	565.0
Crude fat	10.5
Crude fiber	35.6
β-Carotene (vit. A), μg.kg DM^{-1}	982.4
Riboflavin (vit. B2), μg.kg DM^{-1}	24000
Fatty acids (FAs, %)	
Σ Saturated FAs	63.43
Σ Monounsaturated FAs	9.40
Σ Polyunsaturated FAs	26.06
Σ Unsaturated FAs	35.46
PUFAs/SFAs	0.41
UFAs/SFAs	0.56
MUFAs/PUFAs	0.36

Blood biochemistry

At the time of slaughter, blood samples were collected using non-heparinized sterile tubes. The samples were then centrifuged at 3400×*g* for 9 min, and the serum was separated into Eppendorf tubes and stored at -20 °C until further biochemical analysis. Serum contents of total protein, albumin, glucose, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), uric acid, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and alkaline phosphates enzyme (ALP) were measured with a spectrophotometer (Shimadzu UV 1601) using commercial kits produced by Stanbio Laboratory (Boerne, Texas, USA). Serum triiodothyronine (T3) hormone concentration was measured by radioimmunoassay with a kit which was produced by the Institute of Isotopes Co., Ltd. (Budapest, Hungary), and the samples were counted on Packard Gamma Counter (Perkin-Elmer Inc., Branford, CT, USA). Assays of malondialdehyde (MDA), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were performed using commercial kits (Spinreact Co. Girona, Spain). The antibody titers to NDV, AVI, and IBD were determined using a hemagglutination inhibition test enzyme-linked immune sorbent (ELISA) assay (Indical Bioscience GmbH, Leipzig, Germany) as described by [40]. Plasma IgA, IgM, and IgG concentrations were determined using chicken-specific IgA, IgM, and IgG ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, TX, USA).

Statistical analysis

Data of this study for all variables were statistically subjected to ANOVA as a completely randomized design using the SPSS software procedure (version 18.0, SPSS Inc., Chicago, IL, USA). Differences among means were assessed using Duncan's multiple range test (Duncan, 1955). The statistical model used in the analysis was as follows: $Y_{ijk} = \mu + O_i + D_j + OD_{ij} + e_{ijk}$, where Y_{ijk} = an observation on the experimental unit; O_i = the effect of SP dietary supplementation; D_j = the effect of SeNPs dietary supplementation; OD_{ij} = the interaction effect of SP dietary supplementation with the SeNPs dietary supplementation; and e_{ijk} = the residual error.

Results

Growth performance

LBW and BWG are shown in Table 3. On day 35, significant differences ($p < 0.001$) in LBW were found between birds fed supplemented diets and those fed control diet. LBW was increased gradually with increasing dietary levels of SP or SeNPs. The results showed that birds fed diet supplemented with SP plus SeNPs at levels of 10 g/kg and 0.1 mg/kg, respectively, had a higher LBW ($p = 0.005$) than other groups. During the entire rearing period, diet supplements significantly ($p < 0.001$) increased BWG compared with the control diet. In both periods from 22 to 35 days and 1 to 35 days, gradually increase in BWG was observed with increasing incorporated levels of SP or SeNPs. Comparing to all treatments, diet supplementation with 10 g/kg SP in combination with 0.1 mg/kg SeNPs showed the highest values of BWG ($p = 0.002$ and $p = 0.005$) during periods from 22 to 35 days and 1 to 35 days, respectively.

Effect of diet supplements on daily FI and FCR is presented in Table 3. Between days 1 and 21 on treatment, daily FI and FCR were significantly decreased in all treatment groups compared to the control one. These differences were maintained until day 35 but with no significance, although birds fed supplemented diet with10 g/kg SP and 0.1 mg/kg SeNPs in a combination had the numerically lower FCR compared with those fed other diets.

Carcass characteristics

Effect of diet supplements on carcass characteristics and relative weights of lymphatic organs are summarized in Table 4. Diet supplements significantly increased dressing percentage ($p < 0.001$) and carcass yield ($p = 0.001$) and decreased numerically ($p = 0.843$) abdominal fat of heat-stressed broiler chickens at market age compared to control diet. Birds in 5 g SP and 0.1 mg SeNPs group had the highest percentage values of dressing and carcass yield, followed by those in 10 g SP group. The relative weights of the liver, heart, and gizzard were not significantly influenced by dietary treatments.

Serum metabolites

There were no significant differences in serum concentrations of total protein, albumin, uric acid, creatinine, AST, ALT, and ALP as affected by the dietary supplements (Table 5).

Lipid profile

The data shown in Table 6 present the effect of diet supplements on lipid profile and thyroid activity. Dietary supplementation with SP and SeNPs significantly ($p = 0.008$) decreased cholesterol and LDL levels compared to control diet. The lowest blood cholesterol values were observed in birds fed 5 g SP and 0.1 mg SeNPs (160.7 mg. dl^{-1}), followed by those fed 10 g SP and 0.1 mg SeNPs (161.0 mg. dl^{-1}). Birds

Table 3 Effects of dietary supplementation of *Spirulina platensis* (SP) and selenium nanoparticles (SeNPs) on body weight, weight gain, feed intake, and feed conversion ratio of heat-stressed broiler chickens

Treatment		Live body weight (g)			Body weight gain (g/bird/day)			Daily feed intake (g/bird/day)			Feed conversion ratio (g feed/g gain)		
SP (g/kg)	SeNPs (mg/kg)	Initial weight	21 day	35 day	1–21 days	22–35 days	1–35 days	1–21 days	22–35 days	1–35 days	1–21 days	22–35 days	1–35 days
0	0	40.22	812.7	1812.2^{e}	36.78	71.39^{c}	50.63^{e}	46.92^{a}	155.6	90.39	1.28^{a}	2.18	1.79
0	0.1	40.18	816.3	1850.7^{d}	36.96	73.88^{b}	51.73^{d}	44.73^{c}	156.1	89.27	1.21^{bcd}	2.11	1.73
0	0.2	40.05	814.5	1863.7^{cd}	36.88	73.94^{ab}	52.10^{cd}	45.05^{bc}	156.4	89.57	1.22^{b}	2.09	1.72
5	0	40.72	825.2	1863.7^{cd}	37.36	74.18^{b}	52.08^{cd}	45.57^{b}	153.6	88.79	1.22^{bc}	2.07	1.71
5	0.1	39.84	835.0	1876.2^{bc}	37.86	74.40^{b}	52.47^{bc}	45.33^{bc}	154.7	89.09	1.20^{cd}	2.08	1.70
5	0.2	40.25	832.2	1882.2^{bc}	37.71	75.00^{ab}	52.63^{bc}	44.76^{c}	154.9	88.82	1.19^{d}	2.07	1.69
10	0	40.39	837.5	1891.5^{b}	37.96	75.29^{ab}	52.89^{b}	45.48^{b}	154.7	89.17	1.23^{cd}	2.06	1.69
10	0.1	40.13	842.8	1913.5^{a}	38.22	76.48^{a}	53.53^{a}	45.76^{b}	155.7	89.74	1.20^{cd}	2.04	1.68
10	0.2	40.10	847.0	1890.2^{b}	38.42	74.51^{b}	52.86^{b}	45.59^{b}	154.5	89.13	1.20^{d}	2.07	1.69
SEM		0.044	1.879	4.316	0.089	0.244	0.123	0.109	0.646	0.281	0.004	0.011	0.007
Source of variation, *p* value													
SP level		0.355	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.079	0.634	0.515	< 0.001	0.019	0.001
SeNPs level		0.467	0.019	< 0.001	0.012	0.009	< 0.001	< 0.001	0.874	0.927	< 0.001	0.503	0.134
SP level ×SeNPs level		0.349	0.565	0.005	0.502	0.002	0.005	< 0.001	0.994	0.899	0.002	0.381	0.364

Means within a column not sharing a common superscript differ ($p < 0.05$); *SEM* standard error of means

Table 4 Effects of dietary supplementation of *Spirulina platensis* (SP) and selenium nanoparticles (SeNPs) on carcass characteristics of heat-stressed broiler chickens

Treatment		Dressing (%)	Liver (%)	Heart (%)	Gizzard (%)	Abdominal fat (%)	Carcass yield (%)	Thymus (%)	Spleen (%)	Bursa of Fabricius (%)
SP (g/kg)	SeNPs (mg/kg)									
0	0	69.77^{e}	1.796	0.389	1.852	0.500	73.81^{f}	0.148	0.149	0.148
0	0.1	69.54^{e}	1.861	0.398	1.843	0.452	73.64^{f}	0.199	0.163	0.145
0	0.2	69.94^{de}	1.814	0.413	1.868	0.449	74.03^{ef}	0.180	0.180	0.179
5	0	71.18^{bc}	1.931	0.397	1.769	0.451	75.27^{bc}	0.180	0.179	0.126
5	0.1	71.88^{a}	1.834	0.396	1.762	0.414	75.87^{a}	0.216	0.198	0.126
5	0.2	70.37^{d}	1.932	0.372	1.825	0.426	74.50^{de}	0.231	0.177	0.177
10	0	71.54^{ab}	1.958	0.413	1.761	0.413	75.67^{ab}	0.162	0.216	0.162
10	0.1	71.11^{bc}	1.946	0.403	1.772	0.386	75.23^{bc}	0.193	0.228	0.158
10	0.2	70.88^{c}	1.908	0.389	1.838	0.407	75.01^{cd}	0.212	0.194	0.177
SEM		0.160	0.017	0.006	0.017	0.008	0.159	0.008	0.007	0.006
Source of variation, p value										
SP level		< 0.001	0.022	0.631	0.226	0.002	< 0.001	0.234	0.018	0.260
SeNPs level		0.007	0.921	0.831	0.410	0.066	0.058	0.065	0.601	0.028
SP level ×SeNPs level		< 0.001	0.371	0.647	0.982	0.843	0.001	0.937	0.629	0.819

Means within a column not sharing a common superscript differ ($p < 0.05$); *SEM* standard error of means

fed 0.1 mg SeNPs had significantly higher blood triglycerides and VLDL values than other treated birds without significant difference with those fed control birds. However, other treated birds had significantly lower blood triglycerides and VLDL levels compared to control birds. Birds fed 0.2 SeNPs had the lowest levels of both triglycerides ($p < 0.001$) and VLDL ($p = 0.001$) compared to those fed other diets. Supplementing different levels of dietary SP and SeNPs had no significant effect on HDL levels. However, there is a numerical increase with dietary supplementation, whereas HDL level of birds fed both 10 g SP and 0.2 mg SeNPs was increased by 19.45% compared to those fed control diet.

Table 5 Effects of dietary supplementation of *Spirulina platensis* (SP) and selenium nanoparticles (SeNPs) on serum metabolites of heat-stressed broiler chickens

Treatment		T. Proteins (g.dl^{-1})	Albumin (g.dl^{-1})	AST (U.L^{-1})	ALT (U.L^{-1})	ALP (U.L^{-1})	Uric acid (mg.dl^{-1})	Creatinine (mg.dl^{-1})
SP (g/kg)	SeNPs (mg/kg)							
0	0	3.30	1.46	56.20	23.15	174.6	6.84	0.513
0	0.1	3.28	1.50	64.60	21.05	147.1	6.42	0.497
0	0.2	3.36	1.42	61.78	21.61	174.7	6.65	0.658
5	0	3.38	1.41	70.72	18.96	148.5	6.27	0.478
5	0.1	3.16	1.56	73.28	21.89	172.1	6.13	0.613
5	0.2	3.21	1.54	56.23	24.29	141.5	6.63	0.537
10	0	3.00	1.38	69.09	14.52	188.8	5.54	0.623
10	0.1	3.33	1.55	55.50	21.86	178.3	6.75	0.443
10	0.2	3.20	1.54	64.43	23.19	159.9	5.96	0.593
SEM		0.032	0.027	0.891	2.507	4.989	0.018	0.152
Source of variation, p value								
SP level		0.161	0.810	0.658	0.560	0.206	0.375	0.927
SeNPs level		0.878	0.256	0.760	0.159	0.596	0.830	0.107
SP level ×SeNPs level		0.052	0.801	0.398	0.315	0.241	0.428	0.053

Means within a column not sharing a common superscript differ ($p < 0.05$); *SEM* standard error of means

Table 6 Effects of dietary supplementation of *Spirulina platensis* (SP) and selenium nanoparticles (SeNPs) on lipid profile and thyroid activity of heat-stressed broiler chickens

Treatment		Cholesterol ($mg.dl^{-1}$)	Triglycerides ($mg.dl^{-1}$)	HDL—cholesterol ($mg.dl^{-1}$)	LDL—cholesterol ($mg.dl^{-1}$)	VLDL—cholesterol ($mg.dl^{-1}$)	Glucose ($mg.dl^{-1}$)	T3 ($ng.ml^{-1}$)
SP (g/kg)	SeNPs (mg/kg)							
0	0	241.0^{a}	252.9^{a}	41.96	148.4^{a}	50.59^{a}	221.7^{cd}	1.08^{d}
0	0.1	174.0^{d}	254.0^{a}	45.99	77.18^{cd}	50.80^{a}	265.9^{a}	1.72^{ab}
0	0.2	197.7^{c}	201.5^{e}	46.14	111.3^{b}	40.31^{e}	219.2^{cd}	1.38^{bcd}
5	0	198.1^{c}	236.0^{b}	45.42	105.5^{b}	47.19^{b}	211.2^{d}	1.03^{d}
5	0.1	160.7^{e}	204.1^{de}	44.10	75.78^{d}	40.82^{de}	225.4^{bcd}	1.48^{bc}
5	0.2	177.7^{d}	210.2^{cd}	47.05	88.57^{c}	42.04^{cd}	258.3^{a}	1.91^{a}
10	0	210.8^{b}	232.7^{b}	47.48	116.8^{b}	46.54^{b}	253.5^{ab}	1.31^{cd}
10	0.1	161.0^{e}	213.0^{c}	48.81	69.59^{d}	42.61^{c}	218.8^{cd}	1.31^{cd}
10	0.2	177.2^{d}	217.3^{c}	52.09	81.67^{cd}	43.45^{c}	245.7^{abc}	1.34^{cd}
SEM		4.909	3.768	0.626	4.838	0.754	4.548	0.061
Source of variation, p value								
SP level		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.605	0.292
SeNPs level		< 0.001	< 0.001	0.008	< 0.001	< 0.001	0.287	0.001
SP level ×SeNPs level		0.008	< 0.001	0.253	0.001	< 0.001	< 0.001	0.005

Means within a column not sharing a common superscript differ ($p < 0.05$); *SEM* standard error of means

Glucose and thyroid activity

Heat-stressed birds fed dietary 0.1 mg SeNPs had a significantly ($p < 0.001$) higher glucose level than all other treatments, followed by those fed 5 g SP and 0.2 mg SeNPs (Table 6). Diet supplements significantly ($p = 0.005$) affected T3 levels in blood of heat-stressed broiler chickens. Supplementation of SP alone at level 5 g/kg diet reduced levels of glucose and T3. However, birds received dietary 5 g/kg SP plus 0.2 mg/kg SeNPs had a higher level of blood T3 followed by those received dietary 0.1 mg/kg SeNPs.

Immune function

Effect of dietary supplements on immune function of heat-stressed broiler chicken is summarized in Table 7. Comparing to control group, a significant increase for serum IgG level ($p < 0.001$) was observed with birds fed supplemented diets except for those fed 5 g/kg SP. Birds fed both 10 g/kg SP and 0.1 mg/kg SeNPs having the highest IgG level than birds fed other diets. No difference was found for serum IgM level between birds fed 0.1 mg/kg SeNPs and control group ($p > 0.05$). However, other treated groups had increased IgM level ($p < 0.001$), whereas the highest levels were observed with dietary 10 g/kg SP plus 0.2 mg/kg SeNPs and dietary 10 g/kg SP plus 0.1 mg/kg SeNPs, respectively. There was a significant increase for serum IgA level ($p < 0.001$) with dietary supplementation compared to control group. Diet supplements numerically increased HA titers against NDV, AIV, and IBD compared to the much lower values in control group.

Lymphatic organs

There were no significant differences between the relative weight of lymphatic organs, though numerical increases were observed with feeding on supplemented diets compared to control diet (Table 4). Diet supplementation with 5 g/kg SP and 0.2 mg/kg SeNPs increased the relative weight of thymus by 35.93% compared to the control diet. The relative weight of the spleen also increased by 34.65% in birds fed diet supplemented with 10 g/kg SP and 0.1 mg/kg SeNPs compared to those fed control diet. Besides, birds received diet supplemented with 0.2 mg/kg SeNPs exhibited increased relative weight of bursa of Fabricius by 17.39% compared to those received control diet.

Antioxidant status

Antioxidant activity of SP and SeNPs was evaluated by estimating blood SOD, MDA, and GPx, as shown in Table 8. Birds fed diet supplemented with 5 g SP and 0.2 mg SeNPs exhibited higher ($p < 0.001$) levels of SOD and GPx and lower ($p < 0.001$) level of MDA compared to those fed other diets. Interestingly, dietary 5 g SP and 0.2 SeNPs increased GPx level by 64.18% and decreased MDA level by 63.35%in blood

Table 7 Effects of dietary supplementation of *Spirulina platensis* (SP) and selenium nanoparticles (SeNPs) on immune responses of heat-stressed broiler chickens at 35 day of age

Treatment		IgG (mg.dl^{-1})	IgM (mg.dl^{-1})	IgA (mg.dl^{-1})	Antibody titer against[1]		
SP (g/kg)	SeNPs (mg/kg)				NDV	AIV	IBD
0	0	334.5^{f}	42.00^{d}	95.50^{d}	4.00	3.50	267.0
0	0.1	544.0^{d}	62.50^{d}	264.8^{a}	6.67	4.17	308.0
0	0.2	550.0^{d}	113.5^{c}	261.3^{a}	6.33	4.83	302.7
5	0	362.0^{f}	117.5^{c}	155.0^{c}	7.00	4.33	303.0
5	0.1	676.7^{c}	164.0^{b}	177.5^{c}	8.00	5.00	324.0
5	0.2	960.8^{b}	237.2^{a}	299.5^{a}	7.67	5.00	337.3
10	0	474.5^{e}	147.7^{b}	222.0^{b}	6.67	4.33	308.3
10	0.1	1065.5^{a}	243.0^{a}	284.6^{a}	8.00	5.00	333.0
10	0.2	457.3^{e}	244.1^{a}	175.5^{c}	7.67	5.33	325.3
SEM		47.45	14.34	13.13	0.294	0.230	5.213
Source of variation, p value							
SP level		< 0.001	< 0.001	0.141	0.004	0.450	0.012
SeNPs level		< 0.001	< 0.001	< 0.001	0.015	0.265	0.013
SP level ×SeNPs level		< 0.001	< 0.001	< 0.001	0.667	0.991	0.814

Means within a column not sharing a common superscript differ ($p < 0.05$); *SEM* standard error of means

[1] *NDV* Newcastle disease, *AIV* Avian influenza (H9N1), *IBD* infectious bursal disease

of heat-stressed broiler chickens compared to control diet. Unlike supplementation with SeNPs, dietary spirulina improved antioxidant enzyme activity in a dose-dependent manner.

Discussion

Although many studies have investigated the importance of dietary supplementation of spirulina or selenium in broiler chickens, to our knowledge, our study is the first to illustrate the synergistic effect of spirulina and selenium on the performance of heat-stressed broiler chickens. Spirulina has previously been used as a dietary ingredient and a functional dietary component due to its high nutritional value (Table 2) and functional properties [25, 41]. Over the last years, dietary supplementation with different spirulina levels up to 200 g/kg [42] has been investigated, with greatly conflicting findings in terms of its effect on growth performance. The effect of selenium on growth performance in broiler chickens is also somewhat variable.

Table 8 Effects of dietary supplementation of *Spirulina platensis* (SP) and selenium nanoparticles (SeNPs) on antioxidative capacity of heat-stressed broiler chickens

Treatment		MDA (nmol.ml^{-1})	SOD (U.ml^{-1})	GPx (U.ml^{-1})
SP (g/kg)	SeNPs (mg/kg)			
0	0	1.255^{a}	110.5^{f}	12.00^{f}
0	0.1	0.775^{e}	130.3^{cd}	26.25^{cd}
0	0.2	0.850^{de}	127.4^{d}	23.50^{d}
5	0	1.088^{b}	110.0^{f}	18.80^{e}
5	0.1	0.630^{f}	131.0^{bc}	28.13^{bc}
5	0.2	0.460^{g}	135.8^{a}	33.50^{a}
10	0	0.875^{cd}	122.5^{e}	23.88^{d}
10	0.1	0.665^{f}	133.6^{ab}	30.75^{ab}
10	0.2	0.945^{c}	128.1^{cd}	20.00^{e}
SEM		0.046	1.763	1.246
Source of variation, p value				
SP level		< 0.001	< 0.001	< 0.001
SeNPs level		< 0.001	< 0.001	< 0.001
SP level ×SeNPs level		< 0.001	< 0.001	< 0.001

Means in the same column within each classification bearing different letters are significantly different. *SEM* standard error of means, *MDA* malondialdehyde, *SOD* superoxide dismutase, *GPx* glutathione peroxidase

An initial objective of the current study was to identify the effect of treatments duration on the performance of heat-stressed broiler. Numerous studies have reported the promoter function of *S. platensis* and selenium on growth performance in broiler chickens [41, 43–45]. In the current study, on day 35, dietary supplementation with different levels of SeNPs, SP, or their combination significantly improved LBW and BWG of heat-stressed broiler chicken compared to control diet. However, until 21 days of age, there were no significant differences between treated groups and control one. However, mechanisms by which dietary spirulina and selenium enhance growth performance have not been established. These results indicate the importance of the treatment duration that can affect the response to the dietary supplements. In this study, dietary supplements did not negatively affect FI at all experimental periods except on day 21; however, the FCR was better for group fed dietary supplements than the control group. The impaired growth performance of heat-stressed birds [46] may be attributed partially to the evidence that stressed birds use more energy to adapt with the environmental stressor while less energy has been used for growth [47], whereas the improved growth performance of birds fed supplemented diets may due to the positive effect of dietary supplements on feed utilization and nutrient absorption [41, 43]. Another possible explanation for this is that spirulina contains adequate metabolizable energy content [42] and a complete protein with all essential amino acids [41] that might be superior to most vegetable ingredients (Table 2). Besides, amino acids of Spirulina protein are highly digestible [42]. Furthermore, the physiological functions of bioactive substances in spirulina such as phenolic compounds, carotenoids, vitamins, minerals, and others [48] may be involved in the positive effect of spirulina on growth performance of heat-stressed broiler chickens. Selenium is required for the expression and synthesis of about 25 selenoproteins [49] involved in cell protection against stress-induced damage. The exposure to heat stress was found to up-regulate several selenoproteins' expression as a response to the heat stress damage to development of skeletal muscle myotubes [50]. Among these selenoproteins, selenoprotein N1 (SEPN1) involved in skeletal muscle development. Skeletal muscle is the main edible part of broiler chickens, and the impairment of its development mainly affects animal growth performance. Thus, selenium can maintain and improve growth performance via alleviating the adverse effects of heat stress on the developing skeletal muscle.

A number of recent studies have demonstrated the hypolipidemic activity of SP and SeNPs in laboratory animals and chickens [51, 52] which may be due to the ability of these supplements to reduce absorption and synthesis of cholesterol in the host gut. Additionally, the antioxidant activity of the polyphenolic compounds [53] in SP may reduce the level of blood lipids in supplemented birds via inhibit the pancreatic lipase activity [54]. In the current study, our results revealed a significant reduction in blood cholesterol, triglyceride and LDL-cholesterol in birds fed diets supplemented with SP, SeNPs, or their combinations. Additionally, treated diets except for that supplemented with 0.1 mg SeNPs, significantly reduced levels of blood VLDL-cholesterol compared to control diet. The numerically increased serum HDL-cholesterol was also observed in birds fed supplemented diets compared to those fed control diet. In general, these results agree with previous studies [51, 52, 55].

Selenoproteins play a crucial role in metabolism of thyroid hormones and mediate the conversion of tetraiodothyronine (T4) to triiodothyronine (T3). Selenium deficiency reduces mRNA expression levels of selenoproteins and blocks the conversion of T4 to T3 in chicken thyroids [56]. Our results showed that levels of serum glucose and T3 were found to differ significantly among examined groups; birds fed SeNPs had higher level of T3 compared to those fed control diet. However, birds fed both low level of SP and high level of SeNPs had the higher T3 level compared to all other groups, while diet supplementation with 0.1 mg SeNPs singly or dietary combination of 0.2 mg SeNPs and 5 g SP/kg showed the highest levels of glucose.

The current study showed the positive effect of dietary treatments on carcass yield and dressing percentage. This finding may be attributed to the improved growth performance of supplemented birds. These results agree with those of previous studies [57–59].

Genetic selection for rapid growth rate makes broiler chickens more vulnerable to oxidative stress. Moreover, broiler production under high temperature conditions leads to oxidative stress, with both situations leading to biological damage, multiple pathological disorders, and impaired growth performance [3, 8, 60, 61]. Heat stress leads to the accumulation of superoxide anions within mitochondria, resulting in oxidative stress and, consequently, increasing MDA levels, an indicator of lipid peroxidation [19]. Spirulina and/or selenium are known to improve antioxidant activity particularly by increasing blood SOD which involved in conversion of superoxide anions to hydrogen peroxide. Moreover, spirulina and selenium are able to increase levels of blood GPx that catalyze the reduction of harmful hydrogen peroxide to water or the corresponding alcohols [43, 49, 62]. In current study, GPx and SOD significantly increased, and MDA decreased with dietary supplementation. Reduced level of MDA indicates that the cell was not in an oxidative stress condition. Growing studies demonstrated that Spirulina is a rich source of natural antioxidants (e.g., β-carotene, selenium, polypeptide pigment, tocopherol, and phenolic compounds). For example, several studies reported the potent antioxidant effect of phycocyanin, one of the major pigments in spirulina microalgae [43]. On the other hand, previous research has shown that both enzymatic and non-enzymatic antioxidant defense mechanisms are positively affected by selenium-supplemented diets [49].

Selenium contributes to synthesis and expression of selenoproteins including GPx1 and GPx4. Interestingly, selenium utilization by GPx4 provides exquisite resistance to reversible and irreversible over oxidation [63]. Additionally, majority of selenoproteins play an essential role in physiological functions of enzymatic redox reactions and glutathione peroxidases. All antioxidants in vivo work together through a dramatical mechanism to establish strong antioxidant system. According to this hypothesis, both SP and SeNPs synergistically improve the antioxidant status of heat-stressed birds. Hence, in our study, the combined dietary supplementation of 5 g SP and 0.2 mg SeNPs showed the highest SOD and GPx and the lowest value of MDA compared to other diets, indicating a synergistic effect of both supplements.

Heat stress has been associated with abnormalities in immune functions. Additionally, a growing body of evidence suggests that humoral antibody response to different viral antigens do not elevate as required following immunization with vaccines in birds reared under heat stress condition [12, 64]. In addition, several reports have demonstrated that broiler chickens exposed to heat stress had lower levels of IgA, IgM, and IgG [47, 65]. In this study, serum immunoglobulin subclasses IgG, IgA, and IgM and antibody titers against NDV, AIV, and IBD were not increased following vaccination in heat-stressed birds fed unsupplemented diet. However, birds fed the unsupplemented diet had the lowest values of Ig subclasses and HA titers against viral diseases. These results indicate that immunization of heat-stressed broiler chickens adversely affects the production of antigen-specific antibody. Moreover, IgG, secreted by B cells, is the major immunoglobulin subclass and plays a vital role inactivating multiple immune effector systems, including neutralization of viruses. The balance between anti-inflammatory and pro-inflammatory cytokines acts as an essential factor in immune responses. A large and growing body of literature has demonstrated that heat stress induces inflammatory and oxidative stress [12, 47]. Inflammatory and oxidative stress decreases initiation of specific immune responses by revoking antigen processing and antigen-presenting cells potential to stimulate T cells [66]. These findings may be attributed to inflammation or oxidative stress caused by heat stress, which suppresses specific immune responses and antibody titers [46]. On the other hand, exposure to heat stress negatively affects relative weight of lymphoid organs [64]. Results of this study indicate that decreased relative weight of lymphoid organs of heat-stressed birds may be correlated to poor growth performance or due to heat stress-induced dysfunctions such as impaired immune responses or oxidative stress [46]. Hirakawa et al. [12] reported a strong correlation between weight of lymphatic organs and live body weight of birds reared under thermoneutral condition; however, they could not detect this correlation under heat stress condition. One of the aims of this study was to evaluate dietary supplementation with SP and SeNPs on immune responses and the relative weight of major lymphoid organs. Our results indicate that dietary supplementation with SP and SeNPs can alleviate the negative effect of heat stress on immune system and have the potential to enhance immune responses in heat-stressed broiler chickens particularly when birds fed the combined dietary supplementation which indicate the synergistic effect of both supplements. This finding is consistent with previous studies that have suggested that SP and SeNPs enhance immune responses of the broiler chickens [44, 67–70]. A possible explanation for this might be the functional properties of spirulina and selenium such as anti-inflammatory and antioxidant activities.

Conclusion

The addition of SP, SeNPs, or their combinations, particularly at levels 5 g SP plus 0.2 mg SeNPs kg^{-1} and 10 g SP plus 0.1 mg SeNPs kg^{-1}, improved growth performance, carcass yield, immune function, and antioxidant status of broiler chickens which fed a corn-soybean-based basal diet and grown under heat stress condition. The results also showed that birds derived greater benefits from diet supplements when they provided in a combination due to synergistic effects between them. These results suggest the potential use of SP and SeNPs in diets of broiler chickens for alleviating the undesirable effects of heat stress and to improve the productive performance.

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Declarations

Competing interests The authors declare no competing interests.

Ethics approval and consent to participate Animal care and experimental protocol were reviewed and approved by the Institutional Animal Care and Research Ethics Committee.

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