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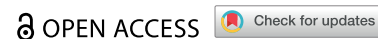


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RESEARCH ARTICLE



Effects of dietary supplementation with zinc oxide or selenium nanoparticles and their combination on rabbit productive performance, nutritional and physiological responses

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ABSTRACT

This research investigated how supplementing growing rabbits with zinc oxide nanoparticles (Nano-ZnO) and selenium nanoparticles (Nano-Se) impacts their growth performance, digestibility, blood biochemistry, and liver mineral content. The study utilised 40 male and 40 female New Zealand White rabbits, all 30 days old, with an average weight of 720 ± 6 g. Rabbits were divided into four treatment diets with 10 replicates of two rabbits each (one male and one female) for 60 days. The diets comprised of 1) control diet, 2) control diet + 40 mg/kg Nano-ZnO, 3) control diet + 0.6 mg/kg Nano-Se, and 4) control diet + 40 mg/kg Nano-ZnO + 0.6 mg/kg Nano-Se. Results showed that dietary Nano-ZnO and Nano-Se in single or combination improved ($p < 0.05$) the growth performance, carcass yield, crude protein, and ether extract digestibility compared with the control group. Rabbits received Nano-ZnO and Nano-Se alone or in mixture showed lower ($p < 0.05$) abdominal fat and serum levels of cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and urea activities than the control. The addition of Nano-ZnO, Nano-Se, or their combination did not affect the concentrations of zinc or selenium in the livers of male and female rabbits. In conclusion, dietary supplementation of Zinc oxide and Selenium nanoparticles alone or in combination improved growth performance and nutrient digestibility of male and female rabbits.

HIGHLIGHTS

- Dietary Nano-ZnO (40 mg/kg), Nano-Se (0.6 mg/kg), or their combination, can improve rabbits' growth performance, feed conversion ratio, and dressing weights.
- Dietary supplementation of Nano-ZnO, Nano-Se, or their mixture had hypolipidemic effects.
- Nano-ZnO, Nano-Se, or their combination impacted the liver and kidney functions; however, the levels of Zn and Se in the liver were unaffected.

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Rabbit; nanotechnology; minerals; digestibility; blood biochemical parameters

Introduction

Over the past decade, nanotechnology has attracted the attention of many scientists owing to its favourable food and agricultural applications. Nanotechnology offers innovative keys for nutrient transfer and health safety and has excellent prospects to advance animal productivity (Horky et al. 2016). This attention is mainly due to the distinctive nanoparticles' physicochemical characteristics, which indicate their small size (1–100 nm), large surface area, great hydrophobicity, and stability.

Nanoparticles can be used as a supplementary source of trace minerals in diets with several new properties that differ from other sources (Abdel-Wareth et al. 2023). Selenium is a crucial trace mineral for growth, immunity, biochemical parameters, hormones, fertility, metabolism, and functions of external and internal organs (Mohapatra et al. 2014; Surai et al. 2017; Amer et al. 2019). Se nanoparticles (Nano-Se) have similar efficacy, more bioavailable, solid adsorption capacity, and low toxicity than other Se sources (Wang et al. 2021). Supplementation with Nano-Se at

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50 mg/kg significantly increased rabbits' growth performance and carcass parameters (Sinha et al. 2011).

Zinc (Zn) is an essential trace mineral that can supplement animal diets and increase growth, nutrient retention, immune system, organ function, enzyme structure, and bone development (Suttle 2010). Supplements with organic and inorganic zinc sources to commercial animal diets provide adequate amounts of zinc to verify average growth, health, and reproductive performance (Bao et al. 2007; Casado et al. 2011). Zinc is vital for general metabolism (King 2011); it improves intestinal health and feed efficacy (Yazhini Prabha et al. 2022), plays a significant function in protein, fat, and carbohydrate metabolism (Kechrid and Bouzerna 2004), and acts as a cofactor for several metalloenzymes (Pandav and Puranik 2015). The use of Nano-ZnO to enhance reproduction is gaining attention since it is bioavailable, has antibacterial properties, and regulates immunity in rabbits (Swain et al. 2015; Mohan and Mala 2019). Increasing the surface area can improve the bioavailability of Nano-ZnO (Hassan et al. 2020). Previous investigations have proven the impacts of low and high zinc levels on rabbit performance. Zinc levels in rabbit diets vary broadly depending on dietary components and zinc supplements, extending from 30 to 400 mg/kg (NRC 1977; Hassan et al. 2017; Ismail and El-Araby 2017).

Therefore, this study highlights the effects of dietary supplementation with Nano-Se, Nano-ZnO, or their mixture on the growth performance, nutrient digestibility, carcass parameters, and blood biochemical and metabolic profile in rabbits.

Materials and methods

Selenium and zinc nanoparticles preparation

Nano-red elemental selenium (Nano-Se) and ZnO nanoparticles were prepared, as previously mentioned in our published articles (Abdel-Wareth et al. 2019; 2022). The structural morphology of the particles was determined using scanning and transmission electron microscope (TEM and SEM) (Figures 1, 2).

Experimental rabbits, design, and feed preparation

This experiment was conducted at the Rabbit Research Unit, Faculty of Agriculture, South Valley University, Qena, Egypt. All experimental procedures were followed and approved by the Experimental Animal Care Committee and the Institutional Committee of Ethics (approval code: SVUAGRI32022).

Animals were cared for using husbandry criteria obtained from South Valley University's standard operating procedures. The study used eighty New Zealand White rabbits—forty males and forty females—each aged 30 days. On average, these rabbits weighed 720 ± 6 grams. Rabbits were assigned to four treatments and 10 replicates per each (one male and one female)/replicate for 60 days. Four experimental diets consisting of 1) control diet, 2) control diet + 40 mg/kg Nano-ZnO, 3) control diet + 0.6 mg/kg Nano-Se, and 4) control diet + 40 mg/kg Nano-ZnO + 0.6 mg/kg Nano-Se. Rabbits were kept in cages measuring 44 cm in width, 50 cm in length, and 35 cm in height, constructed of galvanised wire mesh. These cages were outfitted with automatic drinkers and manual feeders. The rabbits were uniformly housed under comparable housing, sanitation, and environmental conditions throughout the experiment. They were provided with pelleted diets ad libitum and had access to fresh water from automatic nipple drinkers. The components and approximate chemical analysis of the basic diet are mentioned in Table 1 (NRC 1977). The rabbits were reared in an open-house setup characterised by natural ventilation facilitated by windows and ceiling fans. Throughout the experimental phase, the average temperature maintained was 39.5 ± 2 °C, with a relative humidity between 30% and 35%. The room operated on a 16-hour light cycle followed by an 8-hour dark cycle. Daily individual feed intake was meticulously recorded at 8:00 AM, while each rabbit underwent weekly body weight (BW) conducted consistently at 07:00 AM. The feed conversion ratio (FCR) was computed as the daily feed intake divided by the daily body weight gain (DBWG). Incidences of mortality were promptly recorded, and any indications of illness were diligently documented daily.

Digestibility trial

Following the end of the experiment, a four-day digestibility trial was initiated. Each experimental group consisted of ten rabbits, each housed individually in specialised metabolic cages designed for the separate collection of faeces and urine. Throughout this period, the daily intake of feed was meticulously recorded. Faecal samples from each rabbit were gathered, weighed, and preserved at -10 °C until further chemical analysis. Before analysis, these faecal samples underwent a process of partial drying at 60 °C for 48 h. Subsequent analysis of both feed and faecal samples involved the determination of various components, including dry matter (DM), crude protein (CP),

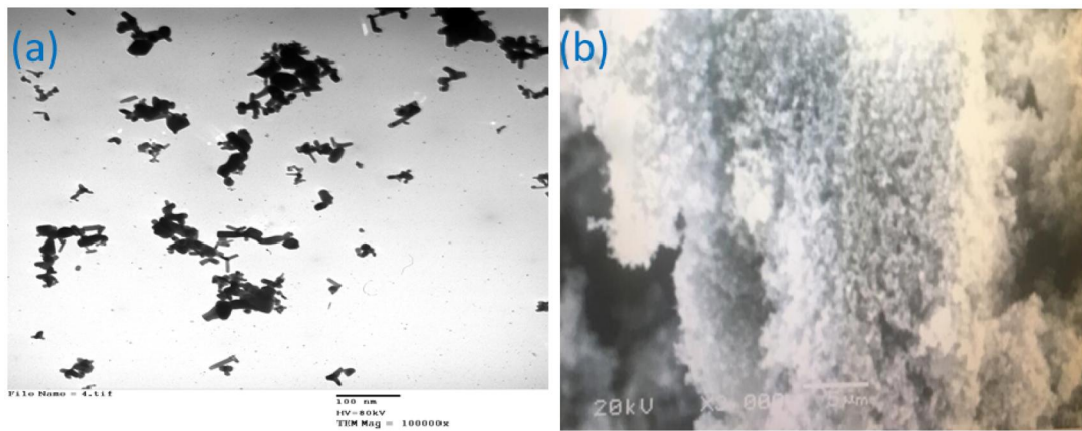


Figure 1. Transmission electron micrographs (TEM) (a) and scanning (SEM) (b) of zinc oxide nanoparticles (Nano-ZnO); 1–100 nm, HV = 80 kv, and TEM mag = 8000 x.

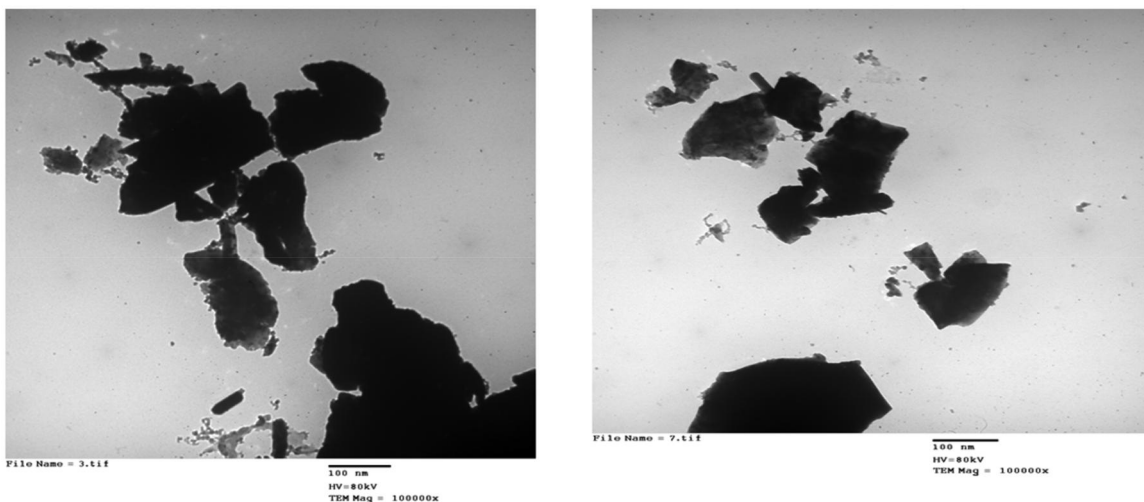


Figure 2. Transmission electron micrographs (TEM) (a) and scanning (SEM) (b) of selenium nanoparticles (Nano-Se); 1–100 nm, HV = 80 kv, and TEM mag = 100,000 x.

ash, and ether extract (EE), following established protocols outlined by the (AOAC 2000). Furthermore, the levels of zinc (Zn), selenium (Se), calcium (Ca), and phosphorus (P) were quantified utilising an Element Analyser equipped with Energy Dispersive X-ray Fluorescence technology (JSX 3222, JEOL, Japan). Gross energy was assessed using adiabatic bomb calorimetry, conducted by Parr Instrument Company based in IL, USA.

Blood biochemical assay

At the end of the experimental period, ten rabbits (five males and five females) from each treatment group underwent anaesthesia *via* intramuscular injection of ketamine and xylazine (AVMA 2013). Subsequently, 10 mL of blood was drawn from the ear vein of each rabbit into EDTA-containing tubes

(K3EDTA; Sigma Company, St. Louis, MO, USA). These blood samples were refrigerated and promptly utilised for hematological parameter assessment following Schalm's method (1962). Additional blood samples were collected in sterile tubes without anticoagulants, centrifuged at 3000xg for 10 min, and the resulting serum was stored at -20°C for subsequent biochemical tests. Liver enzymes (specifically aspartate transaminase (AST) and alanine transaminase (ALT)) and kidney function markers (urea and creatinine) were determined using colorimetric diagnostic kits as per the manufacturer's instructions. Liver samples were obtained from slaughtered rabbits and immediately placed in freezers at -20°C to analyse zinc (Zn) and selenium (Se) content. The Zn and Se concentrations in the rabbits' livers were quantified using an atomic absorption spectrophotometer (Perkin Elmer Analyst 800 model, Shelton, CT, USA).

Table 1. Ingredient and chemical composition of the rabbit diet.

Ingredients	g/kg
Corn	310
Bran of Wheat	200
Soybean meal (440 g/kg CP)	190
Straw of Wheat	120
Hay of Lucerne	50
Bran of Rice	50
Straw of Linseed	28
Meal of Sunflower	25
Limestone	20
Sodium chloride	3
Vitamin-mineral premix ¹	3
DL-Methionine	1
Chemical composition analysed (g/kg)	
Dry matter	934
Digestible energy (DE, MJ/kg DM)	9.5
Crude protein	179
Ether extract	39.4
Crude fibre	121.5
Ash	93.2
Calcium	15.5
Phosphorus	7.63
Zinc	0.09
Se	0.07

¹Source: Ibex International Co., Ltd. Cairo.

Vitamin A 10,000 IU, Vitamin E 50.0 mg, Folic Acid 5.0 mg, Vitamin K 2.0 mg, Vitamin D3 900 IU, Vitamin B1 2.0 mg, Pantothenic Acid 20.0 mg, Vitamin B6 2.0 mg, Vitamin B12 0.01 mg, Choline 1200 mg, Niacin 50 mg, Biotin 0.2 mg, Cu 0.1 mg, Fe 75.0 mg, and Mn 8.5 mg, are all included in the vitamin-mineral premix per kilogram of diet.

Carcass measurements

Following blood collection, the animals were weighed during slaughter to determine their live body weight. The slaughtering process adhered to halal regulations. After slaughtering, the rabbits were bled, and subsequent steps included removing the skin, digestive tract, reproductive organs, urinary bladder, and the distal portion of the legs. The combined weight of the entire head and digestive tract was expressed as a percentage of the total slaughter weight. Additionally, the length of the complete gut was measured. Carcasses were weighed, and various organs (such as testicles, liver, heart, spleen, lungs, kidneys) and the fat surrounding the kidney and shoulder were also weighed and expressed as percentages of the hot carcass weight. The dressing percentage (carcass yield) was calculated by multiplying the hot carcass weight by 100 and dividing it by the body weight.

Statistical analysis

The experimental data were analysed using a randomised complete block design, incorporating a 2 × 4 factorial arrangement involving sex and dietary treatments. The General Linear Model procedures from SAS (SAS Institute, 2005) were employed for this analysis. Specifically, treatment, sex, and the interaction between treatment and sex were considered as

sources of variation. The experimental units for all analyses were the animals. Significance was declared at a threshold of $p < 0.05$. P-values less than 0.001 were expressed as '<0.001' rather than the actual numeric value. To compare means, the Dun-an multiple range tests were applied.

Results

Growth performance

The BW was increased on days 60 and 90 of age by dietary supplementation of Nano-ZnO, Nano-Se, or their mixture, with the highest BW recorded in the mixed diets ($p < 0.001$). On day 90, a significant interaction between the sex and treatments was noticed in body weight ($p < 0.006$). During the 30–60 day and 30–90 day periods, the BWG showed an increase in the rabbits fed diets containing Nano-ZnO, nano-Se, or their mixture with the greatest BWG noted in the mixed supplementation ($p < 0.001$). The FCR was improved due to the dietary inclusion of Nano-ZnO, Nano-Se, or their mixture during the experimental period ($p < 0.001$). The interaction between sex and Treatments showed no effect on the BWG and FCR during the experimental period ($p > 0.05$). The sex of the animals had no impact ($p > 0.05$) on the BWG and FCR. The sex, treatments, and their interaction had no effect ($p > 0.05$) on the total feed intake (Table 2).

Nutrient digestibility

The dry matter, ether extract, and crude protein digestibility were improved by the dietary addition of Nano-ZnO, Nano-Se, or their mixture, with the greatest digestibility documented in the mixed supplementation ($p < 0.01$) (Table 3). The sex and the interaction between sex and treatments did not affect nutrient digestibility ($p > 0.05$).

Carcass criteria

Diets supplemented with Nano-ZnO, Nano-Se, or their mixture significantly improved the dressing percentage ($p < 0.05$). In contrast, the fat-to-BW ratio was decreased ($p = 0.001$) in the groups supplemented with Nano-ZnO, Nano-Se, or their mixture compared to the control group. The sex and interaction between sex and treatments did not affect the dressing-to-live BW ratio ($p > 0.05$). The fat-to-BW ratio was higher in females than males ($p = 0.03$). The sex, treatments, and their interaction did not affect the percentages of

Table 2. Effects of Nano-ZnO, Nano-Se and their combination on growth performance of growing rabbits.

Items	Sex		Treatments				SEM	p-value		
	Male	Female	CON	Nano-ZnO	Nano-Se	Mix		Sex	TRT	Sex x TRT
Body weight, g										
30 days	718	747	714	748	740	730	17.49	0.109	0.562	0.367
60 days	1749	1814	1640 ^c	1801 ^b	1817 ^{ab}	1870 ^a	9.012	0.052	<0.001	0.255
90 days	2753	2833	2605 ^c	2798 ^b	2867 ^a	2902 ^a	21.48	0.056	<0.001	0.006
Body weight gain, g										
30–60 days	34.38	35.56	30.87 ^c	35.08 ^b	35.90 ^{ab}	38.03 ^a	0.579	0.169	<0.001	0.580
60–90 days	33.44	33.96	32.18	33.23	35.00	34.37	0.416	0.519	0.085	0.552
30–90 days	33.91	34.76	31.52 ^c	34.16 ^b	35.45 ^a	36.20 ^a	0.346	0.066	<0.001	0.412
Feed conversion ratio (feed/gain)										
30–60 days	2.56	2.42	2.81 ^a	2.52 ^b	2.39 ^{bc}	2.25 ^c	0.006	0.055	<0.001	0.278
60–90 days	3.09	3.05	3.22 ^a	3.12 ^{ab}	2.97 ^b	2.98 ^b	0.035	0.427	0.031	0.535
30–90 days	2.82	2.78	3.01 ^a	2.80 ^b	2.67 ^c	2.59 ^c	0.034	0.059	<0.001	0.703
Daily Feed Intake (DFI)										
30–60 days	86.96	85.38	86.25	88.08	85.17	85.17	0.552	0.160	0.218	0.916
60–90 days	103.24	102.88	103.20	103.20	103.83	102.00	0.789	0.831	0.894	0.864
30–90 days	95.10	94.13	94.73 ^a	95.64	94.50	93.58	0.552	0.412	0.672	0.951

^{a-b}Means within a row bearing different superscripts differ significantly ($p < 0.05$). SEM, standard error of means. CON: control; Nano-ZnO: nanoparticles of Zinc oxide; Nano-Se: nanoparticles of selenium; Mix: combination of Nano-ZnO and Nano-Se.

Table 3. Effects of Nano-ZnO, Nano-Se, and their combination on nutrient digestibility (%) of growing rabbits.

Items	Sex		Treatments				SEM	P-value		
	Male	Female	CON	Nano-ZnO	Nano-Se	Mix		Sex	TRT	Sex x TRT
Nutrient Digestibility										
DM	63.60	64.02	61.87 ^b	63.69 ^{ba}	64.88 ^a	64.93 ^a	0.03	0.057	0.001	0.367
CP	61.94	62.33	58.47 ^b	62.94 ^a	62.69 ^a	64.46 ^a	0.02	0.677	0.003	0.817
EE	67.39	67.28	63.46 ^b	67.74 ^a	68.85 ^a	69.29 ^a	0.01	0.889	0.004	0.780

^{a-b}Means within a row bearing different superscripts differ significantly ($p < 0.05$). SEM: standard error of means. CON: control; Nano-ZnO: nanoparticles of Zinc oxide; Nano-Se: nanoparticles of selenium; Mix: combination of Nano-ZnO and Nano-Se; DM: dry matter; CP: crude protein; EE: ether extract.

Table 4. Effects of Nano-ZnO and Nano-Se combination on carcass criteria of growing rabbits.

Items	Sex		Treatments				SEM	p-Value		
	Male	Female	CON	Nano-ZnO	Nano-Se	Mix		Sex	TRT	Sex x TRT
Dressing %	53.88	53.35	51.86 ^b	55.03 ^a	52.64 ^{ab}	54.95 ^a	0.478	0.559	0.044	0.845
Liver %	3.59	3.39	3.21	3.62	3.55	3.59	0.073	0.152	0.169	0.609
Heart %	0.36	0.32	0.296	0.357	0.351	0.360	0.014	0.094	0.313	0.472
Kidney %	0.790	0.737	0.791	0.690	0.771	0.802	0.021	0.203	0.242	0.409
Lungs %	0.851	0.751	0.713	0.849	0.857	0.785	0.046	0.344	0.739	0.998
Spleen %	0.053	0.052	0.053	0.056	0.047	0.054	0.003	0.993	0.583	0.017
Fat %	1.21 ^b	1.50 ^a	1.87 ^a	1.36 ^b	1.40 ^b	0.793 ^c	0.100	0.030	0.001	0.830
Caeca %	0.761	0.732	0.662	0.799	0.729	0.795	0.022	0.409	0.042	0.090
Gut %	378.5	381.2	349.7	377	388.2	404.7	0.251	0.855	0.102	0.195
Head %	4.69	4.97	4.48	4.85	4.81	5.19	0.138	0.340	0.428	0.952

^{a-b}Means within a row bearing different superscripts differ significantly ($p < 0.05$). SEM: standard error of means. C: control; Nano-ZnO: nanoparticles of Zinc oxide; Nano-Se: nanoparticles of selenium; Mix: combination of Nano-ZnO and Nano-Se.

head, liver, heart, spleens, kidneys, and small intestine weights ($p > 0.05$) (Table 4).

Sero-biochemical assays and blood haematology

Effects of Nano-ZnO, Nano-Se, and their mixture on blood biochemistry and haematology of rabbits are presented in Tables 4 and 5. Rabbits received Nano-ZnO, Nano-Se, or their mixture exhibited lower serum levels of AST, ALT, cholesterol, triglycerides, creatinine, and urea compared to the control rabbits ($p < 0.05$). Serum total protein level was unaffected by sex, Treatments, or interaction ($p > 0.05$). The sex and interaction between sex and Treatments had no significant

effects on serum biochemical parameters ($p > 0.05$). Furthermore, the sex, Treatments, and interaction did not significantly impact blood haematology criteria.

Concentrations of Zn and Se in the liver

The influence of Nano-ZnO, Nano-Se, or their mixture supplementations on Zn and Se concentrations in the liver of male and female rabbits at 90 days of age is presented in Figures 3 and 4. The addition of Nano-ZnO, Nano-Se, or their mixture did not significantly affect the Zn or Se concentrations in the liver of male and female rabbits ($p > 0.05$).

Table 5. Effects of Nano-ZnO, Nano-Se and their combination on blood biochemistry of growing rabbits.

Items	Sex		Treatments				SEM	p-value		
	Male	Female	CON	Nano-ZnO	Nano-Se	Mix		Sex	TRT	Sex x TRT
AST, u/L	18.03	17.96	20.41 ^a	16.02 ^b	17.21 ^b	18.33 ^b	0.62	0.956	0.008	0.440
ALT, u/L	37.43	38.11	53.12 ^a	40.06 ^b	29.99 ^c	27.89 ^c	2.20	0.644	0.001	0.148
Total protein, mg/dL	5.886	5.956	5.701	6.190	5.980	5.802	1.10	0.756	0.453	0.886
Triglycerides, mg/dL	186.75	184.83	198.67 ^a	186.50 ^b	178.50 ^c	179.50 ^c	1.96	0.274	0.001	0.052
Cholesterol, mg/dL	174.17	176.50	191.67 ^a	170.50 ^{cb}	174.33 ^b	164.83 ^c	2.48	0.403	0.001	0.170
Creatinine mg/dL	1.632	1.748	1.947 ^a	1.437 ^c	1.682 ^b	1.682 ^b	0.05	0.085	0.001	0.054
Urea, mg/dL	54.22	53.80	55.37 ^a	50.95 ^b	52.55 ^b	51.17 ^b	1.12	0.866	0.047	0.859

^{a-b}Means within a row bearing different superscripts differ significantly ($p < 0.05$). SEM, standard error of means. CON: control; Nano-ZnO: nanoparticles of Zinc oxide; Nano-Se: nanoparticles of selenium; Mix: combination of Nano-ZnO and Nano-Se. AST: aspartate transaminase; ALT: alanine transaminase.

Table 6. Effects of Nano-ZnO, Nano-Se and their combination on blood haematology of rabbits.

Items	Sex		Treatments				SEM	p-value		
	Male	Female	CON	Nano-ZnO	Nano-Se	Mix		Sex	TRT	Sex x TRT
Hb (g/dL)	13.11	12.85	12.93	13.28	12.13	13.58	0.17	0.279	0.053	0.831
HCT (%)	42.03	40.96	41.75	42.38	38.75	43.10	0.54	0.269	0.068	0.883
RBCs ($\times 10^6/\mu\text{L}$)	6.84	6.63	6.80	6.97	6.18	7.00	0.10	0.205	0.070	0.855
MCV (fL)	61.49	61.72	61.38	60.85	62.60	61.60	0.40	0.787	0.542	0.551
MCH (Pg)	19.21	19.37	19.02	19.10	19.62	19.42	0.15	0.598	0.465	0.294
MCHC (%)	31.22	31.38	30.97	31.37	31.33	31.52	0.09	0.373	0.183	0.224
RDW-CV	16.68	16.00	16.27	16.32	16.03	16.75	0.17	0.043	0.451	0.416
TLC ($\times 10^3/\mu\text{L}$)	8.35	8.08	8.30	8.28	8.27	8.02	0.18	0.535	0.957	0.787
Platelet count	256.3	264.4	265.0	255.5	261.5	259.5	4.01	0.235	0.779	0.610
MPV	7.88	8.10	8.03	7.75	8.42	7.75	0.22	0.640	0.736	0.729
PDW	11.03	11.66	11.30	11.08	12.07	10.93	0.24	0.203	0.368	0.596
PCT	0.25	0.24	0.255	0.242	0.238	0.237	0.01	0.416	0.818	0.319
Segmented-N	26.25	26.33	25.97	26.83	26.17	27.00	0.59	0.950	0.754	0.799
Lymphocytes ($\times 10^3/\mu\text{L}$)	42.95	43.17	43.83	43.33	43.50	42.17	0.86	0.245	0.197	0.304
Monocytes ($\times 10^3/\mu\text{L}$)	7.92	8.33	8.17	8.67	7.67	8	0.17	0.242	0.260	0.668
Eosinophils ($\times 10^3/\mu\text{L}$)	4.75	4.50	4.33	4.50	4.67	5	0.13	0.379	0.393	0.801

^{a-b}Means within a row bearing different superscripts differ significantly ($p < 0.05$). SEM, standard error of means. CON: control; Nano-ZnO: nanoparticles of zinc oxide; Nano-Se: nanoparticles of selenium; Mix: combination of Nano-ZnO and Nano-Se; RBCs: red blood cells; Hb: haemoglobin; HCT: haematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; TLC: total leucocytic count; MPV: mean platelet volume; PDW: platelet distribution width; PCT: prolactin; Segmented-N: segmented neutrophils.

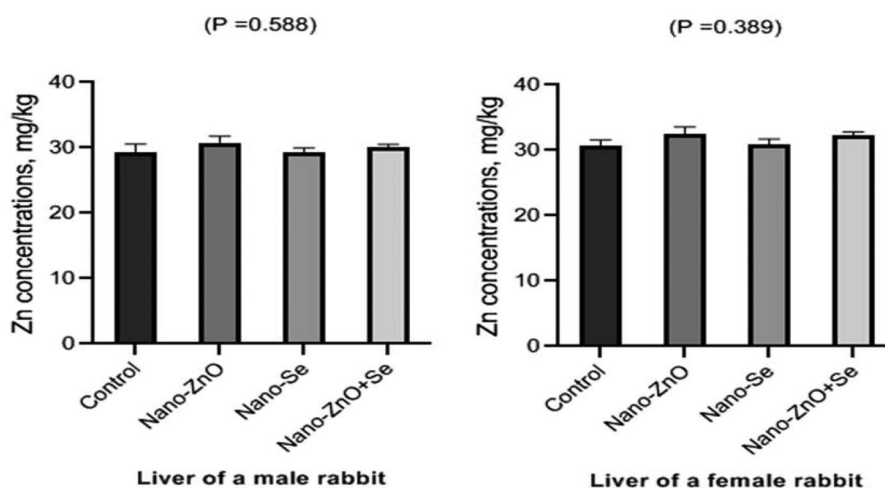


Figure 3. Impact of Nano-ZnO, Nano-Se or their combination supplementations on Zn concentrations in male and female rabbits' liver at 90 days of age.

Discussion

Zinc and selenium have crucial biological functions for animal growth, physiological activities and carcass criteria (Marai et al. 2003; Bao et al. 2007; Suttle 2010).

Zn or Se can be used in nanoparticle size at lower quantities, providing better outcomes than conventional sources owing to their higher bioavailability

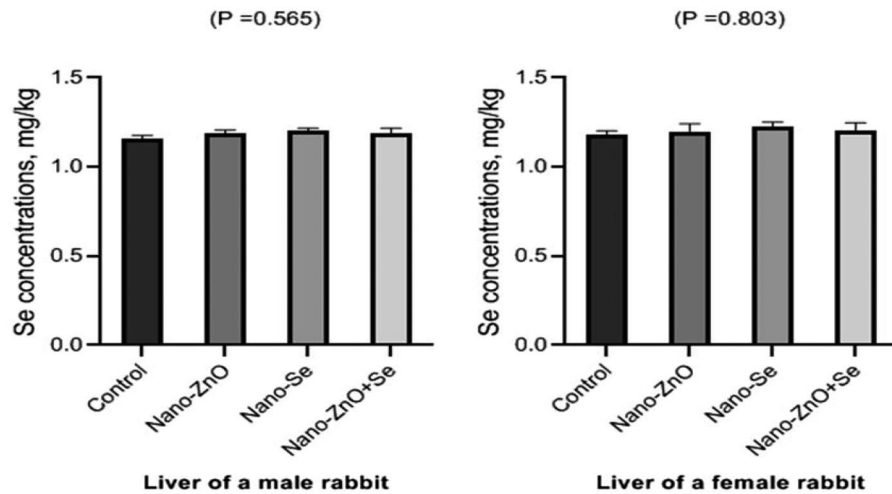


Figure 4. Impact of Nano-ZnO, Nano-Se or their combination supplementations on Se concentrations in male and female rabbits' liver at 90 days of age.

and thus enhancing the rabbits' productive performance (Abdel-Wareth et al. 2022).

The present study showed improved growth performance (BW, BWG, and FCR) by adding Nano-ZnO, Nano-Se, or their combination at 30 to 90 days of age. This improvement may be related to Se and Zn, essential for various animal physiological functions, including antioxidant defense, immune function, reproductive health, thyroid function, and muscle development. Ensuring adequate Se and Zn intake through diet or supplementation is crucial for maintaining the growth performance of rabbits. Se and Zn elements have antioxidant activities and can enhance nutrient digestion and absorption (Brenes and Roura 2010; Verma et al. 2012), increasing rabbits' growth and productivity. Zhang et al. (2011) reported increased BWG and reduced FCR in rabbits by dietary addition of 0.70 mg/kg Se. Dietary addition of Zn or Se significantly increased live BW and feed intake of rabbits under heat stress (Yan et al. 2017; Kamel et al. 2020). Higher BW, BWG, and feed intake were reported in rabbits (Al-Khafaji and Saidi 2019; Emara 2019; Tag-El Din 2019) and broilers (Mahmoud et al. 2016) by dietary addition of Nano-Se. Abd Allah et al. (2020) stated that dietary supplementation of 0.3 mg/kg Nano-Se for growing rabbits increased the BW and BWG. Abdel-Wareth et al. (2019) recorded increased BW and BWG in rabbits by adding Nano-Se at 400 µg/kg with or without 700 mg/kg garlic oil. Sheiha et al. (2020) showed increased BWG and improved FCR of rabbits by dietary addition of 25 and 50 mg of Nano-Se/kg. Similarly, Hassan et al. (2017) suggested that dietary addition of 75 and 100 mg/kg nano-Zn can improve daily BWG and FCR. Uniyal et al. (2017) found that BWG and FCR significantly improved by

supplementing rabbit diets with Nano-ZnO at 20 mg/kg. In the study of Moustafa et al. (2024), all levels of supplemental nano-Se (0.02, 0.03, 0.04 and 0.05 mg/kg diet) increased the BW, daily WG, and improved FCR of rabbits.

Regarding the digestibility in rabbits, the dietary addition of Nano-ZnO, Nano-Se, or their mixture increased the digestibility of DM, CP, and EE. The synergistic response of the Nano-ZnO and Nano-Se combination was detected in the nutrient digestibility compared to the control or single effects. These enhancements might be attributed to the increased bioavailability of Nano-ZnO and Nano-Se, which also can increase nutrient absorption. Furthermore, Se and Zn are essential for the synthesis and metabolism of thyroid hormones. Thyroid hormones play a role in lipid metabolism, including the synthesis, breakdown, and clearance of cholesterol and triglycerides from the bloodstream. Adequate selenium levels can support optimal thyroid function, which in turn may help regulate lipid levels. Hassan et al. (2017) reported increased crude protein and ether extract digestibility in rabbits fed a diet supplemented with 100 mg/kg nano-ZnO. Elsisy et al. (2017) found improved crude protein digestibility in male rabbits fed 100 mg/kg ZnO. In contrast, Tag-El Din (2019) found no significant effects of Nano-ZnO supplementation at 30 and 60 mg/kg on the digestibility of DM, CP, EE, and crude fibre (CF) in rabbits. Moustafa et al. (2024) showed increased digestibility of nitrogen free extract and crude fibre in rabbits by supplemented nano-Se (0.02–0.05 mg/kg diet).

Uniyal et al. (2017) documented that supplementation of different Zn sources (ZnSO₄, Nano-ZnO, and Zn-Me) at 20 mg/kg diet didn't affect the nutrient digestibility of DM, CF, CP, and EE of guinea pigs.

Nano-Se supplementation at 400 µg/kg of diet improved the digestibility of DM, organic matter (OM), CP, and CF in Californian male rabbits (Yang et al. 2012). On the other hand, Amer et al. (2019) reported no significant differences in the digestion coefficient of DM, OM, and CP between the control group and selenium sources groups.

In the current study, diets supplemented with Nano-ZnO, Nano-Se, or their combination improved dressing percentage. At the same time, the fat weights relative to live BW were reduced without any side effects on the liver, spleen, heart, and kidney. Hassan et al. (2017) reported increased hot carcass weight and dressing percentage and reduced liver weight in rabbits fed a diet with 75 and 100 mg/kg Nano-ZnO. Tag-El Din (2019) documented increased carcass traits, internal organs, and heads of rabbits by Nano-ZnO and Nano-Se supplementation. Saleh and Ebeid (2019) reported improved carcass traits and decreased internal organs of broilers by dietary supplementation of Nano-Se. Sheiha et al. (2020) reported increased rabbits' dressing and internal organs weights by Nano-Se addition. Moustafa et al. (2024) reported increased hot carcass weight and dressing percentage by dietary supplemental nano-Se (0.02-0.05 mg/kg diet).

In this study, rabbits fed Nano-ZnO, Nano-Se, or their combination exhibited lower serum levels of ALT, AST, cholesterol, triglycerides, urea, and creatinine without any side effects on hematological parameters. These findings indicated that the dietary addition of Nano-Se might improve liver and kidney functions in rabbits. Se and Zn act as components of enzymes such as glutathione peroxidase, which helps counteract free radicals and decrease oxidative stress. Oxidative stress can contribute to the oxidation of lipids (fats) in the bloodstream, leading to increased levels of cholesterol and triglycerides (McDowell 1992; Paik et al. 1999; Chrastinová et al. 2018). By reducing oxidative stress, selenium may help prevent lipid oxidation and lower blood lipid levels. In addition, Se and Zn have been shown to have anti-inflammatory properties. Chronic inflammation can contribute to dyslipidemia (abnormal lipid levels), including elevated cholesterol and triglycerides (McDowell 1992). By reducing inflammation, selenium may help mitigate this aspect of dyslipidemia. These biochemical upgrades in the serum of male rabbits are because Zn and Se are components of many enzymes that keep the structural integrity of proteins and are essential components of approximately 200 metalloenzymes (McDowell 1992; Paik et al. 1999; Chrastinová et al. 2018) and hormones, such as testosterone, thymulin, somatomedin, and prolactin (Zapsalis et al., 1985). El-Hamid et al. (2018) noted

reduced serum AST in male rabbits that drank water supplied with 75 and 100 mg Zn/liter. El-Katcha et al. (2017) noticed reduced serum AST levels in laying hens fed diets complemented with a 30 mg Nano-ZnO/kg diet. Se and Zn are essential trace elements with significant roles in liver health. Selenium supports antioxidant enzyme function, while zinc contributes to various liver enzymes. Together, they promote optimal liver function and the overall well-being of rabbits. Remember to consult a healthcare professional for personalised recommendations. In the current study, the addition of Nano-ZnO, Nano-Se, or their mixture did not significantly affect the Zn or Se concentrations in the liver of male and female rabbits.

Interestingly, the addition of Nano-Zn or Nano-Se resulted in normal Zn or Se concentrations in the rabbit liver as a control group. Nevertheless, there is a gap in the literature regarding the impact of the combined administration of Nano-ZnO and Nano-Se on Zn and Se levels in rabbit liver. A prior investigation demonstrated that Nano-ZnO displayed potent antimicrobial properties against resistant bacteria, exhibited biocompatibility with animal cells, and maintained long-term stability (Elsisi et al. 2017; Abdel-Wareth et al. 2023). This could possibly describe the synergistic effects detected in our study, wherein the combination of Nano-ZnO and Nano-Se contributed to improving productive performance without remaining in rabbit tissues. Nano-ZnO and Nano-Se have emerged as promising additives in rabbit nutrition. Research indicates that organic selenium sources, such as selenium yeast, demonstrate superior absorption and retention in muscle tissues compared to their inorganic counterparts, like sodium selenite (Amer et al. 2019). The incorporation of organic selenium enhances the antioxidant capacity of meat tissue and contributes to improved meat quality. Nano-ZnO enhances bioavailability, immuno-modulation, and antimicrobial effects which could be the reason for improving serum cholesterol levels and liver function. Nano-Se, converted into selenite, contributes to selenoprotein synthesis, with lower toxicity than other selenium forms.

Furthermore, the zinc levels in rabbits treated with Nano-ZnO serum and testis tissues were notably higher than those in the control group (Abdel-Wareth et al. 2023).

Conclusions

From the obtained results, we suggest that dietary Nano-ZnO (40 mg/kg), Nano-Se (0.6 mg/kg), or their

combination can improve the growth performance, feed conversion ratio, and dressing weights of rabbits by enhancing nutrient digestibility. Dietary supplementation of Nano-ZnO, Nano-Se, or their mixture had hypolipidemic effects by reducing the serum cholesterol and triglyceride levels. Nano-ZnO, Nano-Se, or their combination impacted the liver and kidney functions. However, the levels of Zn and Se in the liver were unaffected. Nanoparticle-supplemented diets represent an innovative advance for sustainable rabbit production due to their high surface area, encapsulation capabilities, and targeted delivery to confirm the effective absorption and utilisation of nutrients.

Ethical approval and consent to participate

The present study obtained approval from the Ethical Committee for live bird sampling at the Department of Animal and Poultry Production, Faculty of Agriculture, South Valley University (SVUAGRI32022). All procedures adhered strictly to the pertinent guidelines and regulations.

Author's contributions

Conceptualisation, A.A.A.A-W., A.H.H.A, Formal analysis, A.A.A.A-W., A.S.N.K., and A.H.H.A, Methodology, A.A.A.A-W., A.S.N.K., and A.H.H.A., S.A.A., E.M.Y., A-W.A.A-W; Writing – original draft, A.A.A.A., S.A.A., A.S.N.K., and A.H.H.A, Writing – review & editing, A.A.A.A-W., S.A.A. and J.L. The manuscript has been read and approved by authors.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data that support this study will be shared upon reasonable request to the corresponding author.

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