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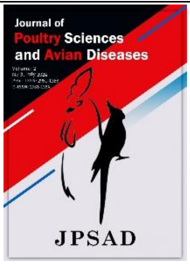

The effect of protein and selenium on broiler performance and blood parameters in summer conditions

Ahmad Mohebbifar[1], Abdolreza Kamyab[2], Shadi Sedgh-Gooya[1], Mehran Torki[1*]

[1] Department of Animal Science, College of Agriculture and Natural Resources, Razi University, Imam Avenue, PO Box 6715685423, Kermanshah, Iran
[2] CEO and Owner at ImEx Gulf, Inc. Plano, Texas, United States

*** Corresponding author email address**: torki@razi.ac.ir

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A B S T R A C T

Heat stress presents a substantial challenge for the poultry industry, negatively impacting broilers' health, growth, and productivity. Optimizing dietary crude protein (CP) levels is a viable approach to reduce the heat generated during birds' amino acid oxidation process. Additionally, supplementing diets with Selenium (Se), a potent antioxidant, can help counteract the detrimental effects of heat stress on broiler growth performance. To explore the effects of varying CP and Se levels on growth performance, carcass characteristics, blood parameters, immune function, and blood leukocyte profiles in broiler chickens under heat stress, a 42-day experiment was conducted. It involved 528 male broiler chicks (Ross 308), divided into six groups with 11 chicks each, fed diets with 100%, 94%, or 88% of the recommended CP levels, and two Se levels (0 and 0.04 mg/kg). The findings indicated that reducing dietary CP levels led to an improvement in daily weight gain (DWG) without affecting feed intake (FI), feed conversion ratio (FCR), or carcass traits. There was a significant interaction between CP and Se levels regarding glucose, albumin, uric acid, cholesterol, triglyceride levels, antibody titers against Newcastle disease virus (NDV), and monocyte counts. Se supplementation increased NDV antibody titers at 14 days, whereas reducing CP lowered these titers at 21 days. The study concluded that lowering dietary CP did not adversely affect growth performance, carcass quality, or blood parameters; it even enhanced DWG compared to a normal protein diet and influenced some blood parameters.

Keywords: *Broiler chickens, Crude protein level, Growth performance, Heat stress, Immune response, selenium.*

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1 Introduction

Broilers, chickens explicitly bred for meat production, play a vital role in the worldwide food sector. Nevertheless, escalating temperatures from climate change pose challenges in preserving their health and optimal productivity. The poultry industry faces a considerable hurdle with heat stress, which detrimentally influences these birds' well-being, growth, and output. This issue transcends animal welfare, impacting the entire supply chain from farmers to consumers and affecting the broader industry (1). Broiler chickens, engineered for optimal meat yield, are particularly vulnerable to elevated temperatures. Their susceptibility is rooted in the fast growth rates achieved through selective breeding and their limited capacity for heat dissipation due to their feather coverage and lack of sweat glands.

Furthermore, the common practice of housing them in crowded conditions within commercial farms intensifies the heat stress they endure (2, 3). High temperatures trigger critical physiological and metabolic adjustments. Moreover, extended periods of heat stress can cause marked reductions in bird performance, negatively impact animal welfare, present obstacles to food safety, and lower the economic efficiency of poultry production (2, 4). Two strategies are pivotal to mitigating elevated temperatures' detrimental impacts on poultry: minimizing heat generation and enhancing heat dissipation. Compared to carbohydrates and fats, proteins are recognized for their higher thermal effect, contributing to an increase in diet-induced thermogenesis (5). In birds, the bulk of heat production during energy metabolism comes from deamination reactions and the conversion of nitrogen into uric acid. (6, 7). However, optimizing dietary crude protein (CP) composition can help decrease the heat produced during amino acid oxidation in birds (8). It has been suggested that decreasing dietary CP can mitigate the harmful effects of heat stress in poultry. In a study, feeding the low CP diet during the starter phase resulted in feed intake (FI), body weight gain (BWG), feed conversion ratios (FCR), and energy efficiency ratios (EER) similar to those for the normal CP diet (9). However, decreasing CP below certain thresholds could adversely impact FI and growth rates (10, 11).

Selenium (Se) is an essential trace mineral in livestock production. It is a crucial constituent of at least 25 selenoproteins (SELs), plays a significant role in thyroid hormone production, and is integral to the antioxidant defense mechanism (12, 13). Research indicates that supplementing broiler diets with Selenium (Se) can mitigate the negative impacts of heat stress on their growth performance (14-16), thyroid hormone metabolism, cellular antioxidant levels, and immune system responses (17). Additionally, Se aids in more efficient body temperature regulation in livestock (18). Given the considerations mentioned earlier, this research aimed to examine how different dietary crude protein (CP) levels and the concurrent intake of Se under conditions of heat stress affect functional attributes, immune system performance, and blood hormone concentrations.

2 Materials and methods

2.1 Birds and housing

A total of 528 male broiler chicks (Ross 308), ten days old, were weighed and evenly distributed into six groups in a randomized manner. Each group consisted of eight subgroups, with 11 chicks each, arranged in a 3 × 2 factorial design. For 42 days, the chicks were housed in metal battery cages, with each battery containing four cages (dimensions: 127×87×45 cm) under conditions of high temperature and humidity. These cages were placed in a building with open sides, allowing environmental conditions to fluctuate naturally with the external summer climate. The indoor temperature exceeded 31°C due to the summer heat, eliminating the need for artificial heating. Ambient temperatures during the day fluctuated between 26°C and 36°C (average 31±0.9°C), with relative humidity levels ranging from 77% to 88% (average 79±4%RH). A constant ventilation rate of 0.12 m/s was maintained throughout the experiment. The lighting was controlled to provide continuous illumination at approximately 20 lux. Chickens had unlimited access to feed and water for the trial, and vaccinations were administered according to the local standard schedule, considering the optimal period for maternal antibody levels. No medications were given during the study.

2.2 Diets

The research tested three different crude protein (CP) levels—100%, 94%, and 88%—alongside two selenium (Se) concentrations (0 and 0.04 mg/kg diet from sodium selenite) across two phases: days 10-24 and 25-42. These treatments were distributed in a 3 × 2 factorial design. The specific CP levels chosen were designed to meet 100%, 94%, and 88% of the Ross 308 recommended levels, equating to 21%, 19.74%, and 18.48% for the starter phase, and 18.00%, 16.92%, and 15.84% for the grower phase, respectively. The NRC (19) regression models were applied to estimate the metabolizable energy contents of corn and soybean meal. The basal diet was formulated to meet the nutrient requirements of the broiler chickens, as recommended by the Ross 308 broiler management guide (20). Table 1 shows the ingredients and chemical composition of the experimental diets.

Table 1. Ingredient and nutrient composition of experimental diets

Age, day	Starter			Grower		
	10-24			25-42		
	CP (%)			CP (%)		
	100	94	88	100	94	88
Ingredient composition (%)						
Corn	55.77	57.63	59.19	65.59	67.14	69.04
Soybean meal	37.44	33.14	30.56	28.71	24.92	21.76
Soybean oil	2.77	2.77	2.77	1.76	1.76	1.76
Wheat Bran	----	2.00	2.00	----	2.03	2.03
Oyster Shells	0.94	0.95	0.95	0.92	0.94	0.94
Dicalcium phosphate	1.96	1.97	2.00	1.85	1.86	1.89
Common salt	0.31	0.31	0.31	0.31	0.31	0.31
NaHco3(S.Bicar)	0.10	0.11	0.11	0.08	0.08	0.09
Vit. & Min. Premix[a]	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.21	0.24	0.27	0.19	0.22	0.26
Lysine-HCL	----	0.13	0.22	0.08	0.20	0.31
L-Threonine	----	0.06	0.10	---	0.05	0.10
Filler	----	0.18	1.10	---	---	1.02
Nutrient composition (as fed basis)						
ME (Kcal/kg)	2950	2950	2950	3000	3000	3000
Crude protein (%)	21.00	19.74	18.48	18.00	16.92	15.84
Calcium (%)	0.90	0.90	0.90	0.85	0.85	0.85
Available phosphorus (%)	0.45	0.45	0.45	0.42	0.42	0.42
Sodium (%)	0.17	0.17	0.17	0.16	0.16	0.16
Chloride	0.22	0.22	0.22	0.22	0.22	0.22
Lysine (%)	1.12	1.12	1.12	0.97	0.97	0.97
Methionine + Cystine (%)	0.85	0.85	0.85	0.76	0.76	0.76
Threonine (%)	0.79	0.79	0.79	0.66	0.66	0.66

[a]Mineral and vitamin premix provided per kilogram of diet: 80 mg Mn (from MnSO4·H2O), 70 mg Zn (from ZnO), 50 mg Fe (from FeSO4·7H2O), 8 mg Cu (from CuSO4·5H2O), 1.5 mg I (from Ca (IO3)2·H2O), and 0.35 mg Se (from Na selenite), 12,500 IU vitamin A (from retinyl acetate), 3,700 IU cholecalciferol, 40 IU vitamin E (from DL- α-tocopheryl acetate), 0.03 mg vitamin B12, 6.4 mg riboflavin, 55 mg niacin (as nicotine amide), 30 mg pantothenic acid (as calcium pantothenate), 3.5 mg menadione (from menadione dimethyl-pyrimidinol), 1.2 mg folic acid, 3 mg thiamine, 7.5 mg pyridoxine, 0.3 mg biotin, 560 mg choline (as choline chloride 60%), and 80 mg ethoxyquin.

2.3 Measurements

2.3.1 Growth performance

The broilers' weight (BW) was recorded at the beginning and conclusion of each phase (days 10, 24, and 42). Feed intake (FI) and feed conversion ratio (FCR) were calculated for each pen based on leftover feed and daily weight gain (DWG) data collected at the end of each phase and for the entire duration of the experiment (days 0-42). Mortality was tracked daily, and any deaths occurring during the experimental period were included in the overall data.

2.3.2 Chemical analysis of blood components and cortisol concentration

At 42 days of age, two chickens from each subgroup were randomly selected for blood sampling through the wing vein into collection tubes. The gathered blood samples were then spun at 3000 rpm (1008g) for 10 minutes, and the resulting sera were preserved at -20°C until needed for analysis. Once thawed to room temperature, serum samples were assessed for glucose, triglycerides, cholesterol, albumin, and uric acid levels using Pars Azmun kits in Tehran, Iran. In addition, before and after being subjected to heat stress conditions, two chickens from each subgroup were again chosen randomly, and blood was drawn from the wing vein. The serum cortisol levels were measured using ELISA kits, following the manufacturer's guidelines.

2.3.3 Assay for Antibody response

On day seven, all the chicks received an injection in the neck with the Newcastle disease virus (NDV) vaccine (Nobilis G + ND; Merck Animal Health). Blood samples were drawn from the broiler chickens' brachial veins on days

14, 21, and 42 following vaccination. The presence of NDV-specific antibodies in the chickens' serum was detected using the hemagglutination inhibition (HI) test and the enzyme-linked immunosorbent assay (ELISA).

2.3.4 *Blood leucocyte profiles*

At 42 days old, blood samples from twelve birds per dietary group were drawn into vials containing EDTA as an anticoagulant. These samples were utilized to prepare blood smears, which were then examined under a light microscope for differential leukocyte analysis. Following the method outlined by Lucas in 1961, 100 leukocytes from each sample were counted (21). The heterophile to lymphocyte (H/L) ratio was calculated based on these counts.

2.3.5 *Carcass traits*

On the 42nd day, 12 broilers from each group were randomly selected, weighed, and then humanely euthanized following Islamic slaughtering practices, which included skinning and removal of all internal organs. The birds underwent a feed withdrawal period of 6 hours prior to slaughter. The carcass weight, measured after removing internal organs, was expressed as a percentage of the bird's live weight, referred to as the dressing percentage. The weights of various parts, including the breast, legs, back, wings, abdominal fat, liver, pancreas, spleen, thymus, and bursa of Fabricius, were recorded and calculated as percentages of the live body weight. The lengths of the gastrointestinal segments—duodenum, jejunum, and ileum—were also estimated.

2.4 *Statistical analyses*

The GLM procedure of SAS (22) was used to conduct ANOVA on the data, which was arranged in a completely randomized design with a 3×2 factorial treatment arrangement. All statements of significance were based on a probability of less than 0.05. Duncan's multiple-range test was utilized to compare mean values (23). A model comprising Yijk = μ + (Ai) + (Bj) + (ABij) + (eijk) was used for analysis. Here, Yijk is the measured characteristic, μ is the overall mean, (Ai) is the main effect of protein levels, (Bj) is the main effect of Se, (ABij) is the interaction between protein level and Se, and (eijk) is the residual error. In case the interaction was notable, the impacts of the primary factors were not considered.

3 Results

Table 2 and Table 3 show how using Se affects the performance of broiler chickens under heat stress at different levels of CP in their diets. The experimental treatments did not significantly affect the FI and FCR of the broiler chickens between 10 to 24, 25 to 42, and 0 to 42 days (P > 0.05). Similarly, DWG and BW between 25 and 42 and the entire experimental period showed no significant differences (P > 0.05). However, the different levels of CP in the diet significantly affected the DWG and BW between 10 to 24 days (P < 0.05). Chickens fed with 88% and 94% CP had better DWG and BW than those fed with a 100% CP diet.

Table 2. Effect of protein level and selenium supplement on broiler performance

Productive performance		Daily weight gain (g/ chick/ day)			Body weight (g/chick)		
CP%		10-24	25-42	10-42	10-24	25-42	10-42
	100	29.32[b]	106.47	67.89	410.57b	2022.94	2046.58
	94	32.15[a]	112.15	72.15	450.17a	2131.24	2197.53
	88	32.02[a]	109.62	70.82	448.29a	2083.68	2117.47
Se (mg/kg)							
	0	31.31	109.02	70.16	438.37	2071.43	2099.68
	0.04	31.02	109.81	70.41	434.31	2087.14	2141.37
				P-value			
CP		0.035	0.314	0.147	0.035	0.312	0.090
Se		0.765	0.795	0.888	0.765	0.785	0.449
CP*Se		0.400	0.958	0.886	0.399	0.961	0.945

CP=Crude protein, Se=Selenium.

a–e Means with no common superscript within each column are significantly (P < 0.05) different.

Table 3. Effect of protein level and selenium supplement on broiler performance

Productive performance		Feed intake (g/ chick/ day)			FCR (g/g)		
CP%		10-24	25-42	10-42	10-24	25-42	10-42
	100	744.130	3027.96	2258.11	1.64	1.95	1.80
	94	775.45	3084.77	2317.84	1.59	1.90	1.74
	88	775.68	2955.48	2253.42	1.61	1.87	1.74
Se (mg/kg)							
	0	776.36	3006.23	2279.47	1.64	1.91	1.78
	0.04	753.81	3039.25	2273.44	1.59	1.90	1.74
				P-value			
CP		0.244	0.261	0.375	0.492	0.623	0.482
Se		0.201	0.606	0.884	0.130	0.872	0.416
CP*Se		0.130	0.276	0.273	0.718	0.480	0.813

CP=Crude protein, Se=Selenium.

a–e Means with no common superscript within each column are significantly (P < 0.05) different.

Table 4 shows the levels of certain substances in the blood for different treatments. The results suggest that the combination of selenium (Se) and different levels of CP has a significant effect on albumin, uric acid, glucose, triglyceride, and cholesterol levels (P<0.05). The treatment that included 100% CP and 0.04 mg/kg Se had the highest albumin levels, while the one with 94% CP and 0.04 mg/kg Se had the lowest. The treatment with 88% CP and 0% Se had the lowest uric acid levels, while the treatment with 94% CP and 0% Se had the highest. Broilers fed with 88% CP and 0.04 mg/kg had higher blood glucose levels than other treatments. The treatment with 88% CP and no Se had the highest concentration of blood triglycerides, whereas the one with 100% CP and no Se had the lowest. The treatment with 88% CP and 0.04 mg/kg had higher cholesterol levels than other treatments.

Table 4. Effect of protein level and selenium supplement on serum biochemical metabolites of broiler chickens

		Albumin (g/dl)	Uric acid (mg/dL)	Glucose (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)
CP%	100	6.43a	5.28a	148.94c	139.43b	1263.38a
	94	5.80b	5.60a	165.18b	184.54a	1132.26b
	88	6.28ab	4.86b	180.97a	184.03a	1365.65a
Se (mg/kg)	0	6.23	5.12	158.77b	177.97a	1279.65
	0.04	6.12	5.38	171.29a	160.70b	1227.87
				P-value		
CP		0.07	0.001	0.001	0.001	0.001
Se		0.63	0.08	0.001	0.001	0.31
CP*Se		0.001	0.001	0.001	0.001	0.001
CP100*Se0		5.83^{bc}	5.23^{b}	142.08^{e}	125.64^{e}	1329.78^{ab}
CP100*Se0.04		7.05^{a}	5.31^{ab}	155.81^{c}	153.21^{d}	1197.03^{b}
CP94*Se0		6.37^{ab}	5.86^{a}	182.51^{b}	191.87^{b}	1256.33^{b}
CP94* Se0.04		5.23^{c}	5.33^{ab}	147.85^{de}	177.22^{c}	1008.20^{c}
CP88*Se0		6.50^{ab}	4.25^{c}	151.74^{dc}	216.40^{a}	1252.86^{b}
CP88* Se0.04		6.07^{bc}	5.5^{ab}	210.19^{a}	151.65^{d}	1478.46^{a}

CP=Crude protein, Se=Selenium.

a–e Means with no common superscript within each column are significantly (P < 0.05) different.

Table 5 shows the effects of CP level and Se supplement on the antibody titer to NDV and cortisol content. After seven days, the lowest antibody titer was observed in birds fed a diet containing 94% CP and 0.04 mg/kg, significantly different from other treatments. Chickens fed 0.04 mg/kg Se showed more antibodies than those fed 0% at 14 days. At 21 days, the highest amount of antibody was observed in the treatment containing 100% CP, which had a significant difference with the treatments containing 94% and 88% CP. Furthermore, there was a significant difference in the main effects of CP and Se on the blood cortisol concentration of broilers before heat stress. The highest cortisol concentration was observed in the treatment containing 100% CP and 0% Se, while the lowest was in the treatment containing 94% CP and 0.04 mg/kg Se.

Table 5. Effect of protein level and selenium supplement on the antibody titer to Newcastle disease virus (log 2) and cortisol content (μg/dl)

		NDV7	NDV14	NDV21	Cortisol Before Stress	Cortisol End of Stress
CP%	100	6.56a	3.56	3.37a	0.20a	0.16
	94	6.00b	3.93	2.62b	0.16b	0.17
	88	6.31ab	3.75	2.87b	0.18ab	0.17
Se (mg/kg)	0	6.29	3.33b	2.87	0.19a	0.17
	0.04	6.29	4.16a	3.04	0.17b	0.16
			P-value			
CP		0.04	0.15	0.001	0.001	0.10
Se		1.00	0.001	0.30	0.03	0.25
CP*Se		0.01	0.07	0.76	0.53	0.45
CP100*Se0		6.37[a]	3.37	3.37	0.222	0.165
CP100*Se0.04		6.75[a]	3.75	3.37	0.191	0.160
CP94*Se0		6.37[a]	3.50	2.5	0.171	0.187
CP94* Se0.04		5.62[b]	4.37	2.75	0.162	0.170
CP88*Se0		6.12[ab]	3.12	2.75	0.193	0.171
CP88* Se0.04		6.50[a]	4.37	3.00	0.178	0.172

CP=Crude protein, Se=Selenium, NDV= Newcastle disease virus antibody in different days.

a–e Means with no common superscript within each column are significantly ($P < 0.05$) different.

The results of the effect of experimental treatments on the blood leukocyte profile of broiler chickens are presented in Table 6. Different levels of CP and Se significantly impacted the population of blood monocytes. At the same time, the impact of different levels of dietary CP on the number of basophils and heterophils was also significant ($P < 0.05$). The treatment containing 100% and 88% CP and 0% Se showed the highest number of blood monocytes, significantly different from the treatment containing 88% CP and 0.04 mg/kg Se, which had the lowest number of monocytes. The broilers fed with 100% CP had the highest number of blood basophils, while the ones on an 88% CP diet had the lowest. The treatment with 88% CP had the highest number of blood heterophils, while the one with 100% dietary CP had the lowest. Eosinophils, lymphocytes, and H/L ratio were not affected by experimental treatments.

Table 6. Effect of protein level and selenium supplement on blood leukocyte profile of broiler chickens

		Monocytes	Eosinophils	Basophils	Heterophil	Lymphocyte	H/L
CP%	100	2.25	3.12	0.93a	34.50[b]	59.18	0.58
	94	2.12	2.81	0.50ab	35.62[ab]	58.93	0.61
	88	2.00	3.25	0.37b	36.87[a]	57.50	0.64
Se (mg/kg)	0	2.41	3.20	0.58	35.45	58.33	0.61
	0.04	1.83	2.91	0.62	35.87	58.75	0.62
				P-value			
CP		0.828	0.834	0.036	0.037	0.475	0.139
Se		0.086	0.635	0.818	0.569	0.732	0.920
CP*Se		0.021	0.500	0.690	0.438	0.432	0.557
Interactions							
CP100*Se0		2.75[a]	3.75	1.000	34.62	57.87	0.601
CP100*Se0.04		1.75[ab]	2.50	0.875	34.37	60.50	0.569
CP94*Se0		1.75[ab]	2.87	0.500	35.75	59.12	0.607
CP94* Se0.04		2.50[ab]	2.75	0.500	35.50	58.75	0.613
CP88*Se0		2.75[a]	3.00	0.250	36.00	58.00	0.630
CP88* Se0.04		1.25[b]	3.50	0.500	37.75	57.00	0.663

CP=Crude protein, Se=Selenium, H/L= Heterophil to lymphocyte ratio.

a–e Means with no common superscript within each column are significantly ($P < 0.05$) different.

According to the findings in Table 7 and Table 8, the experimental treatments did not significantly affect the carcass traits of broiler chickens (P > 0.05). However, there was a discernible difference in the thymus weight based on the diets' selenium (Se) levels. Chickens that were not supplemented with Se exhibited a significantly larger thymus weight (0.68) in comparison to those that received 0.04 mg/kg Se in their diet (0.57) (P < 0.05).

Table 7. Effect of protein level and selenium supplement on broiler carcass characteristics

Items (g/kg of body weight)		Dressing Weight	Fat Pad	Thymus	Spleen	Liver	Bursa of Fabricius
CP%	100	62.51	2.95	0.69	0.22	4.08	0.23
	94	61.53	3.05	0.55	0.25	4.07	0.21
	88	60.72	3.17	0.63	0.25	4.14	0.19
Se (mg/kg)	0	61.09	3.01	0.68[a]	0.25	4.13	0.20
	0.04	62.09	3.10	0.57[b]	0.24	4.07	0.21
			P-value				
CP		0.28	0.81	0.07	0.54	0.94	0.38
Se		0.27	0.76	0.03	0.69	0.73	0.67
CP*Se		0.29	0.79	0.69	0.33	0.12	0.40

CP=Crude protein, Se=Selenium.

a–e Means with no common superscript within each column are significantly (P < 0.05) different.

Table 8. Effect of protein level and selenium supplement on broiler carcass characteristics and the length of different parts of the intestine

							Length(mm)		
Items (g/kg of body weight)		Wings	Legs	Back	Breast	Panceras	Duodenum	Jejunum	Ileum
CP%	100	8.15	29.4	21.21	37.31	0.40	31.68	77.31	80.43
	94	8.26	31.27	21.88	37.93	0.41	31.00	76.87	77.75
	88	8.42	31.00	21.90	38.49	0.42	31.31	78.75	75.56
Se (mg/kg)	0	8.43	30.44	21.22	37.46	0.40	31.20	76.37	77.87
	0.04	8.12	30.68	22.10	38.36	0.42	31.45	78.91	77.95
				P-value					
CP		0.47	0.16	0.82	0.44	0.74	0.84	0.84	0.46
Se		0.08	0.78	0.39	0.23	0.43	0.79	0.36	0.97
CP*Se		0.71	0.84	0.50	0.93	0.59	0.64	0.55	0.94

CP=Crude protein, Se=Selenium.

a–e Means with no common superscript within each column are significantly (P < 0.05) different.

4 Discussion

4.1 Growth performance

During this research, it was noted that chickens that received diets containing 88% and 94% CP between days 10-24 had greater BW and DWG than chickens that were given 100% CP. This trend was also observed throughout the experiment and in the 25-42-day periods, although it was not deemed statistically significant. Previous research has shown that proteins have a higher caloric increment when compared to carbohydrates and fat (6), which leads to an increase in diet-induced heat production. Dietary reductions in CP have been suggested to counteract the harmful effects of heat stress on poultry. The aim is to decrease the energy released during digestion, absorption, and metabolism of nutrients (9). The experimental treatments did not affect FI and FCR, but it was observed that the FCR in chickens fed with diets containing 88% and 94% CP was numerically lower compared to the diet with 100% CP. Therefore, the improvement in BW and DWG can be partially attributed to better utilization of nutrients in the digestive system, intestinal health, and, consequently, higher welfare of the birds. The reduction in protein content increases the net energy content of a diet by reducing the caloric increment, as illustrated by (24). According to Awad et al. (2018), the birds that were fed the low CP diet had a significantly higher protein efficiency ratio (PER) in comparison to the birds that were fed the normal protein diet (10). In a recent study, one of the reasons that FI was not affected by different levels of dietary CP was the equal amounts of amino acids in all diets. FI is influenced not only by protein content but also by protein quality, specifically amino acid balance (25). A study discovered that feeding a low-protein diet fortified with Gly during the starter phase had similar results for FI, weight gain, FCR, and EER as the normal protein diet (10).

Additionally, other studies showed that dietary CP levels did not significantly affect the performance of heat-stressed broiler chickens (26, 27). However, some studies have shown that feeding broilers with low-CP diets negatively impacted their growth performance under heat-stress conditions (28, 29). The breed and age of the experimental animals may have contributed to the disparity in results. Incorporating these discoveries into typical production environments necessitates thorough deliberation. Although devising low-protein diets with well-proportioned amino acids is feasible, it is imperative to ensure that birds receive all the essential amino acids in appropriate quantities.

According to this study, adding Se to the diet of broiler chickens did not significantly affect their performance traits under heat stress. Although previous research has suggested that Se could be used as a nutritional solution to combat heat stress (30, 31), some reviews have not shown any consistent effects of Se supplementation on maintaining performance (32).

4.2 Blood parameters

This study found that reducing dietary CP increased blood glucose concentration when broiler chickens were exposed to heat-stress conditions. However, adding Se to a 100% or 88% CP diet increased glucose content even further, while adding a 94% CP diet decreased blood glucose levels. Stress indicators often include glucose, which animals use as an energy source to cope with stressful events. The increase in serum glucose concentration in low CP-fed groups is due to stress caused by high dietary carbohydrates (33). Moreover, when under stress, corticosterone levels rise, leading to gluconeogenesis, where amino acids are converted to glucose, causing a rise in blood glucose levels (34). Saleh et al. (2021) and Abdel-Moneim et al. (2022) did not find any significant differences in blood parameters, including glucose, when dietary CP was reduced under heat stress conditions, which is in contrast to our findings (35, 36).

Ghazi Harsini et al. (2012) discovered that exposure to heat stress increased glucose concentrations in the serum of chicks; however, supplementing dietary Se at 1 mg/kg helped reduce the adverse effects of high environmental temperature on serum glucose (37). Supplemental dietary Se reduced serum glucose concentrations by decreasing the catabolic effect of corticosterone, which typically increases under heat-stress conditions (38). A recent study showed that this blood glucose regulatory effect by Se was effective in medium CP reduction of the diet (94%). However, it was ineffective in reducing the CP (88%).

This study found that reducing the amount of dietary CP led to a decrease in the concentration of albumin in the blood of broiler chickens. However, adding Se to the diet containing 100% CP increased the blood albumin concentration, while adding Se to the diet containing 88% and 94% CP decreased the blood albumin concentration of broiler chickens under heat stress. Albumin is a protein synthesized by the liver that transports various molecules in the bloodstream, such as hormones, vitamins, and drugs. When protein intake is reduced, the liver may not produce enough albumin, decreasing blood albumin levels. Previous research (39, 40) has shown that dietary reduction of CP by three to four percent decreased serum albumin contents in broilers. In contrast, no significant effect was found in a study on blood albumin concentration in response to different treatments based on low-CP diets under high ambient temperatures (35).

Kim and Mahan (2003) stated that Se shares similar biochemical characteristics with sulfur. It can replace the sulfur molecule in the normal biosynthetic pathways and is actively absorbed by the same amino acid carrier in the intestine (41). Supplementation of organic Se in broiler breeders and layers is actively absorbed and can be directly incorporated into proteins like albumin (42). However, studies have shown inconsistent findings regarding the effect of Se supplementation on serum albumin levels. Some reported a decrease in serum albumin (43, 44), while others found no significant effect (38, 39). These inconsistent findings may be due to various factors, including the type and source of Se used, the supplementation duration, and the broilers' overall nutritional status. Furthermore, the relationship between Se and albumin may be complex and involve various mechanisms.

Birds given a diet consisting of 88% CP showed reduced uric acid levels in their bloodstream compared to other treatments. However, introducing Se to a diet containing 88% CP significantly increased broilers' blood uric acid concentration. Avian species produce uric acid as a major nitrogenous waste product (45). It is reasonable to suggest that a lack of protein, mostly from non-essential amino acids, may lead to a shortage of these substrates in chicken serum. Liu et al. (2016) found that reducing dietary CP levels decreased serum uric acid concentrations in broilers, which is consistent with our results (27). Less uric acid excretion reduces ammonia emissions from poultry waste, a significant environmental concern.

Additionally, these ammonia emissions contribute to respiratory diseases in broilers and conflict with animal welfare (46). In contrast to our result, Badawi et al. (2019) reported no significant changes in blood uric acid concentration among different treatments based on low-CP diets (2, 4, and 6% of the standard CP control diet) (47). On the other hand, this study also highlights potential complexities. Se supplementation in a low-CP diet counteracts the uric acid reduction benefit. Further research is needed to optimize CP levels and Se supplementation for minimal uric acid excretion and sustainable poultry production. A study showed that serum uric acid concentrations increased when chicks were exposed to heat stress. However, supplementing dietary Se at 1 mg/kg alleviated the adverse effects of high environmental temperature on uric acid (37).

Additionally, adding Se nanoparticles to the diet of heat-stressed broiler chickens had no significant impact on uric acid content, according to (36). Of course, these studies were carried out under the influence of the normal amount of CP in the diet of broiler chickens; also, in this study, the addition of 0.04 mg/kg Se to regular and medium CP diets had the same uric acid content as 0% Se. Se plays a role in antioxidant mechanisms and can affect protein metabolism (31). Therefore, changes in protein metabolism due to selenium supplementation in a low CP diet may increase uric acid content.

A recent study found that the interaction between different levels of dietary CP and Se significantly affected the concentration of blood albumin, uric acid, glucose, triglyceride, and cholesterol. However, more information is needed on the combined effect of different dietary CP and Se on the blood parameters of broiler chickens. Our study found that reducing dietary CP increased blood triglyceride content in broiler chickens under heat stress. Adding Se to diets containing 88% and 94% CP reduced triglyceride concentration. Our experiment, as demonstrated by another study, has shown that feeding low-CP diets increases liver lipogenesis and triglyceride (48). Regardless of energy density, birds grown on low-CP diets have higher triglycerides (49).

Moreover, Habibian et al. (2014) have found that Se-Met supplementation can reduce triglyceride concentrations in broilers exposed to high temperatures (50). The addition of Se has considerably decreased serum triglyceride and total cholesterol levels in broilers exposed to heat stress. This effect is attributed to Se's ability to bolster the body's antioxidant defense mechanisms, which helps to reduce oxidative stress and lipid peroxidation. Besides, Se also plays a crucial role in regulating lipid metabolism by influencing enzymes involved in fatty acid synthesis and degradation (30). We may also have a hypolipidemic effect by altering the excretion of cholesterol and bile acids (51). Our investigation revealed that adding Se to diets containing 94% and 88% CP triggered a significant decrease and increase in cholesterol levels, respectively. Abdel-Moneim et al. (2022) conducted a study that showed that feeding heat-stressed broiler chickens with Se nanoparticles decreased triglyceride and cholesterol levels (36). However, it is unclear why adding Se to the 88% CP diet increased cholesterol levels compared to other treatments. When broilers are fed a reduced-CP diet, their bodies may try to compensate by producing more fat to meet their energy needs. Se supplementation may further enhance this process, which could lead to an increase in cholesterol levels.

The measurement criterion commonly used to determine immune status in poultry is the antibody response to foreign antigens, as reported by (52). This study showed that the addition of Se to the diet of broiler chickens at 14 days of age resulted in an increase in the antibody titer to NDV. A similar result was found by (36), who reported that Se supplementation in broilers under heat-stress conditions increased antibody titers to NDV. Selim et al. (2015) reported that Se can help maintain cellular functions against oxidative stress and lipid peroxidation and improve host immunity (53). Our study found that at 21 days of age, the reduction of CP in the broiler diet caused a decrease in antibody titers to NDV. Studies on mice and rats with dietary CP deficiencies generally show that antibody responses are depressed while cellular immunity remains unaffected (54-56). In contrast, Abbasi et al. (2014) found no difference in antibody titers to NDV challenge in chicks fed with low dietary CP (57).

At the age of 7 days, it was observed that the interaction of different levels of CP and Se significantly affected the antibody concentration. Generally, the addition of Se to diets containing different levels of CP resulted in increased antibody titers to NDV, except for 94% of CP + 0.04 mg/kg Se, which had the lowest titer. Unfortunately, we could not find any information about the simultaneous effect of different levels of CP and Se on the concentration of antibodies. According to the literature, Se can help improve abnormal levels of cytokines and oxidative damage induced by heat stress, which can reduce injury (30).

Before experiencing heat stress, adding Se to the diet of broiler chickens resulted in a reduction of blood cortisol concentration. However, after exposure to heat stress, this difference was not significant. Cortisol is a crucial glucocorticoid released from the adrenal cortex in response to various stresses and acts as an immunosuppressant. Previous studies have shown that adding Se to the diet of heat-stressed broilers can benefit their plasma cortisol level (58). Se acts as an antioxidant, protecting the body's cells against damage caused by free radicals and oxidative stress (59). Fan et al. (2009) reported that broilers fed a diet supplemented with 0.1 and 0.4 mg of sodium selenite showed a decrease in corticosterone levels compared to those in the control group (38).

4.3 Leukocyte profile

The results obtained regarding the blood leukocyte profiles of broiler chickens did not follow a consistent trend. Although eosinophil, lymphocyte, and heterophil-to-lymphocyte ratios were not affected by the experimental treatments, the reduction of dietary CP decreased the number of basophils and increased the number of heterophils. Maxwell and Robertson (1998) found that eosinophils decrease in circulation while basophils increase during stress, particularly acute stress (60). Therefore, the decrease in basophil count observed in the diet with 88% CP in this study may be due to the reduction of stress caused by heat stress in the diet with less CP. Houshmand et al. (2012) reported that the CP level did not have an impact on the H/L ratio in broilers (61).

In this study, it was observed that adding Se to the 88% CP diet decreased monocytes. Reductions in the number of monocytes have been reported in stressed animals before (62). However, it is unclear why Se led to a decrease in the number of monocytes in the blood of broilers in this study. Previous studies have reported positive effects of adding Se to the diet of broiler chickens, such as increasing cellular immunity and immunomodulation properties (53, 63). Furthermore, Habibian et al. (2014) conducted a study that revealed Se treatments did not significantly impact the percentages of heterophils and lymphocytes or the H/L ratio on day 32 (50).

4.4 Carcass traits

In the present study, it was found that reducing CP levels did not have any significant effects on carcass traits. Badawi et al. (2019) conducted a study on broiler chicks fed with a reduced 2%, 4%, and 6% of the standard CP control diet. They found no significant difference in the weight percentage of dressing and visceral organs relative to live body weight in all experimental birds (47). These results are consistent with those of Saleh et al. (2021), who found that reducing dietary CP levels did not significantly affect dressing percentages (35). Faria Filho et al. (2005) found that reducing CP levels did not affect carcass and wing yields but did result in a reduced breast yield and increased thigh+drumstick yield (64). Widyaratne and Drew (2011) discovered that the birds fed on a high-CP diet had a substantially greater yield of breast meat than those fed on a low-CP diet (65). However, their findings suggest that even low-CP diets can sustain carcass traits equivalent to high-CP diets if highly digestible ingredients are utilized.

The specific reason for the observed decrease in thymus size in Se-supplemented broilers in this study remains unclear. Nonetheless, it is speculated that the Se-induced reduction in thymus weight in heat-stressed broilers may result from stress alleviation, which helps minimize tissue damage, thereby maintaining the functionality and structure of the thymus. In contrast, research by Habibian et al. (2014) indicated that Se supplementation under heat stress conditions did not affect the weight of broiler chickens' thymus, spleen, bursa, or liver (50). Research by Pečjak et al. (2022) found that Se supplementation in broiler chickens under heat stress conditions had no significant effect on overall carcass yield or the relative weights of the breast, legs, wings, back, heart, liver, pancreas, proventriculus, and intestine (66). The thymus, an essential immune system organ crucial for T cell development in broilers, has been shown to decrease in size under stress, potentially impacting T cell production (67). Se supplementation in the diet has effectively reduced oxidative stress and improved stress tolerance (68). Additionally, Safdari-Rostamabad et al. (2017) showed that NanoSe supplementation increased the thymus's relative size in broilers subjected to heat stress (69).

5 Conclusion

The study's findings indicate that lowering dietary CP levels resulted in enhanced daily weight gain (DWG) and overall body weight, surpassing the results of a standard CP diet. The experimental treatments did not affect feed intake

(FI), feed conversion ratio (FCR), or carcass quality. Introducing Selenium (Se) into a diet with 94% CP saw a reduction in levels of triglycerides, cholesterol, and glucose, whereas a diet with 88% CP led to an increase in uric acid levels. Additionally, Se supplementation boosted antibody levels against the Newcastle disease virus (NDV) at 14 days of age. On the other hand, a reduced protein diet was associated with lower antibody titers at 21 days of age. The study concluded that reducing dietary CP did not detrimentally affect growth performance, carcass traits, or blood parameters. Indeed, it even enhanced DWG compared to diets adhering to normal CP levels.

Moreover, incorporating Se into diets with varying CP levels lowered triglycerides, cholesterol, and glucose levels under heat-stress conditions. Further research is needed to optimize protein levels for different bird types and study the interactions with other diet components. Additionally, the long-term health effects of these dietary changes on broilers should be investigated.

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Conflict of Interest

The authors have no conflict of interest to be declared.

Author Contributions

MT was responsible for formulating the project, developing the key conceptual ideas, outlining the proof, and handling most technical aspects. AK, AM and SS-G conducted the numerical calculations for the proposed experiment and independently validated the trial's numerical outcomes. AM authored the manuscript through collaborative discussions, while SS-G meticulously reviewed and edited it.

Data Availability Statement

The data produced and examined during this study are not openly accessible but can be obtained from the corresponding author upon a reasonable request.

Ethical Considerations

All experimental protocols adhered to the guidelines, which were approved by the Animal Ethics Committee of the Razi University, Kermanshah, Iran, and were according to EU standards for the protection of animals and feed legislation.

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