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RESEARCH ARTICLE
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NUTRITIONAL INTERVENTIONS ALLEVIATE HEAT STRESS EFFECTS ON RABBIT HEALTH AND PERFORMANCE: ROLE OF SPIRULINA PLATENSIS AND SELENIUM NANOPARTICLES

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Abstract

Heat stress as a result of global warming has harmful consequences for livestock and is thus becoming an urgent issue for animal husbandry worldwide. The present trial investigated the efficacy of Spirulina platensis, selenium nanoparticles, zinc nanoparticles, or combinations of these supplements in heat-stressed rabbits. The findings indicate enhanced growth rates, better blood parameters, and stronger immune defenses in rabbits facing heat stress.

The treated rabbits exhibited significantly elevated levels of immunoglobulins (IgA, IgG) and superoxide dismutase activity, with p-values less than 0.05. Meanwhile, serum cortisol, interferongamma, malondialdehyde, and protein carbonyl levels were notably decreased in the treated groups. Specifically, Spirulina platensis (SP) and selenium nanoparticles (SeNPs) at doses of 1 g/kg diet and 50 mg/kg diet, respectively, demonstrated improvements in growth, blood parameters, and immuneantioxidant responses. Moreover, the supplements appeared to reduce the negative effects of heat stress on the rabbits' health and overall well-being.

These results indicate that Spirulina and SeNPs can be potentially used as growth promoter, antioxidant, immunostimulant, and antimicrobial agents in rabbits.

Keywords: Spirulina platensis, Male Rabbits, Nano Selenium, Heat stress, Zinc nanoparticles

Introduction

Rabbits, being highly susceptible to environmental changes, face considerable challenges in maintaining optimal health and performance under heat stress conditions [1]. Heat stress (HS) adversely affects various physiological processes, including growth, immune function, and antioxidant defense mechanisms, ultimately impacting overall productivity in rabbit farming systems [2].

In recent years, dietary supplementation has emerged as a promising strategy to mitigate the detrimental effects of HS on animal health and welfare [3]. Of particular interest are mineral nanoparticles, such as selenium and zinc, known for their antioxidant properties and potential to enhance immune function. Additionally, Spirulina platensis (SP), a microalga rich in essential nutrients and bioactive compounds, has garnered attention for its ability to modulate immune responses and oxidative stress [4].

Given the importance of maintaining rabbit health and performance in the face of rising temperatures, this study aims to investigate the effects of dietary supplementation with mineral nanoparticles (selenium or zinc) and/or SP on rabbits subjected to HS. .

In recent years, numerous scientific fields have witnessed swift advancements in nanotechnology and its associated products. There is evidence that nanominerals interact more effectively. The compared to the organic and inorganic components of an animal's body. Their greater surface area, capacity for solid adsorption, and high catalytic competence are the causes of this [5]. Therefore, in comparison to conventional mineral sources, nanoscale minerals are anticipated to be more bioavailable, interactive, and efficient. The beneficial effects of adding ZnNPs [6] or SeNPs [7] on the wellbeing and general health of stressed-out developing rabbits have been shown in a small number of investigations. In order to enhance development and health, earlier studies by [8], and [9] employed doses of SP (1 g/kg food), SeNPs (50 mg/kg diet), and SeNPs (100 mg/kg diet) in rabbit diets [1011]. Comparative research on these nanominerals combined with SP in stressed rabbits is lacking, nevertheless. Afterward, the purpose of this study was to investigate the effects on growth, feed utilization, blood hematology, serum biochemistry, antioxidant-immune response, and carcass yield of growing rabbits raised in summer conditions when dietary nanominerals [zinc nanoparticles (ZnNPs) or selenium nanoparticles (SeNPs)] were combined with SP.

Materials and Methods

The total 180 weaned male New Zealand White rabbits weighing an average of 716.9±12g and about 35 days old were included in this investigation [12-13]. The study was carried out at The Islamia University of Bahawalpur, Pakistan. These rabbits were divided into six experimental groups, each with thirty rabbits and fifteen duplicates, at random. In accordance with the study protocol, these feed additives were added to the diets during the feed manufacturing procedure.

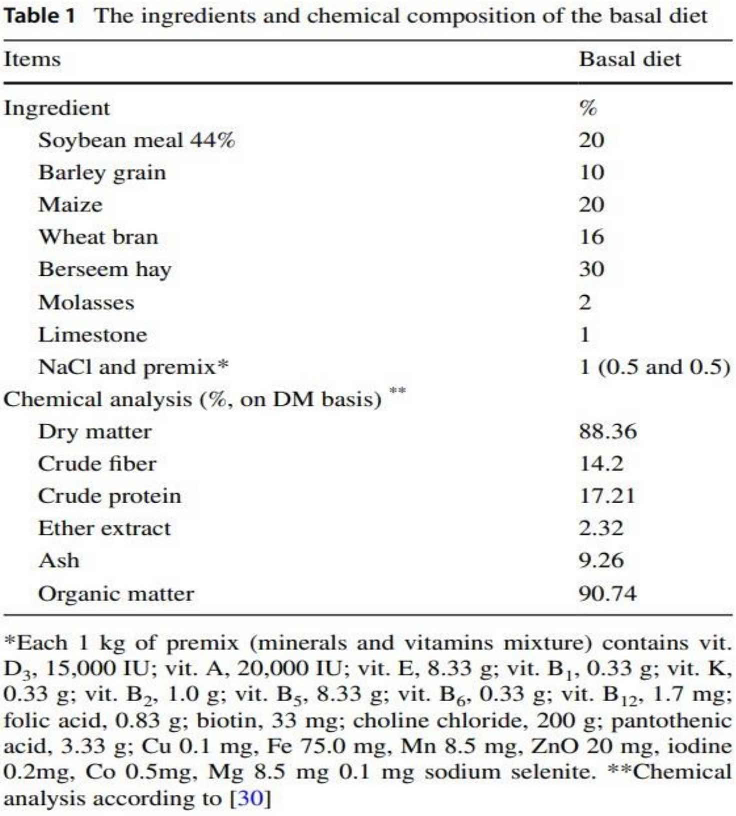

Table 1 The ingredients and chemical composition of the basal diet

Items	Basal diet
Ingredient	%
Soybean meal 44%	20
Barley grain	10
Maize	20
Wheat bran	16
Berseem hay	30
Molasses	2
Limestone	1
NaCl and premix*	1 (0.5 and 0.5)
Chemical analysis (%, on DM basis) **	
Dry matter	88.36
Crude fiber	14.2
Crude protein	17.21
Ether extract	2.32
Ash	9.26
Organic matter	90.74

*Each 1 kg of premix (minerals and vitamins mixture) contains vit. D_3, 15,000 IU; vit. A, 20,000 IU; vit. E, 8.33 g; vit. B_1, 0.33 g; vit. K, 0.33 g; vit. B_2, 1.0 g; vit. B_5, 8.33 g; vit. B_6, 0.33 g; vit. B_{12}, 1.7 mg; folic acid, 0.83 g; biotin, 33 mg; choline chloride, 200 g; pantothenic acid, 3.33 g; Cu 0.1 mg, Fe 75.0 mg, Mn 8.5 mg, ZnO 20 mg, iodine 0.2mg, Co 0.5mg, Mg 8.5 mg 0.1 mg sodium selenite. **Chemical analysis according to [30]

The rabbits were divided into groups according to the dietary treatments they received: a control group (CON) was given a basal diet, while the other groups were given experimental diets fortified with zinc nanoparticles (ZnNPs, 100 mg/kg diet), selenium nanoparticles (SeNPs, 50 mg/kg diet), or a combination of ZnNPs and/or SeNPs and SP, respectively.

All rabbits were fed pelleted diets and kept in conventional cages for the duration of the trial. They always had food and water available to them. Previous research was used to construct the diets based on published nutrient guidelines for rabbits [14-15]. Please refer to Table 1 for comprehensive details on the diet's ingredients and the basal experimental diet's chemical makeup.

Chemicals Used:

A lyophilized culture of Lactobacillus plantarum strain was obtained from Microbiology Department of The Islamia University Bahawalpur. The culture, comprising 2% with approximately 10^5 cfu/mL, was grown in MRS broth medium supplemented with 0.1% skim milk powder and 1% yeast extract. Sodium selenite from Algomhoria Company was introduced to the medium, which was then incubated aerobically at 37°C for 3–4 days in Erlenmeyer flasks without agitation. Subsequently, SeNPs were characterized for size, lyophilized, weighed, and incorporated into the diets per the study protocol.

Zinc oxide was sourced from Algomhoria Company, Zagazig, Egypt. Fresh bacterial strain L. plantarum was cultured on nutrient agar medium at 37°C for 24 hours in 250-mL Erlenmeyer flasks. The suspension was then supplemented with sterilized deionized water containing zinc oxide. After subculturing into a nutrient broth medium and constant shaking at 150 rpm at 37°C for 24 hours, the supernatant was collected following centrifugation at 5000 rpm for 5 minutes from an overnight bacterial culture. The collected ZnNPs were dried at 100°C and utilized in the study based on established methodologies.

Rabbit Growth and Group Mechanism

The rabbits' weights were measured individually using a digital balance, and their feed intake (FI) was recorded throughout the study. Live body weights (BW) and daily body weight gain (BWG) were assessed at 10 and 14 weeks of age. However, only average BW at 6 weeks (start), 10 weeks (4 weeks of treatments), and 14 weeks (end) were documented. Feed intake (FI) and feed conversion ratio (FCR, g feed/g BWG) were calculated for the periods 6–10 weeks, 10–14 weeks, and the overall 6–14 weeks of age.

As the experiment came to a close, ten rabbits at random from each group were selected to have blood drawn from their ears veins using the method described in [31]. The two portions of the blood samples were separated: the serum was separated into clean centrifuge tubes without anticoagulant and centrifuged at 2000 gravity for 20 minutes. The serum was then refrigerated at -20°C for the biochemical studies that followed. The remaining part was drawn into tubes containing EDTA and used to measure several hematological parameters in whole blood. Red blood cells (RBCs), hemoglobin (Hb), hematocrit (Hct), mean corpuscular hemoglobin concentration (MCHC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), platelets, mean platelet volume (MPV), and platelet distribution width (PDW) are examples of parameters.

Results

Effects on Growth Indices

The provided data reveals insights into the effects of various treatments on body weight (BW) and daily weight gain (DWG) across different time points. Analyzing the body weight trends, it becomes evident that there are notable variations among the treatment groups at the initial 6-week mark, with some treatments showing higher initial body weights than others. However, over the course of the study, spanning up to 14 weeks, all treatments exhibit an overall increase in body weight, indicative of growth or development in the subjects. Treatments such as SPSeNPs and SPZnNPs stand out with higher body weights at the 14-week mark, suggesting a potentially enhanced impact on promoting body weight gain compared to other treatments, as shown in Figure 001.

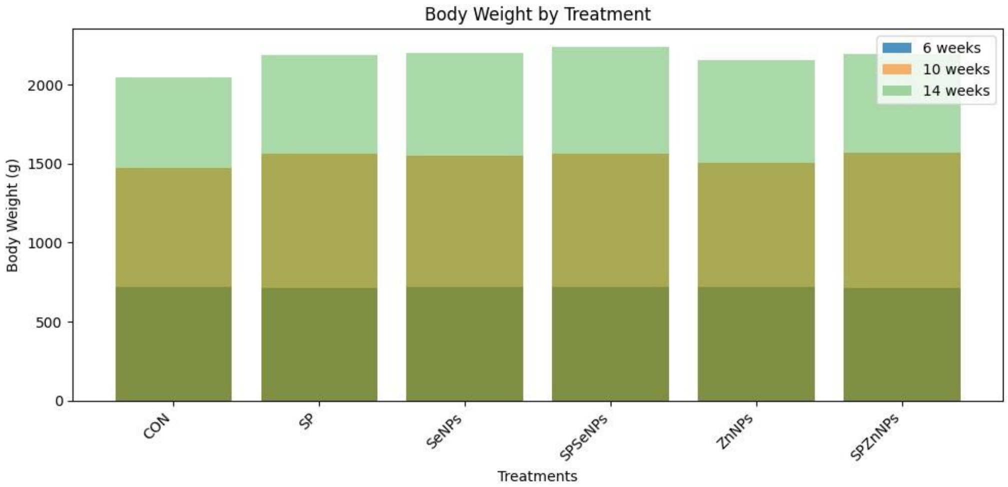

Figure 1: Body Weight Analysis in over applied treatments

In parallel, the analysis of daily weight gain (DWG) provides further insights into the efficacy of the treatments over specific time intervals. Treatments incorporating selenium (SeNPs, SPSeNPs) and zinc (ZnNPs, SPZnNPs) nanoparticles demonstrate relatively higher daily weight gains during certain intervals, indicating potentially more efficient nutrient utilization or growth promotion. Notably, the 6-10 week interval appears to be characterized by the highest daily weight gains across most treatments, implying a period of rapid growth or response to the treatment interventions, the detail are shown in Figure 2.

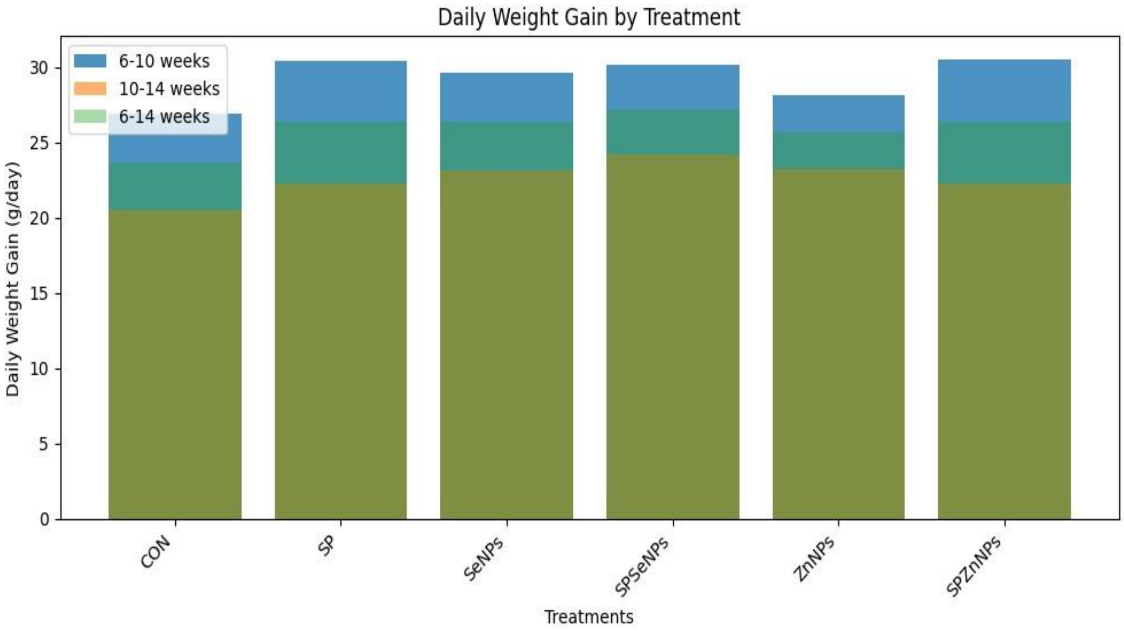

Figure 2: Daily Weight Analysis in over applied treatments

The Figure 3 provided data outlines the dynamics of feed intake and feed conversion ratio across different treatments and time intervals. Analyzing the feed intake trends, it appears that there are subtle variations among the treatment groups across the specified time intervals (6-10 weeks, 10-14 weeks, and 6-14 weeks).

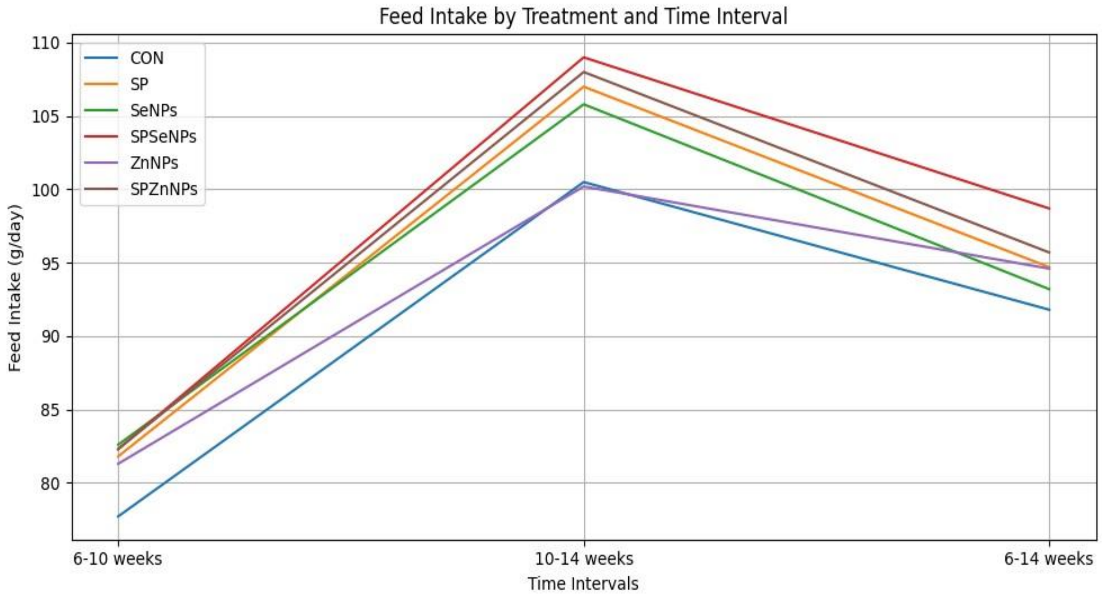

Figure 3: Feed intake analysis with respect to time intervals

Treatments exhibit distinct patterns of feed intake over time, with some treatments showing fluctuations while others remain relatively stable. Notably, treatments like SPSeNPs and SPZnNPs demonstrate relatively higher feed intake compared to the control (CON) and other treatments, particularly during the 10-14 weeks period.

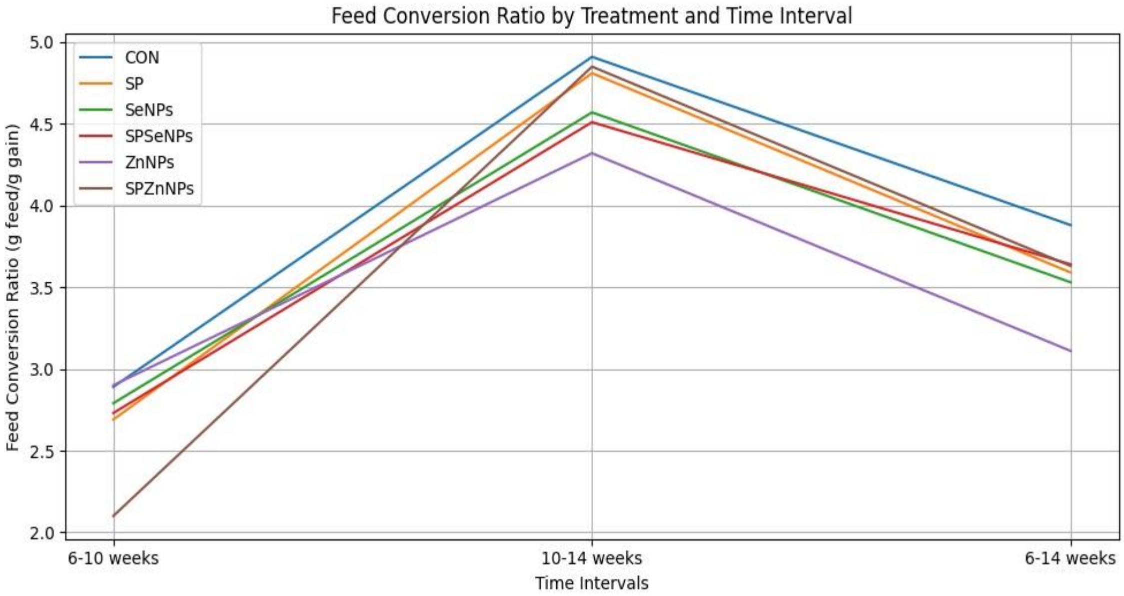

Figure 4: Feed conversation ratio based on time intervals

Regarding the feed conversion ratio as shown in Figure 4, the data reveals insights into the efficiency of feed utilization for weight gain. Across the time intervals, each treatment group showcases its feed conversion ratio, which indicates the amount of feed required to produce a unit of weight gain. Interestingly, treatments incorporating selenium and zinc nanoparticles (SeNPs, SPSeNPs, ZnNPs, SPZnNPs) tend to exhibit lower feed conversion ratios compared to others, suggesting more efficient feed utilization for growth. This indicates the potential effectiveness of these nanoparticle treatments in promoting growth while optimizing feed efficiency.

Table 1: Effects of nanominerals either alone or incorporated with Spirulina platensis (SP) on carcass traits of growing rabbits under summer conditions

	Preslaughter weight (g)	Dressing (%)	Liver (%)	Heart (%)	Kidney (%)	Edible giblets (%)	Lung (%)	Spleen (%)	Tests (%)	Total edible parts (%)
CON	2092	59.98	2.79	0.91	3.98	0.72	0.05	0.2	63.97	36.03
SP	2077.33	60.93	3.12	0.89	4.31	0.67	0.08	0.27	65.24	34.76
SeNPs	2066.33	61.36	3.37	0.83	4.52	0.74	0.12	0.27	65.88	34.12
SPSeNPs	2054.67	61.72	3.45	0.92	4.66	0.68	0.11	0.28	66.38	33.62
ZnNPs	2084	60.05	3.41	0.93	4.6 1	0.67	0.1	0.26	64.65	35.35
SPZnNPs	2095.33	59.85	3.25	0.91	4.51	0.7	0.13	0.22	64.36	35.64
SEM	15.836	1.49	0.14	0.06	0.17	0.06	0.02	0.01	1.49	1.49
P-Value	0.4216	0.9136	0.0428	0.8976	0.0231	0.9571	0.3004	0.2122	0.8499	0.8499

Table 1 provided results offer insights into the pre-slaughter characteristics and organ yields of the subjects under different treatment conditions. Across the treatment groups, variations in pre-slaughter weight, dressing percentage, and organ yields are observed. Notably, treatments incorporating selenium (SeNPs) and zinc nanoparticles (ZnNPs) show marginal differences in pre-slaughter weight compared to the control (CON) and standard protein (SP) treatments. The dressing percentages, reflecting the percentage of edible carcass weight to live weight, remain relatively consistent across treatments, suggesting uniformity in carcass yield. However, treatments involving selenium nanoparticles (SeNPs, SPSeNPs) exhibit slightly higher percentages of liver and kidney weights compared to other treatments, indicating potential effects on organ development. Moreover, treatments incorporating zinc nanoparticles (ZnNPs, SPZnNPs) demonstrate higher spleen percentages, suggesting potential influences on immune system development.

Table 2: Efects of nanominerals either alone or incorporated with Spirulina platensis (SP) on erythrogram and white blood cells of growing rabbits under summer conditions

	Hb (g/dL)	RBCs (106/mm3)	Hct (%)	MCV (fL)	MCH (g/dL)	MCHC (%)	Platelets (103/mm 3)	MPV (fL)	POW (fL)	WBCs (103/mm 3)
CON	8.94	4.77	28.66	63.57	22.23	34.97	186.6	7.21	24.38	6.44
SP	11.22	5.46	32.74	65.07	22.62	34.78	182.4	6.711	19.82	9.98
SeNPs	11.82	5.25	33.72	64.38	22.56	35.06	201.4	6.99	21.96	10.74
SPSeNPs	12.31	5.38	42.26	54.4	24.29	30.44	173	9.93	20.04	8.83

ZnNPs	11.74	5.36	33.18	61.93	21.93	35.44	204	7.2	25.76	14.9
SPZnNPs	11.38	5.51	32.58	64.31	22.45	34.94	216	6.75	24.96	9.62
SEM	1.95	0.11	5.2	3.46	1.14	1.8	27.32	1.37	2.97	1.88
P-Value	0.01	0.036	0.005	0.286	0.756	0.392	0.883	0.567	0.595	0.043

Similarly, The results in Table 2 highlight minor differences in pre-slaughter weight among treatments, with selenium and zinc nanoparticle treatments showing subtle variations. Dressing percentages remain consistent across treatments, indicating uniform carcass yields. Selenium treatments exhibit slightly higher liver and kidney weights, while zinc treatments show increased spleen percentages, suggesting potential influences on organ development and immunity.

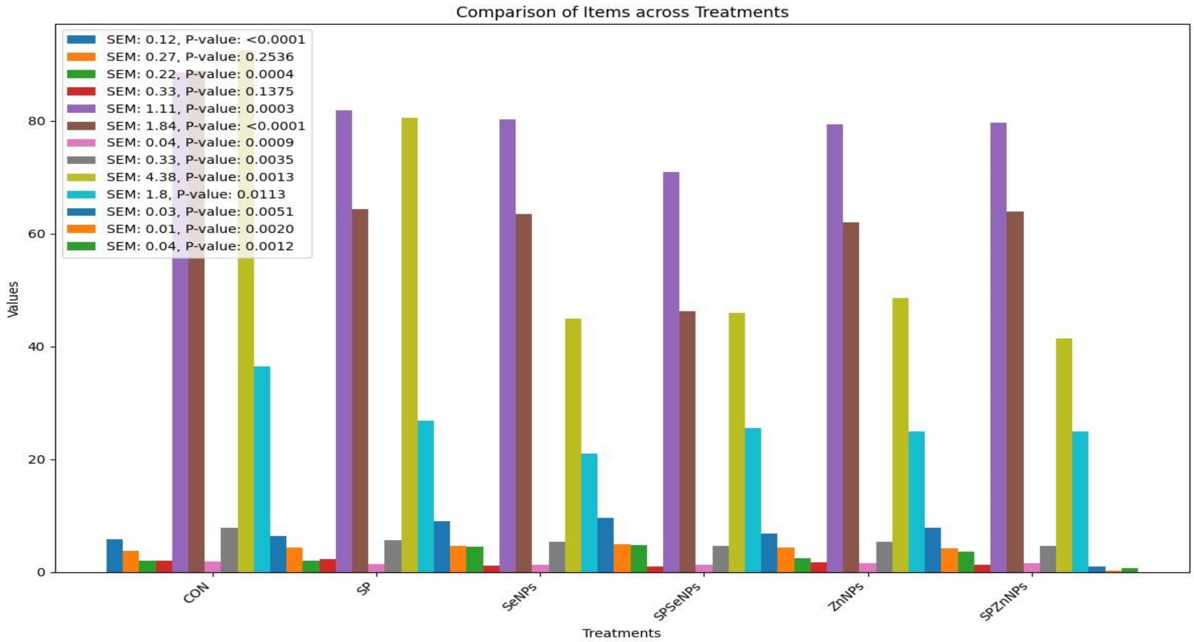

Figure 5: Effects of nanominerals (SeNPs or ZnNPs) either alone or incorporated with Spirulina platensis (SP) on blood biochemical of growing rabbits under summer condition

The results in Figure 5 provided various biochemical parameters across different treatments. Treatments incorporating selenium (SeNPs, SPSeNPs) and zinc nanoparticles (ZnNPs, SPZnNPs) exhibit notable differences in total protein (TP), glucose (GLU), triglycerides (TG), creatinine, uric acid, alanine transaminase (ALT), and aspartate transaminase (AST) levels compared to the control (CON) and standard protein (SP) treatments. Notably, treatments containing selenium nanoparticles tend to show higher TP and GLU levels but lower TG levels, suggesting potential metabolic modulation effects.

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