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Effect of Zinc Oxide Nanoparticles on some biochemical parameters and body weight in Barki fattening lambs

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Abstract

Zinc Oxide nanoparticles (ZnO-NPs) have promising positive effects on animal performance, feed utilization, antioxidant status, and immune response. This study aimed to evaluate the effects of the ZnO-Nps on some biochemical parameters of male Barki lambs fed on adlibitum and restricted diets. 300 post-weaning Barki lambs were selected and randomly grouped into 6 groups; Group 1(G1), group 2(G2), and group3 (G3) received adlibitum ration with 0 mg/kg, 15mg/ kg, and 30mg/kg ZnO NPs, respectively, while G4, G5, G6 were fed on a restricted ration containing 0 mg/kg, 15mg/ kg and 30 mg/kg ZnO-NPs, respectively. A significant reduction in alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and uric acid levels between treated G3 (30 mg ZnO-Nps). Lambs fed on rations supplemented with 15mg/kg ZnO-NPs, showed a significant difference in total Cholesterol, Triglyceride, and HDL levels compared with the control groups (G1 and G4). Both 15 mg/kg and 30 mg/kg of ZnO-NPs showed significant differences in zinc, and iron while selenium levels were not significantly affected. Serum malondialdehyde (MDA), and nitric oxide (NO) levels showed significant reduction between both treated groups (15 mg and 30 mg ZnO-Nps) and the control group. While, glutathione (GSH), and sodium oxide dismutase (SOD) levels were significantly increased. ZnO-NPs enhanced the growth rate and weight gain of fattening Barki lambs without adverse impacts on the liver and kidney functions at levels of 15mg/kg and 30mg/kg. These results were not significantly affected by the feeding method used

Keywords: zinc oxide nanoparticles, biochemical parameters, Barki lambs.

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Introduction

Sheep (*Ovis aries*) are widespread domestic species of small ruminants. A multitude of breeds are adapted to many purposes in diverse environments and are reared mainly for their meat, milk, wool, and hides. There are more than 1.2 billion sheep in the world (Gilbert et al., 2018). In Egypt, the most common sheep breeds are Ossimi, Rahmani, and Barki. The Barki sheep are adapted to the harsh desert conditions in the Mediterranean zone and produce a considerable amount of meat, milk, and wool, and represent 8.5% (470,000 heads) of the total sheep population in Egypt. Growth performance has an important role in regarding the supply of red meat for human consumption (Abousoliman et al., 2020).

Minerals are vital components in animal nutrition during all stages of the production system. They perform digestive, reproductive process and growth functions inside the animal body (Raje et al., 2018). The bioavailability of minerals from their inorganic sources is quite low so these minerals are added 20-30 folds higher than the normal requirement of animals. The bioavailability can be reduced by minerals or vitamin antagonists (Mahmoud et al., 2020).

Nanotechnology is in some way an innovative field of science. Applications of nanotechnology pass in many parts of our life, such as in the therapeutic field, nutrition, disease diagnosis, chemical industries, and biological research (Hassanein et al., 2021).

Nanoparticles can be primarily categorized into organic and inorganic materials based on their chemical characteristics. In the livestock sector, the nutritional values of animal feed can be enhanced by using organic nanoparticle supplements (Abd El-Hack et al., 2017).

Zinc is known as an essential trace element for most of the biological functions in the animal’s body and as an important nutrient in living organisms (Yusof et al., 2019). The larger particle zinc oxide is not commonly used in livestock feed supplementation due to its low bioavailability. However, ZnO-Nps can provide a better surface-to-volume ratio, than larger particulate ZnO or zinc methionine supplements (Geetha et al., 2020). ZnO-Nps can promote productive performance, act as antibacterial agents, and influence the immunity and reproduction of animals. ZnO-NPs can be used at low doses, resulting in better outcomes than conventional zinc sources (Hussein et al., 2020).

Using Zinc nanoparticles (ZnO-Nps) in livestock nutrition regimes showed promising results in enhancing the performance, and nutrient bioavailability, as well as improving the immunity status of animals and the quality and composition of animal products while reducing environment-related hazards. The international animal production sector continuously faces the pressure of ever-increasing costs of raw materials, including prices of minerals. This necessitates seeking alternative sources of minerals having better bioavailability, efficacy, and lower antagonism (Abdelnour et al., 2021).

This study aimed to evaluate the effect of the ZnO-Nps in restricted and adlibitum rations on weight gain, liver and kidney functions, antioxidant enzymes, serum protein, lipid profiles, and some trace elements in male Barki lambs.

Materials and Methods

Animal grouping

300 post-weaned (three months of age), apparently healthy Barki lambs were randomly selected to be used in this experiment. The study was carried out on a farm belonging to agricultural researchers, in Giza, Egypt during the post-weaning period of lambs. The weaned lambs will be randomly allocated into 6 groups, as follows and the rates of diet consumption will be calculated daily. G1, G2, and G3 (50 for each): fed adlibitum on the basal diet (commercial ration according to nutrient requirements of sheep that meet all nutritional requirements of weaning lambs) without adding of ZnO-Nps (control group1), 15mg/kg, and 30 mg/kg respectively. While G4, G5& G6 (50 for each) were fed on restricted amounts of ration without adding ZnO-Nps. while, G5 and G6 were fed on restricted amounts of ration without adding ZnO-Nps at 15mg/kg, and 30 mg/kg of the basal diet.

The studied groups were fed on a balanced ration (70%barseem + 30% hay(dares) as well as a dry ration consisting of; 46.5 meal; 15% yellow corn; 35% bran; 1% salt; 1.5% Limestone; and 1% vitamins.

Laboratory analysis:

The blood samples were collected from the jugular vein of each animal after clipping and disinfecting the area of the vein. About 7.5 ml of blood was put in a centrifuge tube without anticoagulant. The separated sera used for the detection of some liver enzymes such as AST, and ALT (u/l), as well as, some kidney function tests including urea (mg/dl), uric acid (mg/dl), and creatinine (mg/dl). Some antioxidants and oxidants include (SOD) (u/ml), (GSH) (mg/dl), catalase (Cat) (u/ml), total antioxidant capacity (TAC) (mg/dl), (MDA) (mol/m), and (NO) (mg/dl). Protein profiles include total protein (mol/m), total albumin (mol/m), and total globulin (mol/m). Lipid profiles include cholesterol (mg/dl), triglyceride (mg/dl), low-density lipoprotein (mg/dl), and high-density lipoprotein (mg/dl). The biochemical profiles were identified by a scanning spectrophotometer (CECIL 3000) and commercial kits produced by Bio-diagnostic Co. (Diagnostic and research reagents). Serum levels of some minerals such as iron (mg/dl), selenium (ug/dl), and zinc(mg/dl) were determined by the atomic absorption assay (Sens AA GBC Scientific Equipment) according to AOAC (2015).

Statistical analysis.

The obtained results were statistically evaluated by application of a one-way ANOVA test, standard error of the mean (SEM) of the mean and the least significant difference for the mean (LSD), Tukey’s test, and significance (at $p < 0.05$) using IBM-SPSS (SPSS Inc., Chicago, USA).

Results

There were significant increases in body weight and weight gain between both treated groups (15 mg and 30 mg ZnO-Nps) and the control group (0 ZnO-Nps) at 9 and 12 months of age, also significant differences between 15mg and 30 mg/kg ZnO-Nps (Fig.1)

Treatments at 9 months there is a significant reduction in ALT levels in the group (adlibitum +15 mg ZnO-Nps) compared with the control group (Table 1a).

On the other hand, results shown in revealed that there was a non-significant reduction in the serum ALT group (adlibitum+30 mg/kg ZnO-NPs) compared with the control group. Followed by a significant reduction in the group (adlibitum+15 mg/kg ZnO-NPs) compared with the control at 9 and 12 months of age (Tables 1a). There is a significant reduction in serum AST in both treatment groups compared with the control group at 9 and 12 months of age. Also, there are significant differences between the control groups that had received adlibitum feed and those fed with restricted ration at all measured ages 9 and 12 months. While no significant differences were observed between the 2 feeding methods at the same age in groups supplemented with ZnO-Nps (Tables 1a, b).

Our results showed a significant reduction in serum urea levels in both treatment groups compared with the control group at 9 and 12 months of age, while there is no significant effect of the two different feeding methods during the experiment period. Serum creatinine levels showed a significant reduction between treated groups (30 mg ZnO-Nps). Also, serum uric acid levels were decreased significantly in both treated groups (15 mg and 30 mg ZnO-Nps) compared with the control group at 9 and 12 months of age (Tables 1a, b).

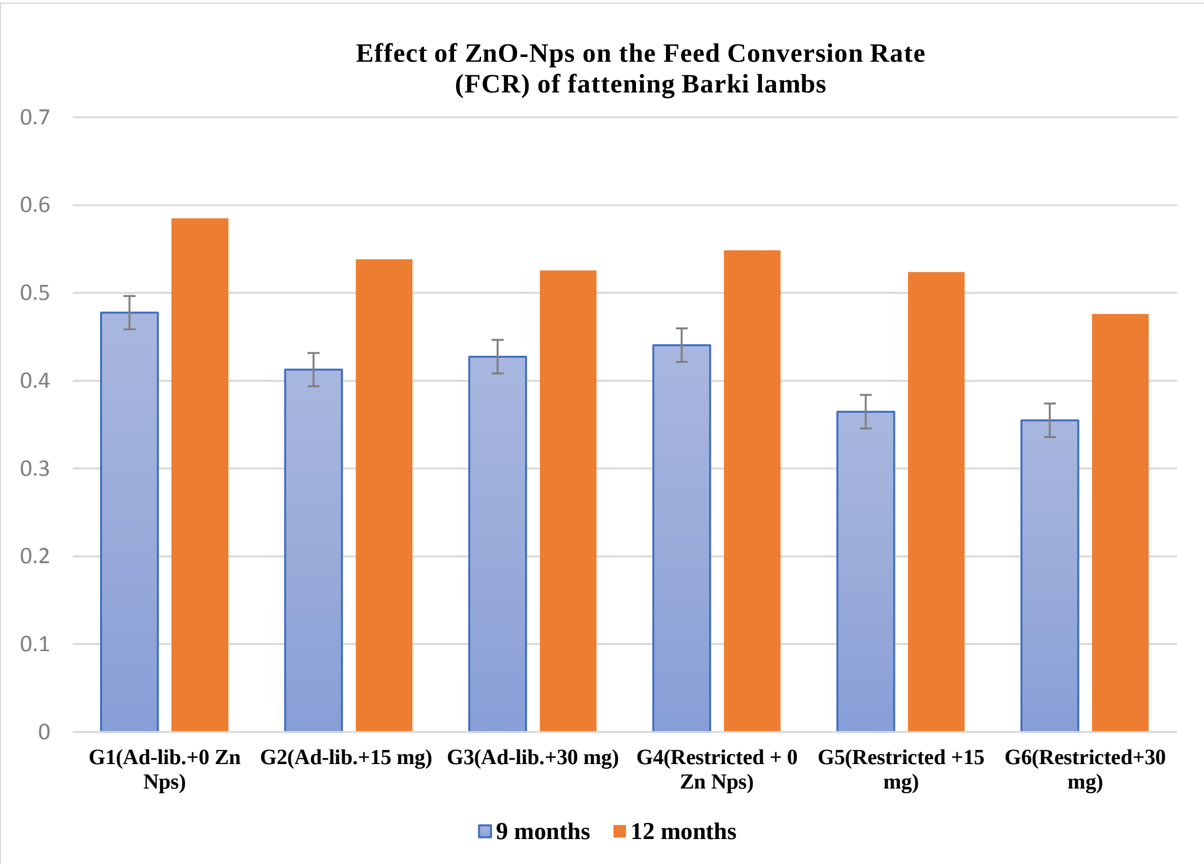

Figure 1: Effect of Zn Nps on average feed conversion rate (FCR) in the Barki lambs

Table 1a: Effect of ZnO- Nps on liver and kidney function tests of Barki lambs fed on ad libitum ration.

	ZnO Nps	9 months (x)	12 months (y)
ALT	0mg/ kg (a)	38.4 ± 1.5$^{bc, y}$	36.2 ± 2.6 $^{bc, x}$
	15 mg/ kg (b)	35.2±1.64 $^{ac, y}$	32.6±1.58 $^{ac, x}$
	30 mg/ kg (c)	37.6±1.34$^{ab, y}$	34.6±1.14 $^{ab, x}$
AST	0mg/ kg	30.6±2.3$^{bc, y}$	26.6±1.14 $^{bc, x}$
	15 mg/ kg	25.2±2.2 $^{ac, y}$	21.4±0.9 $^{ac, x}$
	30 mg/ kg	26.2±2.3$^{ab, y}$	23±2.2 $^{ab, x}$
Urea	0mg/ kg	30±1.58 ^{bc}	29.6±1.52 ^{bc}
	15 mg/ kg	26.6±1.14$^{ac, x}$	24.4±1.14 $^{ac, x}$
	30 mg/ kg	23.8±0.84^{ab}	22.6±1.14 ^{ab}
Creatinine	0mg/ kg	1.94±0.03$^{c, x}$	1.77±0.063$^{bc, x}$
	15 mg/ kg	1.89±0.063^{ac}	1.80±0.018 ^{ac}
	30 mg/ kg	1.73±0.025 $^{ab, x}$	1.59±0.033 $^{ab, x}$
Uric acid	0mg/ kg	6.32±0.055	6.716±0.065
	15 mg/ kg	4.246±2.2	5.29±0.085
	30 mg/ kg	5.246±0.16	5.04±0.055

Lower case superscript (a,b,c) for significant difference between ZNO-Nps concentrations (vertical), x, y for significant difference between 9 months and 12 months for the same group.

Table 1b: Effect of ZnO- Nps on liver and kidney function tests of Barki lambs fed on restricted ration.

liver/ kidney function	ZnO Nps	9 months	12 months
ALT	0mg/ kg	39.8 ± 0.8 ^{bc}	36 ± 2.24 ^{bc}
	15 mg/ kg	37.6±1.14 $^{ac, y}$	39.2± 0.8 $^{ac, x}$
	30 mg/ kg	39±1 ± 2.6 ^{ab}	38.6 ± 1.14 ^{ab}
AST	0mg/ kg	33.6±1.14 $^{bc, y}$	21.8±1.3 $^{bc, x}$
	15 mg/ kg	26±0.7 $^{ac, y}$	30.8±1.3 $^{ac, x}$
	30 mg/ kg	25.2±1.3 ab, y	21.8±0.8 ab, x
Urea	0mg/ kg	31±1.58bc	24.4±0.55 bc
	15 mg/ kg	25.2±0.8ac, x	30.6±1.14 ac, x
	30 mg/ kg	24.4±1.14 ab, y	22.8±2.17 ab, x
Creatinine	0mg/ kg	1.904±0.071 c, x	1.72±0.044 bc, x
	15 mg/ kg	1.78±0.031 ac	1.77±0.07 ac
	30 mg/ kg	1.61±0.028 ab, x	1.64±0.024ab, x
Uric acid	0mg/ kg	6.142±0.05	5.038±0.12
	15 mg/ kg	5.64±0.088	6.328±0.084
	30 mg/ kg	5.23±0.12	4.86±0.182

Lower case superscript (a,b,c) for significant difference between ZNO-Nps concentrations (vertical), x, y for significant difference between 9 months and 12 months.

Zinc and iron levels were significantly increased in the serum of both treated groups (15 mg and 30 mg ZnO-Nps) compared with the control group at both 9 and 12 months of age, and also significant differences between 15mg and 30 mg ZnO-Nps. It was noticed that the groups that had received by restricted feeding method showed a significant increase in their iron blood levels than those that received adlibitum rations. While serum selenium levels showed a non-significant increase. (Tables 2a, b). Serum MDA and NO levels were significantly reduced, in both treated groups (15 mg and 30 mg ZnO-NPs), compared to the control group at both 9 and 12 months of age. While, GSH and SOD levels increased significantly, with significant differences between 15mg and 30 mg ZnO-NPs. (Tables 2a, b).

Table 2a: Effect of ZnO- Nps on serum mineral and antioxidant levels of Barki lambs fed on ad libitum ration.

Serum mineral	ZnO Nps	9 months	12 months
Zinc	0mg/ kg	4.06±0.114 bc	4.16±0.114 bc
	15 mg/ kg	5.17±0.049 ac, y	5.64±0.04 ac, x
	30 mg/ kg	6.31±0.05 ab, y	6.95±0.095 ab, x
Iron	0mg/ kg	63.2±2.17 ab, y	72.6±2.7 ac, x
	15 mg/ kg	78.4±1.3 ac, y	80.2±1.1 ab, x
	30 mg/ kg	76.4±3.65 bc, y	83±1 ab, x
Selenium	0mg/ kg	0.224±0.015	0.23±0.01
	15 mg/ kg	0.206±0.013	0.20±0.02
	30 mg/ kg	0.18±0.016	0.19±0.02
MDA	0mg/ kg	21.58±0.75 bc	21.9±1.0 bc
	15 mg/ kg	13.66±0.11 ac, y	10.4±0.16 ac, x
	30 mg/ kg	10.18±0.22ab	9.76±0.36 ab
NO	0mg/ kg	4.68±0.18 bc	4.98±0.24 bc
	15 mg/ kg	4.2±0.16	4.02±0.19
	30 mg/ kg	4±0.19	3.6±0.28 ab,x
GSH	0mg/ kg	42.98±0.72 bc	43.96±3.2 bc
	15 mg/ kg	52.26±0.56 ab, y	54.8±1.02 ab, x
	30 mg/ kg	56.6±1.38 ac, y	61.9±0.95 ac, x
SOD	0mg/ kg	7.32±0.08 bc	7.46±0.11bc
	15 mg/ kg	12.34±0.29 ac, y	13.6±0.15 ac, x
	30 mg/ kg	13.54±0.51 ab, y	14.64±0.27 ac, x
Catalase	0mg/ kg	27.19±0.98 bc	28.68±0.66 bc
	15 mg/ kg	43.3±1.02 ac, y	47.86±0.5 ac, x
	30 mg/ kg	48.03±0.7 ab, y	52.07±0.42 ab, x
TAC	0mg/ kg	116.8±4.32 bc	120.6±1.34 bc
	15 mg/ kg	138.6±3.65 ac, y	148±3.39 ac, x
	30 mg/ kg	147.4±4.56 ab, y	160.2±6.1 ab, x

Lower case superscript (a,b,c) for significant difference between ZNO-Nps concentrations (vertical), x, y for significant difference between 9 months and 12 months.

Table 2b: Effect of Zn0- Nps on serum mineral and antioxidant levels of Barki lambs fed on restricted ration.

	ZnO Nps	9 months	12 months
Zinc	0mg/ kg	4.36±0.1 bc, y	4.44±0.1 bc, x
	15 mg/ kg	5.28±0.056 ac, y	5.79±0.04 ac, x
	30 mg/ kg	6.5±0.197 ab, y	7.058±0.2 ab, x
Iron	0mg/ kg	66.6±1.14bc, y	85.6±2.3 bc, x
	15 mg/ kg	80.2±1.09 ac, y	77.8±3.7 ac, x
	30 mg/ kg	81±1 ab, y	87.6±1.67 ab, x
Selenium	0mg/ kg	0.198±.0084	0.224±0.01
	15 mg/ kg	0.216±0.011	0.19±0.01
	30 mg/ kg	0.188±0.008	0.19±0.02
MDA	0mg/ kg	22.9±0.31bc	21.74±0.68 bc
	15 mg/ kg	13.36±0.21 ac, y	10.12±0.26 ac, x
	30 mg/ kg	9.4±0.55 ab	8.48±0.19 ab

NO	**0mg/ kg**	**5.08±0.16^{c}**	**5.22±0.15 ^{bc}**
	15 mg/ kg	**4.34±0.21**	**3.82±0.13 ^{c}**
	30 mg/ kg	**3.8±0.2^{ab}**	**3.52±0.22 ^{a}**
GSH	**0mg/ kg**	**43.9±1.02 $^{bc, y}$**	**46.5±1.82 $^{bc, x}$**
	15 mg/ kg	**54.96±1.1 $^{ac, y}$**	**57.96±0.89 $^{ac, x}$**
	30 mg/ kg	**57.46±1.5 $^{ab, y}$**	**66.54±0.43 $^{ab, x}$**
SOD	**0mg/ kg**	**7.32±0.13 ^{bc}**	**7.84±0.09 ^{bc}**
	15 mg/ kg	**12.68±0.27 ^{ac}**	**13.84±0.3 ^{ac}**
	30 mg/ kg	**13.96±0.15 $^{ab, y}$**	**15.08±0.2 $^{ab, x}$**
Catalase	**0mg/ kg**	**27.9±0.4 ^{bc}**	**30.48±0.96 ^{bc}**
	15 mg/ kg	**43.67±0.87 $^{ac, y}$**	**49.4±0.53 $^{ac, x}$**
	30 mg/ kg	**49.3±0.89 $^{ab, y}$**	**54.52±0.56 $^{ab, x}$**
TAC	**0mg/ kg**	**119.4±1.82 $^{bc, y}$**	**126±2.3 $^{bc, x}$**
	15 mg/ kg	**146.4±2.04 ^{a}**	**152.8±1.92 ^{ac}**
	30 mg/ kg	**152±2.12$^{a, y}$**	**163.4±3.65 $^{ab, x}$**

Table 3a: Effect of Zn0- Nps on some serum Proteins of Barki lambs fed on ad libitum.

	ZnO Nps	**9 months**	**12 months**
Total serum protein	**0mg/ kg**	**6.06±0.17**	**6.18±0.084**
	15 mg/ kg	**6.35±0.098**	**6.42±0.084**
	30 mg/ kg	**6.62±0.084**	**6.74±0.089**
Albumin	**0mg/ kg**	**4.1±0.071**	**4.18±0.084**
	15 mg/ kg	**4.1±0.071**	**4.1±0.071**
	30 mg/ kg	**4.18±0.084**	**4.132±0.084**
Globulin	**0mg/ kg**	**1.96±0.15**	**2±0.1**
	15 mg/ kg	**2.25±0.14**	**2.32±0.11**
	30 mg/ kg	**2.44±0.15**	**2.608±0.12**
A/G ratio	**0mg/ kg**	**2.04±0.13 ^{bc}**	**2.13±0.13 ^{bc}**
	15 mg/ kg	**1.81±0.12 ^{ac}**	**1.78±0.1^{ac}**
	30 mg/ kg	**1.72±0.14 $^{ab, y}$**	**1.59±0.09 $^{ab, x}$**

Table 3b: Effect of Zn0- Nps on some serum Proteins of Barki lambs fed on restricted ration.

Total serum protein	**0mg/ kg**	**6.18±0.08**	**6.42±0.08**
	15 mg/ kg	**6.35±0.09**	**6.22±0.08**
	30 mg/ kg	**6.52±0.084**	**6.62±0.08**
Albumin	**0mg/ kg**	**3.98±0.11**	**4.12±0.08**
	15 mg/ kg	**3.98±0.11**	**4.12±0.08**
	30 mg/ kg	**4.18±0.084**	**4.08±0.08**
Globulin	**0mg/ kg**	**2.2±0.12**	**2.3±0.12**
	15 mg/ kg	**2.37±0.17**	**2.1±0.14**
	30 mg/ kg	**2.34±0.15**	**2.54±0.13**
A/G ratio	**0mg/ kg**	**1.8±0.15 ^{b}**	**1.84±0.05 ^{c}**
	ZnO Nps (15 mg/ kg)	**1.69±0.15^{a}**	**1.9±0.12 ^{a}**
	ZnO Nps (30 mg/ kg)	**1.79±0.16 $^{ac, y}$**	**1.61±0.1 $^{ab, x}$**

Lower case superscript (a,b,c) for significant difference between ZNO-Nps concentrations (vertical), x, y for significant difference between 9 months and 12 months.

Table 4a: Effect of Zn0- Nps on serum lipid profile levels of Barki lambs fed on ad libitum ration.

	ZnO Nps	9 months	12 months
Total cholesterol	0mg/ kg	184.2±1.9 [bc]	185.2±2.4 [bc]
	15 mg/ kg	163.8±1.78 [ac]	160.4±1.7 [ac]
	30 mg/ kg	159.2±0.84 [ab, y]	211.2±133.5 [ac, x]
Triglyceride	0mg/ kg	88.4±2.1 [bc]	87±2.1 [a]
	15 mg/ kg	69.4±0.9 [ac, y]	58.8±2.17 [ac, x]
	30 mg/ kg	63.4±1.34 [ab, y]	53.6±1.14 [ab, x]
HDL	0mg/ kg	29.68±0.51 [bc]	29.74±0.87 [bc]
	15 mg/ kg	32.5±1.02 [ac, y]	37.88±1.13 [ac, x]
	30 mg/ kg	35.06±0.32 [ab, y]	40.42±0.99 [ab, x]
LDL	0mg/ kg	136.8±2.0 [bc]	138.1±2.8 [bc]
	15 mg/ kg	117.44±1.9 [ac, y]	110.81±2.3 [ac, x]
	30 mg/ kg	113.94±6.1 [ab, y]	162.2±0.6 [ab, x]
Risk 1	0mg/ kg	6.21±0.12 [bc]	6.23±0.25 [b]
	15 mg/ kg	5.04±0.16 [a]	4.24±0.14 [a]
	30 mg/ kg	4.54±0.05 [c]	5.25±0.4 [b]
Risk 2	0mg/ kg	4.6±0.1 [ac]	4.6±0.22 [b]
	15 mg/ kg	3.6±0.15 [ac, y]	2.9±0.13 [ac, x]
	30 mg/ kg	3.3±0.19	4.0±0.12 [b]

Table 4b: Effect of Zn0- Nps on serum lipid profile levels of Barki lambs fed on restricted ration.

	ZnO Nps	9 months	12 months
Total cholesterol	0mg/ kg	176.8±2.95 [bc]	175.8±1.3 [bc, x]
	15 mg/ kg	161.2±1.3 [ab, y]	154.2±0.84 [ac, x]
	30 mg/ kg	153.6±2.51 [ac, y]	147.6±2.3 [ab, x]
Triglyceride	0mg/ kg	81±1 [bc]	81±1 [bc]
	15 mg/ kg	65±0.71 [ac, y]	56.8±1.3 [ac, x]
	30 mg/ kg	59.8±1.48 [ab, y]	52±2.1 [ab, x]
HDL	0mg/ kg	26.58±1.3 [bc]	27.3±0.65 [bc, x]
	15 mg/ kg	34±0.5 [ac, y]	40±2.1 [a, x]
	30 mg/ kg	38±0.8 [bc], [y]	42.98±1.3 [ab, x]
LDL	0mg/ kg	134.0±3.3 [bc]	132.3±1.6 [bc]
	15 mg/ kg	114.2± 1.18 [ac, y]	102.9± 1.4 [ac, x]
	30 mg/ kg	105.98±4.0 [ab, y]	96.3±3.6 [ab, x]
Risk 1	0mg/ kg	6.67±0.4 [bc]	6.45±0.18 [bc]
	15 mg/ kg	4.74±0.06 [a]	3.86±0.19 [a]
	30 mg/ kg	4.04±0.02	3.44±0.12
Risk 2	0mg/ kg	5.1±0.33[bc]	4.9±0.17[bc]
	15 mg/ kg	3.4±0.06[a, y]	2.6±0.17[a]
	30 mg/ kg	2.8±0.16[a]	2.2±0.14[a]

Discussion

Zinc dietary intake is essential for building the physiological function and augmentation of the immune system (Zhao et al., 2014). Zinc may be supplemented in the form of inorganic, organic, or nano sources. Zinc Oxide nanoparticles can adequately be a higher potential than their conventional Zn sources (Swain et al., 2019).

The obtained results showed that there was an improvement in weight gain and feed conversion rates but there were no significant differences between treatment groups (Fig.1). Riyazi et al. (2019) 0 mg/kg of Zn as Zn-NPs compared with other higher Zn levels (40 and 60 mg/kg dry matter, DM) during an in vitro fermentation study. These positive effects are also seen in vivo with adult and/or growing animals; in ewes, the dietary supplementation of Zn-NPs significantly increased the digestibility of DM, organic matter, nitrogen, and crude fiber-free extract compared with Zn larger particle and control ewes (Mohamed et al. 2017). In growing animals, the inclusion of Zn-NPs in the diets of lambs enhanced the digestibility and feeding value of the diet (Vardanjani et al., 2020). This result disagreed with Zaboli et al. (2013) reported that average daily gain was significantly improved to 0.080 and 0.080 in kids supplemented with 20-40 ppm nano zinc oxide compared with 0.065 in the negative control group.

Blood biochemistry parameters are important markers of physiological status (El-Kholy et al., 2017). Belewu and Adewumi (2021) reported that ZnO-NPs at 40 and 80 mg/kg caused a significant reduction ($p<0.05$) in ALT (6.5 and 7.0 IU/L) as compared to the control (13.0 IU/L). These findings disagreed with those obtained by Swain et al. (2019) found that 90 days feeding of male goats on diets containing 25 and 50 mg/kg ZnO-NPs had a non-significant effect on serum ALT levels (17.3 and 19.9 IU/L) compared to the negative control (21.9 IU/L) (Table1a-b). While Belewu and Adewumi (2021) reported that a higher concentration of ZnO-NPs 80 mg/kg caused a significant reduction ($p<0.05$) in AST (31.00 IU/L) as compared to the control (60.50 IU/L). While ZnO-NPs at 40 mg/kg caused a significant increase (73.00 IU/L) as compared to the control (60.50 IU/L) (Belewu and Adewumi (2021). However, Swain et al. (2019) found that 90 days feeding of male goats on diets containing 25 and 50 mg/kg ZnO-NPs had a non-significant effect on serum AST levels (198 and 207 IU/L) compared to the negative control (197 IU/L). Blood enzymes such as ALT, and AST (IU/L) were similar in all the groups. The values obtained by Swain et al. (2019) study were in the physiological ranges suggested by Kaneko et al., (2008). Mandal et al., (2008) in crossbred calves, and Hassan et al. (2011) in adult Bakri sheep. Chen et al. (2011) demonstrated that Zinc Nanoparticles (Zn-Nps) are much more active and can be rapidly transformed into respective ions in gastric juice. So large amounts of metal ions are generated and subsequently brought to the liver and kidney for metabolism and excretion, which might cause damage to hepatic and renal tissues.

Results in the Table (1a,b) agreed with Zaboli et al. (2013) who had reported that feeding goat kids on diets containing 60 ppm ZnO-Nps had a non-significant effect on serum urea levels (31.32 mg/dL) compared with (32.1 mg/dL) initial urea level disagree with those obtained by Belewu and Adewumi (2021) who reported that west African dwarf (WAD) goats fed on 40 and 80 mg/kg (0.004, 0.008%) ZnO NPs showed a significant reduction in blood urea nitrogen (4.43 and 7.42) compared to (13.17) in the negative control. Also, Novoselec et al. (2017) reported a similar effect after using Nano-

Zn, ZnO, and Zn-Met at a dose of 28 mg/kg DM in sheep diet caused a reduction of serum BUN contents.

Zaboli et al. (2013) reported that feeding goat kids diets containing 60 ppm nano zinc oxide had a non-significant effect on serum creatinine levels (1.12 mg/dL) compared with (0.91 mg/dL) initial creatinine level. Also, Swain et al. (2019), had found that 90 days feeding of male goats on diets containing 25 and 50 mg/kg ZnO -NPs had a non-significant effect on serum creatinine levels (1.37 and 1.21 mg/dL) compared to the negative control (1.1 mg/dL). Similar results were reported by Anil, et al. (2020) for BUN and creatinine levels in crossbred calves. Najafzadeh et al. (2013) found that the activity of creatinine was significantly increased in lambs fed 20 mg zinc nanoparticles per kg body weight daily for 25 days compared with the control. On the other side, AL Tamimi et al. (2021) explained that ZnO-NPS caused a significant increase in creatinine, blood urea nitrogen, and uric acid in the treated group at a concentration (300 mg/kg) for 30 consecutive days. ZnO-NPS caused a significant increase in creatinine, blood urea nitrogen, and uric acid, the level of the renal markers in serum are changed under the influence of kidney disorders, and the renal markers (that present inside the proximal cells of nephrons) are released in the blood when the kidney damages, therefore the increased concentration of them indicates cell damage (Layasadat et al., 2018). ZnO NPS caused toxic effects on the kidney resulting in the potential for kidney damage, thus increasing the biomarkers of the kidney (urea and creatinine), there are several mechanisms by which the nanoparticles can cause cell toxicity, including the production of (ROS), O.S, genotoxicity, lipid peroxidation, and stimulation the pathway of inflammatory (Mokhtar et al., 2019) dependent on dose the ZnO-NPS can stimulate renal toxicity) Layasadat et al. (2018) (Generation O.S). A low dose of ZnO-NPS can stimulate more renal toxicity, but the mechanism of this result is unclear (Layasadat et al., 2018). The most important markers that are used to evaluate kidney function, are BUN and Cr because it's mainly released from the kidneys, however, creatinine is a more sensitive indicator of kidney function (Shivaraj et al., 2010). The exposure and dosage of ZnO- NPS are factors that may affect the levels of BUN and Cr in the blood (Najafzadeh et al., 2013).

Similarly, Najafzadeh et al. (2013) found increased serum zinc levels in lambs fed 20 mg/kg body weight zinc nanoparticles daily for 25 days. Also, Zaboli et al. (2013) reported a significant increase in serum Zn levels (1.85 µg/dl) in kids fed on 60 ppm ZnO-Nps (30 nm size) compared with (0.79 µg/dl) in the negative control group. A similar result had been reported in kids by Singh et al. (2008) and Swain et al. (2021) in male goats, who found that serum Zn levels had significantly increased from 0.47 and 0.44 to 0.80 and 0.90 mg/L, after 90 days of feeding on diets containing 25 and 50 mg/kg ZnO-NPs, respectively. Similar results were reported by Anil, et al. (2020) in crossbred calves, Jia et al. (2009) in Cashmere goats, and Garg et al., (2008) in lambs as well. This might be correlated to better bioavailability of Zn-Nps than ionized zinc, resulting in better absorption, distribution, and uptake of zinc as suggested by Kumar et al., (2006).

However, contrary to these findings, nano-zinc supplementation at 40 mg/kg increased serum Zn at 35 days after supplementation but not at 70th days in Markhoj goats (Zaboli et al., 2013).

There is a significant increase in serum iron levels between treated groups (Table 2a-b). This result disagreed with Swain et al. (2021) who reported that 90 days of feeding on diets containing 25 and 50 mg/kg ZnO-NPs had nonsignificant serum iron levels (3.73 mg/L) for both concentrations compared to the negative control (3.40 and 3.55 mg/L).

These results agreed with those reported by Zaboli et al. (2013) and Uniyal et al. (2017) where serum mineral (Ca, P, Cu, Fe, Mn, and Co) levels were not affected in kids supplemented 20-40 ppm nano zinc oxide, however, serum zinc levels were significantly higher in groups fed 20 ppm ZnO-Nps.

Results observed that there is a significant reduction in MDA levels between treated groups. This result disagreed with those reported by Layasadat et al. (2018) who revealed that ZnO-NPs had increased the concentration of MDA due to the oxidative stress which stimulated lipid oxidation. Layasadat et al. (2018) stated that the elevation of MDA levels and reduced antioxidant enzyme activity in tissues promote lipid peroxidation formation, indicating insufficient protection of antioxidants against excessive production of free radicals.

Results showed that there is a significant increase in serum SOD. This result disagreed with Zaboli et al. (2013) who had reported that feeding goat kids diets containing 60 ppm nano zinc oxide had a significant effect on SOD levels (242.54 U/dL) compared with (265.5 U/dL) initial SOD level.

This result is constant with those reported by Ahmadi et al. (2013), Uniyal et al. (2017), and Zhao et al. (2014) who observed that supplementation of Zn-Nps at 20, 60, and 100 ppm levels significantly higher total antioxidant capability, catalase activity and SOD activity as compared to control fed 60 ppm of ZnO. In contrast, Muralisankar et al. (2014) reported that activities of enzymatic antioxidants SOD, and CAT showed no significant alterations in 10-60 mg /kg ZnO-Nps supplemented feed for 90 days. However, 80 mg ZnNps/ kg supplemented feed showed significant elevations in SOD and CAT.

MDA is an important index for lipid peroxidation and oxidative damage caused by ROS. Duzguner and Kaya (2007), Attia et al. (2013), and Reda et al. (2021) confirmed the hypothesis that appropriate concentrations of ZnO-NPs stimulate SOD activity, and enhanced SOD will suppress ROS generation, thus decreasing MDA levels.

Results shown in the Table revealed that there is a significant increase in serum (TAC). These results agreed with those reported by Mohamed et al. (2017) and Riazi et al. (2019) who revealed that Zn nanoparticles improved the antioxidant capacity in sheep. Prasad et al. (2014), Bąkowski et al. (2018), and Suresh et al. (2015) explained that Zn is a strong antioxidant metal decreasing free radicals. It has also been reported that nano-ZnO can increase antioxidant activity and decrease free radicals due to the increased specific surface area and thus the higher number of active sites. This disagreed with Sharma et al. (2012) who had reported oxidative stress due to the effect of ZnO-Nps.

Results in Table (2a-b) showed that there were non-significant differences in total serum protein and albumin between treated groups. This result agreed with those obtained by Belewu and Adewumi (2021) who reported nonsignificant variations in total protein (5.70 and 5.35), between the 40 and 80 mg/kg ZnO NPs, respectively as compared to the control (5.75).

However, contrary to these findings, Swain et al. (2019), had found that 90 days feeding of male goats on diets containing 25 and 50 mg/kg ZnO-NPs had a significant increase ($P<0.05$) in total serum protein (6.85 and 6.9 g/dL, respectively) compared to the negative control (6.78 g/dL), without any significant effect on serum albumin. While the 50 mg/kg ZnO showed a statically significant increase in serum globulin. Also, Anil, et al. (2020) reported that the serum zinc, total protein, serum albumin, and serum globulin were significantly ($P<0.01$) higher after 90 days of ZnO-Nps supplementation in crossbred calves.

Results in Table (3a-b) revealed that there was a significant reduction in the serum A/G ratio. Contrary to this result, Swain et al. (2019), found that Zn-NPs at concentrations of 25 and 50 mg/kg had non-significant effects on serum A/G ratio levels (1.16 mg/dL) for both treatments compared to the negative control (1.17 mg/dL). Zn-Nps 25 (3.17 ± 0.02) remained intermediate in globulin level. Similar to globulin, total protein (g/dL) was more ($P<0.001$) in Zn-Nps 50 (6.90 ± 0.01). The albumin/globulin ratio was similar ($P>0.05$) in all the groups which ranged from 1.16 ± 0.01 (Zn-Nps 50 and 25).

Total serum cholesterol and triglyceride levels were significantly decreased in both treated groups (15 mg and 30 mg ZnO-Nps) at both 9 and 12 months of age. While, serum HDL and LDL levels significantly increased between both treated groups (15 mg and 30 mg ZnO-Nps) and the control group at 9 and 12 months of age, and also significant differences between 15mg and 30 mg ZnO-NPs. Also, the different feeding methods had affected significantly where the restricted ration feeding showed higher LDL levels than those of the adlibitum groups (Table 4a-b). Abd El-Hack et al. (2017) demonstrated that the change in cholesterol levels in the blood serum may be due to zinc’s role in enzymatic actions, in that zinc forms an integral part of several enzymes (metalloenzymes) that are severed in lipid digestion and absorption.

Conclusion

The use of nano zinc particles in livestock nutrition regimes showed promising results in enhancing the growth rate and weight gain performance status in fattening Barki lambs without any adverse impacts on the liver and kidney functions or the total serum proteins and selenium levels. Also, it caused an improvement in the antioxidant status, as well as the levels of iron. There was no significant difference in biochemical parameters of lambs received 15 mg/kg and those received 30 mg/kg at 9 months or 12 months of age. These results did not been affected by the change in feeding method.

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