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GROWTH PERFORMANCE AND CARCASS RESPONSE OF RABBITS FED ON DIETS SUPPLEMENTED WITH DIFFERENT LEVELS OF SILVER NANO PARTICLES.

Hoda M. El-Hosseiny[1] ; G. F. Shaheen[1,*] ; Kout-Elkloub M. Moustafa[2] ; G.E. Younan[3] ; E. M. El- Kotamy[1] ; A. Ghoniem[1] ; M. M. El-Nahrawy[1] ; Mona E. Farag[1] ; A. A. Abdou[1] and M. E. Sayed-Ahmed[1]

1 Department of Animal Nutrition Research. 2 Department of Poultry Nutrition Research 3 Department of rabbits, Turkey and waterfowl Breeding Research, Animal Production Research Institute (APRI), Agricultural Research center (ARC), Dokki, Giza, Egypt.

[*]E-mail-G.F Shaheen, gamal.shaheen@arc.sci.eg.

ABSTRACT

Fifty male New Zealand White rabbits, aged 6 weeks and average live body weight of 710.74 ± 10 g, were randomly assigned to five groups (10 rabbits each) and fed basal diets containing varying levels of Silver Nano Particles (Ag NPs) for a 14-weeks experimental period. This study evaluated the efficacy of using different levels of Ag NPs as dietary supplementation in growing rabbits.

A completely randomized design was employed, with the first group G1 (control) receiving the basal diet without supplementation. Rabbits in the other groups G2, G3, G4, and G5 were fed the basal diet supplemented with Ag NPs at levels of 1.5, 2.0, 2.5, and 3.0 mg/kg of diet, respectively.

The obtained results indicated that the highest values of live body weight, daily weight gain, hot carcass weight, and dressing percentage were observed in rabbits fed diets supplemented with Ag NPs across all levels compared to the control group. However, nutrient digestibility and nutritive values were not significantly affected by the nanoparticle supplementation. Additionally, feed conversion ratios, net revenue, and economic efficiency values improved in the groups receiving Silver Nano Particles when compared to the control group.

Our findings suggest that the Silver Nano Particles in rabbit diets positively influenced performance and economic efficiency compared to the control group. The optimal results were obtained at 2 mg Ag NPs/kg of diet.

Key Words: Rabbits, Ag NPs, Productive performance and Economic efficiency.

INTRODUCTION

Nanomaterials and nanoparticles are key components of nanotechnology, typically ranging in size from 1 to 100 nanometers in at least one dimension **(Ahmadi and Kordestany, 2010)**. The size of these particles is crucial because it influences the action of nanoparticles **(Mikhailova, 2020)**. Nanoparticles smaller than 300 nm can circulate within the body, while those smaller than 100 nm can more easily and quickly reach target cells, tissues, and organs **(Choi and Hu, 2008; Prabhu and Poulose, 2012)**. This small size also helps reduce the presence of residual compounds and decreases the withdrawal time of drugs in livestock tissues **(Traoncarelly *et al.,* 2018)**. In medicine, nanoparticles can be used to target specific tissues while minimizing side effects and toxicity **(Sadr *et al.,* 2023)**. Nanoparticles can be administered through drinking water, feed, injections, or inhalation **(Salleh *et al.,* 2020)**. Recently, nanotechnology has been widely applied in animal nutrition to help explain metabolic and physiological mechanisms, improve fiber digestion, promote health, and enhance animal production overall **(Rajendran *et al.,* 2014)**. When used as supplements, nano-minerals offer several advantages over traditional minerals, including higher bioavailability at lower doses, larger surface areas, higher surface activity, and stronger adsorption capabilities **(Chaudhry and Castle, 2011 ; Shi *et al.,* 2011; Albanese *et al.,* 2012 and Rajendran *et al.,* 2014)**. Silver nanoparticles (Ag-NPs) play a significant role in animal production due to their disinfectant and antimicrobial properties. Ag-NPs had a potent inhibitory effect against *E. coli* growth in in vitro trail **(Awaad *et al.,* 2021)**. While in in vivo trail, decreased the number of harmful bacteria represented as *E. coli* and had no effect on microflora represented as lactobacillus **(Moustafa *et al.,* 2015).** Also, it used as drugs to combat bacteria, fungi, and viruses **(Sawosz *et al.,* 2007 ; Zhang *et al.,* 2016 and Ali, 2019)**. Moreover, Ag-NPs are known to have anticancer properties **(Hamed *et al.,* 2017 and Salem *et al.,* 2021)** and are seen as alternatives to antibiotics in the fight against drug-resistant bacteria **(Hill and Li, 2017 and Zhang *et al.,* 2016)**. Silver nanoparticles help reduce ammonia and nitrogen oxide emissions **(Xu *et al.,* 2013)**, aid in regulating rumen fermentation and overcoming reproductive challenges in animals **(Gopi *et al.,* 2017)**. **Moustafa *et al.,* (2015)** investigated adding silver nanoparticles to broiler chicken diets (2, 4, 6, 8 and 10 ppm/kg) improved body weight, body weight gain and feed conversion ratio . In addition, cholesterol & total lipids were significantly decreased ,and increased total serum antioxidant capacity . Also, increase the European Production Efficiency Index (EPEI) and generally, the best level was 4 mg/kg diet. **Ahmed *et al.,* (2023)** found that adding Ag-NPs to chicken diets enhanced performance, improved nutrient utilization, and boosted economic efficiency. Other studies found that supplemented with Ag-NPs had no significant effect on chicken performance when it added at a ratio of 0, 4, 8 or 12 ppm in drinking water by **Ahmadi and Rahimi (2011),** at 0,10 and 25 ppm by **Pineda *et al.,* (2010),** at 50 ppm by **Vadalasetty *et al.,* (2018)**. **Al-Sultan *et al.,* (2022)** suggested that high doses of Ag-NPs (20 mg/kg) might reduce protein digestion and inhibit the absorption of sugars and amino acids, leading to lower body weight and organ mass in broilers. Similarly, **Tammam *et al.,* (2023)** noted no significant impact of silver or graphene nanoparticle supplementation on carcass characteristics or immune organs in broilers. While **Loghman *et al.,* (2012),** observed liver damage at high doses (12 ppm) of Ag-NPs of broiler chicks. In rabbits, **Kotb *et al.,* (2024)** reported that supplementing diets with Ag-NPs, especially at 1 mg/kg, improved body weight, weight gain, and feed efficiency. Lower doses (0.25 mg/kg) enhanced liver and kidney function, lipid profile, immunity, antioxidant capacity, and digestive enzyme activity while promoting beneficial gut bacteria with rabbits. **Abdelsalam *et al.,* (2019)**, found no significant impact on daily gain or feed intake when rabbits were injected with Ag-NPs at doses of 0.5 mg and 1 mg/kg body weight. However, they noted that plasma cholesterol and triglyceride levels were lower in treated rabbits compared to the control ration, and the accumulation of silver in the blood and meat increased with higher doses. There is a lack of findings on experimental utility of Nano particles, especially Silver nitrate for rabbit production. So, the present study was aimed to evaluate the effect of using different levels of Ag NPs as dietary supplementation on nutrient digestibility, growth performance, carcass traits, some blood plasma biochemical, histological features of liver, kidney and economic efficiency of growing New Zealand White rabbits during the fattening period (6-14 weeks of age).

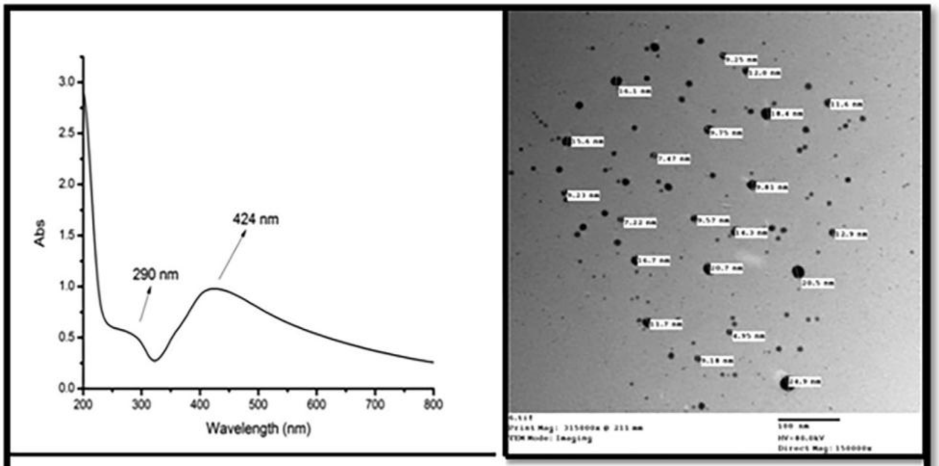
MATERIAL AND METHODS

Ethical Approval

This study was conducted at the Sakha Experimental Research Station in Kafr El-Sheikh, under the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt. The study followed all ethical guidelines for animal welfare, in line with the European Union's regulations (2010/63/EU) on the protection of animals used in experiments. The study was officially registered with the serial number (372429).

Preparation and characterization:

Silver nanoparticles (Ag-NPs) were chemically prepared in the Poultry Nutrition Department's laboratories at the Animal Production Research Institute. The process involved reducing silver nitrate (AgNO3) using diluted aqueous solutions that contained cetyl trimethyl ammonium bromide and hydrazine hydrate as a dispersing agent to produce Ag-NPs. A colour change was observed during the mixing process, indicating successful nanoparticle formation, as described by **Khan *et al.*, (2011) and Moustafa *et al.*, (2015)**. To confirm the synthesis of Ag-NPs, a UV-visible spectrophotometric analysis was performed. This analysis, conducted at the National Research Center in Dokki, Egypt, showed a peak wavelength of 424 nm (Fig.1), a key indicator that silver nitrate (AgNO3) had converted to nanoparticles (Ag-NPs). Additionally, the size and distribution of the particles were examined using a transmission electron microscope (TEM), revealing that the nanoparticles ranged in size from 5 to 25 nm and their size distribution was relatively wide (Fig.1). Flame atomic absorption spectrometry (Agilent Technologies 200 Series AA, Agilent Technologies, Santa Clara, CA) was used to measure the concentration of Ag-NPs at wavelength 328.1 nm. When silver particles reach nano-size, they exhibit a gray colour due to surface plasmon resonance **(Govindaraju *et al.*, 2009)**, which was confirmed with an absorption peak at 424 nm. The analysis, conducted using the JEM 1400 microscope at Cairo University, confirmed the successful synthesis of Ag-NPs. The concentration of Ag-NPs was found to be 2.15 g/L. Different feed concentrations of Ag-NPs were then prepared at levels of 1.5, 2, 2.5, and 3 mg/kg of diet.



UV-Visible spectrophotometer analysis of Ag-NPs synthesized. Abs: absorbance

Transmission Electron Microscopy (TEM) image of Ag-NPs.

Figure 1. UV-visible spectrophotometer analysis and Transmission Elctron Microscopy of Ag-NPs.

Animals and management:

Fifty weaned male New Zealand White rabbits, each around six weeks old with an average live body weight of 710.74±10 g, were randomly divided into five groups (10 rabbits per group). Each group was fed a different level of Silver Nanoparticles (Ag-NPs) for the entire experimental period, which lasted at 14 weeks of age. The rabbits were housed individually in battery cages (30 × 60 × 40 cm) located in a naturally ventilated building. The environmental conditions included a temperature range of 22-25°C, relative humidity of 60-70%, and a lighting schedule of 14 hours of light and 10 hours of darkness. The experiment took place from November to December , and all rabbits were maintained under the same management, hygiene, and environmental conditions throughout the study.

Experimental diets:

Five experimental treatments were used, with a completely randomized design. The first group (G1), serving as the control and it was fed a basil diet without any Ag-NPs supplements to meet maintenance and production requirements of rabbits, as mentioned in **NRC guidelines (1977).** The other four groups (G2, G3, G4, and G5) received the same basal diet with added Ag-NPs at levels of 1.5, 2.0, 2.5, and 3.0 mg/kg of diet, respectively. The rabbits were fed *ad-libtum* and fresh water was available at all times through an automatic system. The composition and calculated analysis of the basal diet are presented in Table 1.

Table 1: The composition and calculated analysis of the basal diet.

Ingredient	%	Calculated analysis	
Berseem hay	30.05	Crude protein (%)	17.75
Barley	24.60	Digestible energy kcal/kg	2500
Wheat bran	21.50	Crude fiber (%)	15.38
Soybean meal (44% CP)	17.50	Ether extract (%)	2.27
Molasses	3.00	NFE (%)	54.16
Di-calcium phosphate	1.60	Ash (%)	10.44
Limestone	0.95	Calcium (%)	1.24
Sodium chloride (NaCl)	0.30	Total phosphorus (%)	0.80
Vitamin & mineral mixture*	0.30	Lysine (%)	0.98
DL-Methionine	0.20	Methionine (%)	0.46
		Methionine + Cysteine (%)	0.76
		Sodium (%)	0.16

*Each kilogram of diet contained : Vitamin A, 6000 IU; Vitamin D_3, 900 IU; Vitamin E, 40 mg; Vitamin K_3, 2 mg; Vitamin B_1, 2 mg; Vitamin B_2, 4 mg; Vitamin B_6, 2 mg; Pantothenic acid, 10 mg; Vitamin B_{12}, 0.01 mg; Niacin, 50 mg; Folic acid, 3 mg; Biotin, 0.05 mg; Choline, 250 mg; Fe, 50 mg; Mn, 8.5 mg; Cu, 5 mg; Co, 0.1 mg; Se, 0.1 mg; I, 0.2 mg and Zn, 50 mg.

Digestibility trials:

At the end of the experimental period, a digestibility trial was conducted to assess the rabbits' nutrient digestibility. This involved five separate digestion experiments, one for each group, using all rabbits from each group when they reached 14 weeks old. Each rabbit was housed individually in metabolic cages (60 × 50 × 40 cm) designed to separate feces from urine. Feces were collected daily and treated with a 2% boric acid solution to trap ammonia for five days. After collection, the feces were dried at 60°C for 48 hours, ground into fine powder, mixed thoroughly to ensure uniformity, and stored for analysis according to **AOAC (1995)** guidelines.

Growth performance:

The rabbits live body weight (LBW) and feed intake were recorded monthly, weight gain (difference between final and initial weight/period), and feed conversion ratio (FCR) (dividing daily feed intake by daily weight gain) were calculated.

Carcass traits:

At the end of the study, three rabbits from each group were randomly taken and fasted for 16 hours before slaughter. Carcass traits were evaluated, including carcass weight, edible organs (liver, heart, kidney, spleen), total non-carcass fat %, fur, and dressing %, following the method described by **Hassan *et al.*, (2016)**.

Blood sampling:

During slaughtering, blood samples (5 ml/each sample) were taken individually from three rabbits in each group (15 samples). Blood samples were collected in dry clean centrifuge tubes containing a few drops of heparin solution and centrifuged at 3000 rpm for 20 minutes to separate blood plasma and stored in a deep freezer at approximately -20^{o}C until the time of analysis to estimate blood parameters **(Kairalla *et al.*,2022)**.Blood plasma samples were determined by spectrophotometer (Spectronic 21 DUSA) using commercial diagnostic kits supplied by Bio Merieux, France, following the same steps described by manufacturers. The blood plasma was measured for total protein , albumin , glucose, urea, creatinine, AST, ALT, cholesterol, HDL, LDL, triglycerides, T_3 and T_4.

Histological study:

Representative samples were taken from the medial part of the liver and kidney of slaughtered male rabbits from each group (1 sample /rep.). Samples were placed in 10% buffered formalin and paraffin-embedded. Sections were stained with hematoxylin and eosin (H&E) stains and histologically examined using a light microscope (Optico XSZ-107B Binocular, China) at x200 magnifications **(Bancroft *et al.*, 1996)**.

Economic efficiency:

Economic efficiency was calculated by comparing the income from weight gain to the cost of feed consumed at 14 weeks of age.

Net revenue (LE) = Total revenue – Total feed cost.

Economic efficiency % = (Net revenue (LE)/ Total feed cost (LE)) *100.

Ingredient prices and the market price of live rabbits (100 Egyptian Pounds LE, per kilogram) were based on local market conditions in January 2022.

Statistical Analysis:

The data were analyzed using a one-way analysis of variance (ANOVA) based on a Completely Randomized Design, applying the General Linear Models procedure **(SAS, 2009)**. The model used for statistical was as follows:

$$Yij=\mu+Ti+eij$$

Where:
Yij = The observation,
µ = Overall mean,
Ti = Effect of treatments (i: 1 to 5),
eij = random error, assumed to be normally distributed.

Duncan's multiple range test **(Duncan, 1955)** was used to detect significant differences among treatment means.

RESULTS

Nutrients digestibility and nutritive values:

Results in Table 2 show that the rabbit fed $G_{3,}$ supplemented with Ag NPs, had the highest nutrient digestibility percentages across all nutrients. Rabbits followed this in group G_4, then group G_2 and finally the animals fed the control rations G_1. The lowest digestibility values were in the G_5 group, fed the highest level of Ag NPs. However, the differences in nutrient digestibility among these groups were not statistically significant. Similarly, the nutritive values, expressed as Total Digestible Nutrients (TDN) and Digestible Crude Protein (DCP), were not significantly impacted by the supplementation of Ag NPs at any level when compared to the control group.

Table 2: Nutrients digestibility and nutritive values of rabbit's diet with Nano Silver supplementation.

Item	**Experimental groups**				
	G_1	**G_2**	**G_3**	**G_4**	**G_5**
Digestibility (%)					
DM	**64.84±1.68**	**65.51±3.17**	**66.41±5.20**	**65.65±2.84**	**64.20±3.58**
OM	**71.83±0.94**	**72.66±3.74**	**73.68±3.79**	**72.96±2.24**	**71.56±3.54**
CP	**65.17±2.30**	**65.25±3.39**	**66.09±3.73**	**65.71±4.53**	**64.01±3.28**
EE	**76.17±4.18**	**76.39±5.80**	**76.84±5.50**	**76.57±6.84**	**75.90±2.49**
CF	**52.33±1.40**	**52. 43±2.13**	**53.69±1.77**	**52.96±1.80**	**52.14±2.45**
NFE	**78.17±4.43**	**78.83±3.72**	**79.51±4.78**	**78.93±4.23**	**78.08±5.29**
Nutritive values (%)					
TDN	**65.85±1.14**	**66. 23±2.70**	**66.96±2.38**	**66.46±1.40**	**65.57±3.16**
DCP	**11.57±0.24**	**11.58±0.84**	**11.73±0.75**	**11.66±0.87**	**11.63±0.91**

G_1: Control contained zero Ag NPs of diet. G_2: Contained 1.5 mg/kg Ag NPs of diet. G_3: Contained 2 mg/kg Ag NPs of diet. G_4: Contained 2.5 mg/kg Ag NPs of diet. G_5: Contained 3 mg/kg Ag NPs of diet. DM: Dry matter. OM: Organic matter. CP: Crude protein. EE: Ether extract. CF: Crude fiber. NFE: Nitrogen free extracts. TDN: Total digestible nutrients. DCP: Digestible crude protein.

Growth performance:

The Results presented in Table 3 indicated that rabbits fed diets supplemented with Ag NPs had significantly higher average live body weight

(LBW) ($p \leq 0.05$) compared to those in the control group (G_1) by the end of the 14-weeks. Whereas, the best average LBW was recorded with G_3, during the same period. Average daily gain (g/head) was increased ($p \leq 0.05$) with Ag NPs supplementation within different levels and rabbit ages in comparison with those in the control group (G_1). Rabbits receiving 2 mg/kg of Ag NPs (G_3) showed the highest daily weight gain compared to other groups. The results also reveal that the average daily feed intake (g/head/day) was a slight increase in rabbits supplemented with Ag NPs compared to the control group during the periods from (6 to 10) and (10 to 14) weeks of age. Moreover, a significant improvement in feed conversion ratio ($p \leq 0.05$) was observed in rabbits fed diets supplemented with silver nanoparticles compared to the control group during the experimental period. The best feed conversion ratios were observed in group G3, which fed diets supplemented with 2 mg/kg of silver nanoparticles compared with the other treatments. Overall, rabbits fed 2 mg/kg of Ag NPs (G_3) demonstrated improved feed intake and the best feed conversion ratio compared to all other treatments.

Table 3 Rabbits performance values as affected by different levels of Nano silver supplementation at different ages

Item	Experimental groups					
	G_1	G_2	G_3	G_4	G_5	±SEM
Live Body Weight, g						
Initial Weight	717	708.5	696.0	712.7	719.5	35.47
at 10 weeks	1311.0^{b}	1393^{ab}	1444.5^{a}	1439.5^{a}	1360.5^{ab}	27.99
at 14 weeks	1921.16^{c}	2111^{ab}	2213.5^{a}	2157^{ab}	2024^{b}	40.42
Daily weight gain, g/head						
6-10 weeks	21.19^{c}	24.45^{ab}	27.95^{a}	25.96^{ab}	23.03^{b}	0.99
10-14 weeks	21.79^{d}	25.64^{ab}	26.72^{a}	25.63^{ab}	23.77^{c}	1.45
6-14 weeks	21.49^{c}	25.05^{ab}	27.33^{a}	26.09^{ab}	23.50^{b}	0.75
Daily feed intake, g/head/day						
6-10 weeks	61.82	62.25	62.21	62.14	61.73	0.90
10-14 weeks	102.84	105.32	106.50	105.68	104.61	1.03
6-14 weeks	82.33	83.79	84.36	83.91	83.17	0.78
Feed conversion ratio						
6-10 weeks	2.92^{a}	2.55^{cb}	2.23^{c}	2.39^{bc}	2.68^{b}	0.11
10-14 weeks	4.72^{a}	4.11^{ab}	3.99^{b}	4.12^{ab}	4.40^{a}	0.24
6-14 weeks	3.83^{a}	3.35^{bc}	3.09^{c}	3.22^{bc}	3.54^{b}	0.09

$^{a\ ,b,c,d}$ means in the same row with different superscripts are significantly different ($P \leq 0.05$).

G_1: control contained zero Ag NPs of diet. G_2: contained 1.5 mg/kg Ag NPs of diet. G_3: contained 2 mg/kg Ag NPs of diet. G_4: contained 2.5 mg/kg Ag NPs of diet. G_5: contained 3 mg/kg Ag NPs of diet. Feed conversion ratio= feed intake, g / daily weight gain, g

Carcass traits:

The results in Table 4 indicate that both the hot carcass weight and dressing percentage were significantly ($p \leq 0.05$) higher in rabbits supplemented with Ag NPs compared to the control group. The highest values for these parameters were observed in Groups G3 and G4, supplemented with 2 and 2.5 mg Ag NPs per kg of diet, respectively. For the relative weights of the kidney, liver, heart, spleen, giblets, and fur, no significant differences were noted among the experimental groups, except for the spleen, which showed a significant difference ($p \leq 0.05$) between groups. Conversely, the relative weights of the head and total non-carcass fat were the lowest in the control group (G1) compared to the Ag NP-treated groups, with significant differences ($p \leq 0.05$).

Table 4: Carcass parameters of growing rabbits as affected by different levels of Nano silver supplementation.

Item	Experimental groups					
	G_1	G_2	G_3	G_4	G_5	±SE
Per-slaughter (g)	1900^{ab}	1990^{a}	1890^{b}	1953^{ab}	1941^{ab}	50.26
Hot-carcass, %	42.04^{c}	50.42^{ab}	53.45^{a}	52.28^{a}	51.74^{ab}	1.34
Dressing, %	55.19^{c}	64.00^{ab}	65.47^{a}	65.29^{a}	61.37^{b}	1.27
Liver %	2.52	2.61	2.47	2.73	2.57	0.15
Kidney %	0.84	0.61	0.76	0.81	0.64	0.08
Heart %	0.37	0.39	0.34	0.34	0.28	0.03
Spleen %	0.06^{ab}	0.1^{a}	0.1^{a}	0.08^{ab}	0.04^{b}	0.02
Giblets weight %	3.37	3.61	3.57	3.88	3.49	3.89
Non-carcass fat weight %	0.58^{b}	1.05^{ab}	1.86^{a}	1.55^{a}	1.22^{a}	0.46
Head %	5.55^{b}	6.46^{ab}	6.66^{a}	6.66^{a}	6.80^{a}	0.16
Fur %	18.00	18.5	18.18	17.40	15.25	1.03

$^{a\ ,b}$ means in the same row with different superscripts are significantly different ($P \leq 0.05$).

G_1: control contained zero Ag NPs of diet. G_2: contained 1.5 mg/kg Ag NPs of diet.
G_3: contained 2 mg/kg Ag NPs of diet. G_4: contained 2.5 mg/kg Ag NPs of diet.
G_5: contained 3 mg/kg Ag NPs of diet.

Table 5: Some blood plasma parameters of male rabbits fed diets containing different levels of Nano silver particles.

Item	Experimental groups					
	G_1	G_2	G_3	G_4	G_5	±SE
Total protein (g/dl)	6.55^{a}	6.00^{b}	5.80^{b}	5.93^{b}	6.47^{ab}	0.22
Albumin (g/dl)	3.94	4.69	4.72	4.36	4.72	0.26
Globulin (g/dl)	2.51^{a}	1.31^{b}	1.08^{b}	1.57^{ab}	1.75^{ab}	0.35
Cholesterol (mg/dl)	131.00^{a}	108.0^{ab}	104.0^{ab}	101.67^{ab}	90.00^{ab}	5.34
HDL (mg/dl)	40.25^{a}	35.65^{b}	30.50^{b}	30.43^{b}	30.25^{b}	6.56
LDL (mg/dl)	49.35^{bc}	79.15^{a}	49.47^{bc}	38.30^{c}	41.00^{c}	7.64
Triglycerides (mg/dl)	123.33^{a}	114.30^{ab}	113.67^{ab}	113.00^{ab}	111.67ab	12.20
Glucose (mg/dl)	98.0^{b}	104.00^{ab}	129.33^{a}	107.00^{ab}	121.67^{a}	7.78
Urea (mg/dl)	45.67^{b}	52.00^{a}	56.03^{a}	55.50^{a}	52.30^{a}	7.94
Creatinine (mg/dl)	1.46	1.36	1.31	1.31	1.35	0.14
AST (U/l)	43.67^{a}	29.33^{ab}	29.00^{ab}	27.33^{b}	25.00^{b}	4.20
ALT (U/l)	87.33	66.67	71.50	87.00	78.00	12.78
T_3 (ng/ml)	1.20	0.96	0.94	0.73	0.74	0.18
T_4 (ng/ml)	3.49	2.00	2.83	2.37	2.10	0.68

$^{a\ ,b,c}$ means in the same row with different superscripts are significantly different ($P \leq 0.05$).

G_1: control contained zero Ag NPs of diet. G_2: contained 1.5 mg/kg Ag NPs of diet. G_3: contained 2 mg/kg Ag NPs of diet. G_4: contained 2.5 mg/kg Ag NPs of diet. G_5: contained 3 mg/kg Ag NPs of diet.

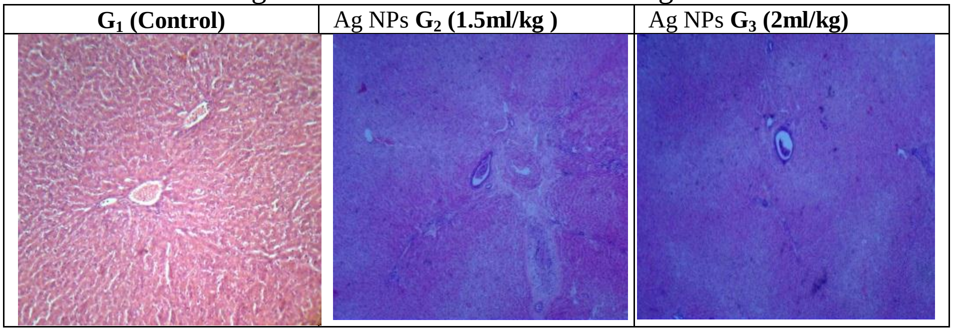
Blood parameters:

Results in Table 5 show that the total plasma protein, globulin, cholesterol and triglycerides concentrations were lowest ($p \leq 0.05$) in the groups treated with Ag NPs compared to the control group. However, there were no significant effects of Ag NP supplementation on plasma albumin, creatinine, ALT, T_3 and T_4 levels. On the other hand, the plasma LDL (except in groups G_4 and G_5), glucose, and urea concentrations were numerically higher in the Ag NP-treated groups compared to the control group, with significant differences ($p \leq 0.05$) among them. In contrast, HDL and AST concentrations significantly decreased ($p \leq 0.05$) in the Ag NP-treated groups compared to the control.

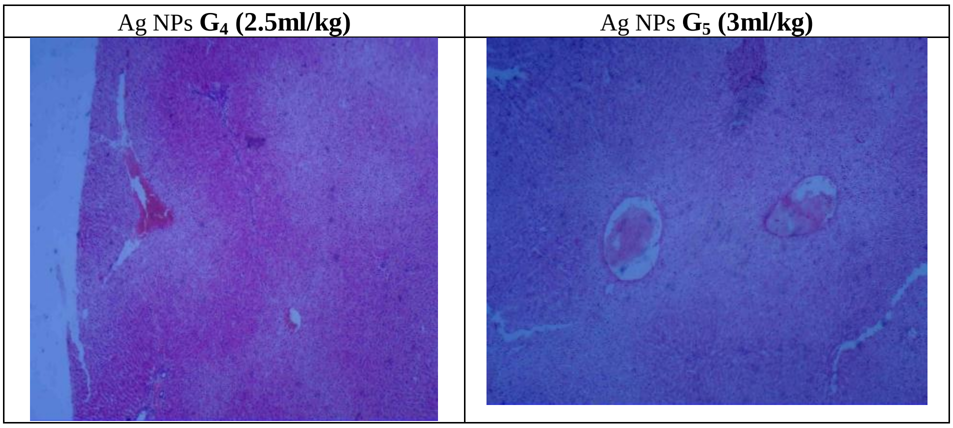
Histological Study:

The histological analysis of the liver from slaughtered rabbits (Plate 1) indicated that the liver architecture in the Ag NP-treated groups (G2, G3, G4 and G5) was normal, with no pathological lesions observed, similar to the control group (G1). Likewise, the histological examination of the kidneys (Plate 2) showed typical renal cortex and medulla architecture across all treated groups, with no differences compared to the control group.

Plate 1: The histological examination of liver of slaughtered rabbits.



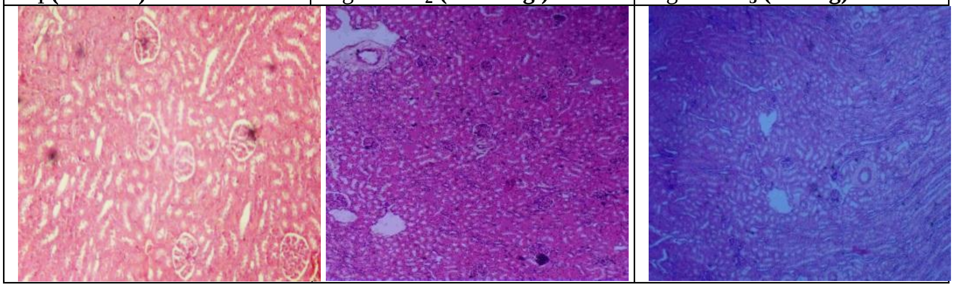
G_1 (Control) | Ag NPs **G_2 (1.5ml/kg)** | Ag NPs **G_3 (2ml/kg)**



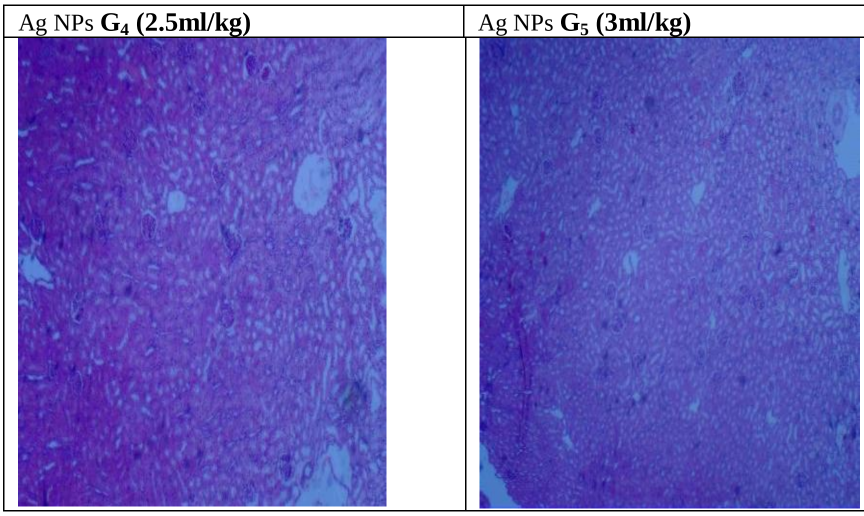
Ag NPs **G_4 (2.5ml/kg)** | Ag NPs **G_5 (3ml/kg)**

(×200, H&E stains)

Plate 2: The histological examination of Kidneys of slaughtered rabbits.



G_1 (Control) | Ag NPs **G_2 (1.5ml/kg)** | Ag NPs **G_3 (2ml/kg)**



Ag NPs **G_4 (2.5ml/kg)** | Ag NPs **G_5 (3ml/kg)**

(×200, H&E stains)

Table 6: Economical efficiency for growing rabbits feeding diets containing different levels of Ag NPs.

Item	Experimental groups				
	G_1	G_2	G_3	G_4	G_5
Total body weight gain (kg)	**1.208**	**1.403**	**1.518**	**1.444**	**1.305**
Price of 1 kg body weight (l.E)	**100**	**100**	**100**	**100**	**100**
Selling price/rabbit (LE) A	**120.8**	**140.3**	**151.8**	**144.4**	**130.5**
Total feed intake (kg)	**4.627**	**4.692**	**4.724**	**4.700**	**4.657**
Price of 1kg feed (LE)	**14**	**14**	**14**	**14**	**14**
Total feed cost/rabbit (LE) B	**64.78**	**65.69**	**66.14**	**65.80**	**65.20**
Net revenue (L.E)[1]	**56.02**	**74.61**	**85.40**	**78.60**	**65.30**
Economic efficiency [2]	**0.87**	**1.14**	**1.29**	**1.20**	**1.00**
Relative EEF, %	**100**	**131.04**	**148.28**	**137.93**	**114.94**

[1] Net revenue = A-B, [2] Economical efficiency= Net revenue / B

Based on prices of the Egyptian market during the experimental period (2022).

The price of one kg live body weight was100 Egyptian Pound (LE).

G_1: control contained zero Ag NPs of diet. G_2: contained 1.5 mg/kg Ag NPs of diet.
G_3: contained 2 mg/kg Ag NPs of diet. G_4: contained 2.5 mg/kg Ag NPs of diet.
G_5: contained 3 mg/kg Ag NPs of diet.

Economic efficiency:

Table 6 indicates that rabbits fed diets supplemented with Ag NPs at various levels exhibited notable profit margins compared to the control group at the marketing age of 16 weeks. The relative economic efficiencies (%) were as follows: 131.04 for G2, 148.28 for G3, 137.93 for G4, and 114.94 for G5. The highest relative economic efficiency was observed in Group G3, which was treated with a 2.5 mg/kg diet of Ag NPs, reaching 148.28%. This suggests that incorporating Ag NPs into the diets of growing male rabbits is economically feasible.

DISCUSSION

Nutrient digestibility and nutritive value:

Silver nanoparticles (Ag NPs) are still being researched in the livestock, rabbit, and poultry sectors. The present study was aimed to evaluate the impact of Ag NPs as dietary supplementation on different parameters, including nutrient digestibility, growth performance, blood consistency, carcass traits, histological examination, and economic efficiency in growing New Zealand White (NZW) rabbits. The findings revealed that the nutrient digestibility and nutritive values (%), including Total Digestible Nutrients (TDN) and Digestible Crude Protein (DCP), showed insignificant improvement across all groups treated with Ag NPs compared to the control group. This could be attributed to the potential of Ag NPs to enhance the population of beneficial microbes in the gut. Notably, the highest level of Ag NPs (3 mg/kg diet, G_5) yielded the least favorable outcomes, indicating a dose-dependent effect. Research by **Albanese *et al.*, (2012)** suggests that Ag NPs can stimulate digestive enzyme activity, thereby improving nutrient digestibility **(Kumar *et al.*, 2020; Bolandi *et al.*, 2021 and Kotb *et al.*, 2024)**. Previous studies **(Cho *et al.*, 2005 and Sawosz *et al.*, 2007)** highlighted the antimicrobial properties of silver nanoparticles, which can adversely affect both Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains. **Sotiriou and Pratsinis (2010)**, noted that Ag NPs release silver ions (Silver + ions from their surface) in contact with water, which exhibit strong antimicrobial effects by disrupting cellular respiration and compromising bacterial cell membranes **(Vadalasetty *et al.*, 2018)**. Moreover, Ag NPs may alter the phosphotyrosine profile of bacterial peptides, hindering growth **(Shrivastava *et al.*, 2007).** However, **Awaad *et al.*, (2021)**, indicated that the effectiveness of Ag NPs is dose-dependent and is more pronounced against Gram-negative bacteria. On the other hand, the antimicrobial activity of metal nanoparticles can be attributed to cell membrane disruption, cell wall damage, and cytoplasmic leakage, ultimately leading to microbial death **(Gajbhiye *et al.*, 2009 and Hassan *et al.*, 2014; 2015)**.

Growth performance:

The current study demonstrated that average live body weight (LBW) in rabbits supplemented with Ag NPs was significantly higher ($p \leq 0.05$) compared to the control group (G1) at 14 weeks. The percentage increases in

LBW were 9.88% for G1, 15.22% for G2, 12.28% for G3, and 5.35% for G4. Additionally, Ag NPs supplementation in rabbit diet at different levels increased ($p \leq 0.05$) average daily gain (g/head) of G1, G2, G3 and G4 groups as compared to the control group. The increasing rate was 16.57, 27.29, 21.41 and 9.35 %, respectively at 14 weeks. The highest LBW and average daily gain were observed in groups G_3 and G_4, supplemented with 2 mg and 2.5 mg Ag NPs per kg of diet. Rabbits in groups G_1, G_2, G_3, and G_4 also exhibited significant improvements ($p \leq 0.05$) in total feed intake (g/head/week), average daily feed intake (g/head/day), and feed conversion ratio compared to the control group (G_1) during the 6-14 week period. The best feed conversion ratio (g feed intake/g daily weight gain) was noted in Group G_3 (2 mg/kg diet). These improvements in growth performance may be attributed to the stimulation of digestive enzyme activity by Ag NPs **(Kumar *et al.,* 2020; Bolandi *et al.,* 2021 and Kotb *et al.,* 2024)**, an increase in beneficial microbial populations, enhanced nutrient digestibility and absorption **(Sadr *et al.,* 2023)**, and reduced ammonia and nitrogen emissions **(Xu *et al.,* 2013). Moustafa *et al.,* (2015) and Saleh and El Magd (2018)** noted that chicks receiving Ag NPs at 50 ppm exhibited increased body weight gain and muscle weight, along with improved feed conversion.

In contrast, **Abdelsalam *et al.,* (2019)** found that although final body weight increased with Ag NPs injection at 0.5 mg/kg body weight, while, body weight gain, feed intake, and feed conversion ratio showed no significant changes. **El-Faham *et al.,* (2017)**, reported that Ag NPs in drinking water at 0.5 or 10 ppm did not significantly affect live body weight, daily weight gain, or feed conversion in rabbits and broilers. **Hang and Tra (2013)** also observed significant increases ($p \leq 0.05$) in live body weight when rabbits received Ag NPs in drinking water.

Carcass traits:

The results presented in Table 4 indicate that carcass traits, particularly hot carcass weight and dressing percentage, were significantly affected ($p \leq 0.05$) by the supplementation of Ag NPs compared to the control rations. The highest values were observed in the G3 and G4 groups, which were supplemented with Ag NPs at 2 and 2.5 mg/kg of diet, respectively. This improvement in carcass traits can be attributed to the enhanced digestion coefficients and nutritional value (as shown in Table 2), which positively impacted both live body weight and average daily gain, ultimately leading to increased hot carcass weight and dressing percentages. The relative organ weights of the kidneys, liver, heart, giblets, and fur were numerically similar to those in the control group, except for the spleen, which exhibited significant differences among the treated and control group. These findings align with **Abdelsalam *et al.,* (2019)**, who reported similar relative weights for the liver, kidney, and heart in rabbits injected with Ag NPs at 0.5 or 1 mg/kg body weight. Conversely, **Hassan (2018)**, found that Ag NPs supplementation in the drinking water or feed of broilers significantly affected dressing percentage, liver, and heart weights.

Additionally, **Al-Sultan *et al.*, (2022)**, demonstrated that broiler diets supplemented with Ag NPs at various concentrations (2.5, 5, 10, or 20 ppm) improved the relative weights of warm dressing, heart, liver, and gizzard. However, **Kotb *et al.*, (2024)**, reported no significant effects on carcass traits such as dressing percentage and organ weights (except for liver percentage) due to Ag NPs supplementation in rabbit diets. Similarly, **El-Faham *et al.*, (2017)** found no significant impact on the carcass traits of quail, broilers, and rabbits when Ag NPs were supplemented in drinking water at concentrations of 0.5 or 10 ppm. **Ahmadi *et al.*, (2013) and Abdelsalam *et al.*, (2019),** also reported no significant effects of Ag NPs on liver, heart, kidney, or spleen percentages and dressing percentages or carcass traits. **Tammam *et al.*, (2023)**, found no significant differences in carcass traits (including carcass percentage, giblets, total edible parts, and abdominal fat percentage) or organ parts (breast, thigh, drumstick, and wings percentages) when Ag NPs were used in broiler drinking water.

Blood parameters:

Blood plasma metabolites

The results presented in Table 5 ,regarding blood plasma metabolites, indicate that plasma total protein and globulin levels were significantly ($p \leq 0.05$) decreased of the treated groups receiving Ag NPs compered with the control rations. Plasma albumin concentrations showed an insignificant increase compared to the control group. **Ashour *et al.*, (2004)**, noted that albumin concentration is linked to an animal's ability to store and synthesize protein. Additionally, **Jones and Park (1979)**, reported that changes in albumin levels reflect liver function, as the liver is responsible for albumin synthesis, whereas globulin is formed by lymphoid tissue. Our results are partially consistent with those of **Sultan *et al.*, (2022)**, who found that supplementation of 2.5 mg Ag NPs/kg of broiler diet significantly increased total protein levels, while albumin and globulin showed insignificant increases. In contrast, **Kotb *et al.*, (2024)**, reported significant increases in total protein, albumin, and globulin levels in rabbits fed diets supplemented with Ag NPs at 0.25 and 0.75 mg. **Abdelsalam *et al.*, (2019)**, stated that blood plasma content of total protein, albumin, and globulin were not significantly affected when rabbits were injected with Ag NPs at 0.5 or 1 mg/kg body weight. **Moustafa *et al.*, (2015)**, demonstrated that broiler diets supplemented with Ag NPs at 2, 4, 6,8 and 10 ppm significantly affected blood globulin level, while total protein and albumin were not significantly impacted. **Syrvatka *et al.*, (2014)** reported that silver nanoparticle supplementation did not alter any blood parameters.

Blood Lipid Profile

The results presented in Table 5 indicate that the concentrations of plasma total cholesterol and triglycerides were significantly decreased in rabbits treated with Ag NPs compared to the control group. These findings align with those of **Abdelsalam *et al.*, (2019)**, who reported that rabbits injected with Ag NPs at a dosage of 0.5 mg exhibited reduced plasma concentrations of total cholesterol and triglyceride levels. **Saleh and El-Magd (2018)**, also noted significant reductions in plasma triglycerides and total cholesterol when broiler chickens were fed a diet containing 50 ppm/kg of Ag NPs, without affecting total protein levels. Additionally, **Kotb *et al.*, (2024)** found that serum total cholesterol and LDL concentrations significantly decreased in rabbits receiving 0.75 mg Ag NPs/kg diet compared to the control and other treatment groups. Conversely, **Al-Sultan *et al.*, (2022),** reported a significant increase in blood total cholesterol levels and an insignificant increase in triglyceride concentrations in broilers fed diets supplemented with Ag NPs at 2.5 mg/kg. Furthermore, **Ahmadi (2012)**, observed no significant changes in lipid profiles due to Ag NPs supplementation. Also. **Sawosz *et al.*, (2009)**, reported that silver nanoparticles did not affect the activities concentrations of cholesterol.

Our study also revealed a significant ($p \leq 0.05$) decrease in plasma HDL concentrations in rabbits fed diets containing Ag NPs at all levels compared to the control group. Conversely, LDL concentrations significantly ($p \leq 0.05$) increased with a 1.5 mg/kg diet compared to the other treated and the control groups. This finding is consistent with **Ahmadi (2012)**, who noted that chickens fed diets containing small amounts (8 and 12 ppm/kg) of Ag NPs exhibited increased LDL cholesterol and decreased HDL cholesterol concentrations.

Liver Function

In terms of liver function, the data indicated that plasma ALT and AST activities decreased in rabbits treated with Ag NPs compared to the control group. The decrease in AST was significant ($p \leq 0.05$), while the decrease in ALT was not significant. This transaminase activity suggests no damage to hepatic cells due to Ag NPs, in accordance with **Mezey (1976)**, who stated that any damage to hepatic cells or their membranes results in increased AST and ALT levels in the blood serum. **Dosoky *et al.*, (2021)**, supported this indicating that decreased liver enzyme activity, such as ALT and AST, correlates with protein metabolism and varying levels of transaminases. Our findings are consistent with those of **Moustafa *et al.*, (2015)**m who demonstrated that broiler diets supplemented with Ag NPs at 2, 4, 6,8 and 10 ppm significantly affected AST level, while ALT were not significantly impacted. **Kotb *et al.*, (2024)**, found significant reductions in serum AST and ALT levels with Ag NPs supplementation at up to 0.75 mg/kg diet. **Ahmadi (2012)**, also reported decreased AST and ALT activities in the blood plasma of chickens receiving Ag NPs at 20, 40, and 60 ppm/kg of feed. In contrast, other studies **(Sawosz *et al.*, 2009; Andi *et al.*, 2011; Saleh and El-Magd, 2018)**, indicated that Ag NPs supplementation in broiler chickens had no significant effects on ALT and AST activity levels.

Kidney Function

Regarding kidney function, the data in Table 5 revealed that Ag NPs supplementation in rabbit diets significantly ($p \leq 0.05$) increased plasma urea values, while plasma creatinine levels were decreased (though insignificantly) compared to the control group. These results are partially consistent with those reported by **Kotb *et al.,* (2024)**, who found that rabbits fed diets including Ag NPs at 0.50 and 0.75 mg/kg exhibited significantly lower creatinine concentrations. They also reported that urea concentration was significantly decreased at 0.25 mg Ag, while 0.50 and 1 mg Ag NPs led to increased serum urea levels. Conversely, **Sultan *et al.,* (2022)** observed insignificantly higher urea concentrations in broilers fed Ag NPs at mg/kg, while **Dosoky (2021)**, noted decreased urea and creatinine concentrations in broiler chicks fed Ag NPs hydrocolloid. **Lohakare and Abdel-Wareth (2022)** found no effect on urea or creatinine levels in broiler diets.

Hormonal and Glucose Parameters

The data in Table 5 also showed that plasma thyroid hormones (T3 and T4) concentrations were insignificantly decreased in rabbits treated with Ag NPs compared to the control group. However, glucose concentrations significantly ($p \leq 0.05$) increased in rabbits treated with Ag NPs compared to the control group. These findings contrast with those of **Sawosz *et al.,* (2009)**, who reported that silver nanoparticles did not affect the activities of glucose concentrations.. Overall, the blood plasma metabolites analyzed in this study were within the normal levels for healthy rabbits, as indicated by **Őzkan *et al.,* (2012).**

Histological Examination:

The histological examination of the liver and kidneys from slaughtered rabbits revealed normal hepatic lobule and renal cortex structures across all treated groups. This observation is attributed to the blood plasma parameters remaining within normal physiological ranges, indicating that the developing rabbits demonstrated remarkable adaptive abilities to maintain high productive performance levels without compromising liver and kidney function. **Hassan (2009)**, emphasized that the normal histological structure correlates with the AST and ALT activity results in the blood serum of rabbits, which were within normal values for growing rabbits (Table 5).

Economic efficiency:

From an economic standpoint, feeding growing rabbits diets supplemented with Ag NPs led to reduced feed costs and increased net revenue and relative economic efficiency compared to the control group. The highest relative economic efficiency was observed in the G3 (148.28%) and G4 (137.93%) groups, which were treated with 2 and 2.5 mg Ag NPs/kg, respectively. These improvements can be attributed to enhanced growth performance, dressing percentage, and overall health care of the rabbits. **Saleh and El-Magd (2018)**, attributed growth improvements to Ag NPs' positive effects on the balance of beneficial and harmful bacteria in the intestines and their impact on digestive enzyme activity, which improved nutrient absorption and promoted a healthier digestive tract. Other studies **(El-Sabry *et al.*, 2018 and Salem and Fouda., 2021)**, have reported that nanoparticles increased survivability, production performance, and overall physical function across various animal species.

CONCLUSION:

The present study concluded that supplementation of Silver Nanoparticles (Ag NPs) in rabbit diets at varying doses positively affected growth performance, carcass quality, health status, and economic efficiency, without adverse effects on blood metabolism or histological findings. Optimal results were obtained at 2 mg Ag NPs/kg of diet. There is a notable lack of studies exploring the experimental benefits of nanoparticles, particularly silver nitrate, as feed additives for rabbits. Thus, further research is warranted to determine a safe dosage that balances the health status and productivity of the growing rabbits while minimizing any potential harmful effects. Additionally, caution should be exercised regarding high doses of Ag NPs, as they can lead to toxicity due to accumulation in blood plasma and meat.

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أداء النمو وأستجابة الذبيحة للأرانب التي تتغذى على علائق مضاف اليها مستويات مختلفة من جزيئات نانو فضة

هدى محمد الحسيني[1]، جمال فاروق شاهين[1]، قوت القلوب محمد مصطفى[2]، جورج يونان[3]،
عصام القتامي[1]، عبد الغني غنيم[1]، مصطفى محمد النحراوي[1]، منى أحمد فراج[1]،
على أحمد عبده[1]، محمد السيد سيداحمد[1]

1- قسم بحوث تغذية الحيوان ، 2- قسم بحوث تغذية الدواجن، 3- قسم بحوث تربية الأرانب و الرومي والطيور المائية
معهد بحوث الإنتاج الحيواني ، مركز البحوث الزراعية ، الدقي، الجيزة، مصر.

أجريت الدراسة بأستخدام عدد 50 ذكر من أرانب النيوزيلندى الابيض، متوسط أعمارهم 6 أسابيع ووزنهم الحي 710.74 ± 10 جرام، تم توزيعهم بشكل عشوائي على 5 مجموعات (10 أرانب لكل مجموعة) وتم تغذيتهم بأعلاف أساسية تحتوي على مستويات متفاوتة من جزيئات الفضة النانوية (Ag NPs) لفترة تجريبية مدتها 14 أسبوعًا. أجرى هذا البحث بهدف تقييم فعالية استخدام مستويات مختلفة من جزيئات الفضة النانوية كمكملات غذائية للأرانب النامية. حيث غذيت المجموعة الأولى G1 (المجموعة الضابطة) على علف الأساسي بدون مكملات. و تم تغذية الأرانب في المجموعات الأخرى G2 وG3 وG4 وG5 على العلف الأساسي(المجموعة الضابطة) مضاف إليه جزيئات الفضة النانوية بمستويات 1.5 و2 و2.5 و3 مجم / كجم من العلف على التوالي.

وكانت أهم نتائج هذه التجربة ما يلى:

وجد أن أعلى قيم لوزن الجسم الحي و معدل زيادة اليومية ووزن الذبيحة ونسبة التصافى كانت في الأرانب التي تغذت على مكملات جزيئات الفضة النانوية فى جميع المستويات مقارنة بالمجموعة الضابطة. ومع ذلك، لم تتأثر معاملات الهضم والقيم الغذائية بشكل كبير بمكملات جسيمات النانو. كذلك تحسنت الكفاءة الغذائية و الاقتصادية في المجموعات التي تلقت جزيئات الفضة النانوية بالمقارنة بالمجموعة الضابطة.

من هذه الدراسة ينصح بأضافة جزيئات الفضة النانوية في علائق الأرانب كأضافات غذائية حيث أثرت بشكل إيجابي على الأداء الأنتاجى والكفاءة الاقتصادية مقارنة بالمجموعة الضابطة. وقد وجد أن اضافة 2 مجم من جزيئات الفضة النانوية / كجم من العلف أعطى أفضل النتائج.