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Impact of nano zinc on hormonal profile, immunity and body/testicular measurements of Ossimi lambs

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ABSTRACT

Objective: The aim of this study was to evaluate the effect of using different levels of ZnO nanoparticles (ZnO NPs) into drinking water (10, 20 and 30 mg/kg DM intake) for 90 days on growth, immunity, anti-oxidants status and physiological performance of lambs. **Method:** The current study was carried out at Fayoum Governorate, Egypt at a private farm called "Al Sharif farm". Healthy 16 Ossimi ram lambs, 5 months old and approximately 30 kg weight, were randomly divided into 4 equally groups (control and 3 treatment groups). The ZnO NPs were prepared by chemical precipitation method using zinc sulfate heptahydrate and characterized by X-ray diffraction technique. ZnO NPs were orally performed in water to treated lambs by drenching or dosing gun with doses of (10, 20, and 30 mg/kg DM intake) for 90 days. Ram lambs were kept under the same managerial and feeding conditions. Fresh water was provided *ad libitum* throughout the day. Ram lambs were biweekly weighed to follow their growth rates, calculate zinc (Zn) quantities and to adjust feed requirements. Body and testicular measurements were estimated. Blood samples were monthly withdrawn and the obtained serum was used to determine Zn levels, immunity response as immunogloblin (IgG), antioxidants status (total antioxidants; TAC, catalase; CAT), in addition to estimates the levels of serum hormones (GH and testosterone; T). **Results:** Our results indicated that providing ZnO NPs to ram lambs led to insignificant increases in body weight and score, testicular measurements while body measurements were significantly increased in ZnO NPs treated lambs. Serum Zn concentration was elevated by 2.69, 4.36, and 15.70% for 10, 20 and 30 mg/kg DM treated lambs as compared to the control. Moreover, treating lambs increased CAT, TAC, T and GH ($p \leq 0.05$). **Conclusion:** It can be concluded that Zn supplementation to ram lambs improved testosterone, GH, antioxidants and immunity status and consequently growth performance of Ossimi ram lambs.

Keywords: Antioxidants, body measurements, growth, hormones, Sheep, Zn nanoparticles.

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1. INTRODUCTION

Sheep (*Ovis aries*) are widespread domestic species of small ruminants since there are more than 1.2 billion sheep spread over the world **(Gilbert *et al*., 2018)**. A multitude of breeds are adapted to many purposes in diverse environments and are reared mainly for their meat, milk, wool, and hides. Nanotechnology is one of the most modern techniques used for many purposes and may support and maintain the sustainability of the livestock sector. Nanotechnology has contributed to the development of nano vehicles for nutrients to ensure efficient delivery leading to improvement of digestion, absorption and metabolism of nutrients. Because of low solubility of zinc oxide (ZnO) (**Wedekind and Baker, 1990**), using of ZnO nanoparticles (ZnO NPs) in ruminant feeding has been started **(Singh *et al*., 2018)**, since using nano particles (< 100 nm in diameter), rather than micro forms, with increased surface area allows greater solubility leading to better utilization in animals. Some researchers have indicated that ZnO NPs have been shown to exhibit strong protein adsorption properties, which can be used to modulate metabolism or cellular responses. One more feature of ZnO NPs is their ability to remain in circulation for a longer time and take charge as a dietary modulator for hydrolase activity. In addition, it also exhibit strong gastrointestinal protective, antibacterial and antioxidative properties **(Bedi and Kaur, 2015; Chik-kanna *et al*., 2019; Geetha *et al*., 2020)**. Furthermore, it was reported that because of their higher bioavailability than the ordinary ZnO, the use of trace minerals such as ZnO NPs can decrease mineral excretion and environmental pollution **(Padmavathy and Vijayaraghavan, 2008; Hosseini-Vardanjani *et al*., 2020)**.

Minerals in the form of nano are interesting surrogates to enhance bioavailability and reduce pollution consequently stimulating antioxidant activity, and enhancing growth, reproductive performance and immune response. Another important role of nano particles is to help for improving assisted reproductive technologies outputs by enrichment media for cryopreservation of spermatozoa, oocytes, and embryos with antioxidant nano particles. Zn is also an essential mineral nutrient for animals and humans and is considered a component includes in the synthesis of many enzymes and hormones **(Wang *et al*., 2018)**. It also can promote growth through its action as an antibacterial agent, and modulates the immunity and reproductive status of animals **(Swain *et al*., 2016)**. Since Zn is not stored in the body, a constant dietary supply is required to achieve its proper physiological responses (**Zalewski *et al*., 2005)** such as normal growth **(Case and Carlson, 2002)**, synthesizing DNA, cell division and gene expression **(Prasad, 1991)**, ossification **(Roughead and Kunkel, 1991)**, stimulating the body immunity **(Zhao *et al*., 2014 and Parashuramulu *et al*., 2015)** and these were achieved through energy and protein production, protecting membranes from bacterial endotoxins and lymphocyte replication and antibody production **(Nockels, 1994).** Zn also plays an important role in physiological functions in the animal or human body such as bone development, coagulation, and biofilm stability along with the participation in the metabolism of carbohydrates, fats, proteins, nucleic acids, and vitamins **(Chai and Tian 2013; Chi *et al*., 2019)**.

Finally, Although Zn is a very important element for most of the body functions and is needed to continuously provide to farm animals, traditional form of ZnO has a low solubility and/or bioavailability as well as its ability to produce more pollution. So, the current study assigned to evaluate the nano form of ZnO that has higher solubility,

bioavailability and protective impacts on growth performance of Ossimi ram lambs.

For why ZnO NPs insetead of traditional ZnO, the following table illustrates the difference between them.

Table 1. Differences between ZnO nanoparticles (ZnO NPs) and traditional ZnO

Traditional ZnO	ZnO NPs	Reference
Low solubility	Greater solubility	**Wedekind and Baker (1990)**
Normal surface area	Increased surface area	**Swain *et al.* (2016)**
Less utilization in animal	Better utilization in animals	**Singh *et al.* (2018)**
Exhibit lower protein adsorption properties	Exhibit strong protein adsorption properties	**Geetha *et al.* (2020)**
Remain in circulation for a shorter time	Remain in circulation for a longer time	**Chik-kanna *et al.* (2019)**
Lower bioavailibility	Higher bioavailability	**Singh *et al.* (2018)**
increase mineral excretion and environmental pollution	Decrease mineral excretion and environmental pollution	**Singh *et al.* (2018)**

The current study aimed to evaluate the effect of supplementing ZnO NPs in drinking water (10, 20, and 30 mg/kg DM intake) for 90 days on growth and physiological performance of lambs.

2. MATERIAL AND METHODS

Sixteen healthy Ossimi ram lambs, 5 months old and approximately 30 kg in weight, were randomly divided into 4 equally groups, control (G1) and 3 treated groups. Three levels of ZnO NPs, 10 (G2), 20 (G3) and 30 (G4) mg/kg DM, were tested to evaluate its effect for 90 days. ZnO NPs were supplemented in drinking water by using drenching or dosing gun to ensure manipulating all the dose quantities without any loss where lambs consumed all daily provided ZnO NPs solution.

Ram lambs were kept under the same managerial and feeding conditions where it fed a diet based on soybean meal, yellow corn, $CaCO_3$, salt table and vitamins and mineral mixture. in addition to wheat straw according to **NRC (2007)**. Fresh water was provided ad libitum throughout the day. Ram lambs were biweekly weighed to adjust Zn quantities, feed requirements and to follow up their growth rates. ZnONPs were prepared by chemical precipitation method using zinc sulfate heptahydrate (99.99% Pure, Sigma-Aldrich, USA) as precursor salt and and sodium hydroxide (99 - 100%, Sigma-Aldrich, USA) according to **(Kumar *et al*., 2013)**, while chemical structure for prepared ZnO NPs was assessed using X-ray Diffraction (XRD) technique.

Blood samples were collected monthly to obtain the serum which used to determine serum Zn, hormones (growth hormone; GH and Testosterone; T) levels, and immunoglobulin (IgG) in addition to determine antioxidants status (total antioxidants; TAC, catalase; CAT). Special commercial kits were used to quantify blood serum according to the procedures outlined by the manufactures. GH was measured spectrophotometrically with ElISA kit plate at a wave length of 450 nm, while total T was quantitatively analyzed by i-CHROMA Reader system**, (biocheck, Inc. Foster City, CA 94404, U.S.A)**. Serum TAC and CAT levels were determined using Spectrophotometer Diasys star Dust MC-15 at wave length 505 and 510 nm according to **Koracevic *et al.* (2001)**, **Aebi (1984)**, and **Fossati (1980)**, respectively, while IgG was estimated by using enzyme linked immunsorbent assay (ELISA) with ELISA kit with plates. Bender, Med Systems Gm bH. Campus Vienna Austria at wave lengths 450 and 570. Serum Zn was calorimetrically estimated by with 5 Brom PAPS at wave length 560nm, MDSS-GMbH schiffgraben 41 30175 Hannover, Germany, according to **Johnsen and Eliasson (1987)**. Additionally, growth or morphometric measurements (whole body length, shoulder body length, body height and chest and abdominal circumferences) as well as testicular measurements

(Scrotum circumference (SC) and testicular length (TL) were biweekly measured by using flexible tap.

Statistical analysis was performed using SPSS software program, version 22, (SPSS, 2015, IBM, Chicago) with general linear model and complete randomizing design. Level of statistical significance was set at $p \leq 0.05$ where the following model was used for the current experiment: $Y_{ijk} = \mu + T_i + p_j + (T_i \times p_j) + e_{ijk}$ where, Y_{ijk} is dependent variable in the study, μ is overall mean, T_i is the effect of treatment or ZnO NPs level (i=control, Zn10, Zn20, and Zn30), P_j is the effect of period (j=0, 30, 60 and 90d) for serum measurements or (j= 0,15, 30, 45, 60, 75 and 90d) for body measurements., while ij is effect of interaction of $T_i \times P_j$ and e_{ijk} is the error.

3. RESULTS AND DISCUSSION

3.1. Serum zinc (Zn) concentration

It has been shown in **Table 2** that serum Zn levels were not significantly elevated gradually with the increase of orally supplemented Zn NPs levels in treated ram lambs compared to control group. These increases were 2.69, 4.36 and 15.70% for 10, 20 and 30 mg ZnO NPS/kg DM–treated lambs as compared to the control. The overall means of serum Zn were 106.53 ±7.68, 109.40 ±4.66, 111.17 ±7.03 and 123.25 ±7.99 mg/dl for control, 10, 20 and 30 mg ZnO NPs/kg DM-treated ram lambs, respectively. Periods of 0, 30 and 60d seemed to be the same and were significantly higher than 90 d. No significant changes were detected for the interaction (treatment × period). Zn concentration in blood plasma or serum is the most widely used indicator of Zn status. Plasma Zn concentrations normally respond to Zn supplementation, especially in lambs consuming diets with a low or marginal Zn level **(Suttle, 2010; Wieringa *et al.*, 2015**).

The higher serum Zn levels in the ZnO NPs treated groups might be due to the greater absorption of ZnO NPs in small lambs **(Singh *et al.*, 2018)**. It has been shown that nano particles are absorbed in duodenum by active transport and nano-elemental forms can cross the small intestine and further distribute into the blood **(Hillyer and Albrecht, 2001)**. The current findings were in agreement with those of **Najafzadeh *et al.* (2013)**, **Abdelgayed *et al.* (2022)**, **Mallaki *et al.* (2015)**, **and Aliarabi *et al.* (2015)** in lambs and male goats (**Jia *et al.*, 2008)**. In contrast, no significant difference was observed in the plasma Zn concentration in adult sheep supplemented daily with 75 or 150 mg of Zn either as chelated Zn or inorganic Zn **(Ryan *et al.*, 2002)** and calves supplemented with 80 or 120 mg Zn/kg DM as Zn-sulfate on a basal diet containing 29.7 mg Zn/kg DM during 60- and 90-day feeding trials **(Ramulu *et al.*, 2015)**.

Table 2. Serum zinc (Zn) and immunoglobulin (IgG) concentration in orally ZnO nanoparticles supplemented Ossimi ram-lambs

Item	Period (d)	G1 (Control)	G2 (Zn 10)	G3 (Zn 20)	G4 (Zn 30)	Overall mean
	0	117.73±11.23	118.33±12.56	115.87±22.01	115.07±8.12	**116.75±6.47 A**
	30	105.23 ±8.57	119.83±9.88	124.00 ±1.00	121.50±4.85	**117.64±3.65 A**
Zn (mg/dl)	60	130.10±18.95	99.83±6.77	106.88±17.89	152.90±26.78	**122.43±10.06 A**
	90	73.07 ±4.52	99.60±2.72	97.93 ±7.51	103.53 ±5.08	**93.53 ±3.88 B**
	Average	**106.53 ±7.68**	**109.40±4.66**	**111.17±7.03**	**123.25 ±7.99**	**112.59 ±3.49**
	0	33.42 ±1.03	27.33±1.04	37.67±3.12	38.00 ±4.95	**34.11 ±1.96**
	30	30.33 ±2.78	30.67±2.49	32.07±0.41	32.50 ±4.17	**31.39 ±1.28**
IgG (mg/dl)	**60**	25.33 ±0.85	42.07 ±9.96	32.07±0.41	34.50 ±4.44	**33.49 ±2.89**
	90	34.67 ±2.01	40.95 ±11.51	41.25±5.17	35.50 ±4.92	**38.09 ±3.16**
	Average	**30.94 ±1.25**	**35.25 ±3.83**	**35.76±1.69**	**35.13 ±2.14**	**34.27±1.23**

Means having the different superscripts (capital letters) in the same column are significantly differed ($p \leq 0.05$).

3.2. Immunoglobulin G (IgG)

As shown in **Table 2**, the highest levels of IgG were observed in all ZnO NPs treated lambs groups compared to the control group. The average serum IgG concentrations were 30.94±1.246, 35.25±3.834, 35.76±1.690 and 35.13±2.135 mM for control, 10, 20, and 30 mg ZnO NPs/kg DM, respectively, where the increasing rates were 13.95, 15.59, and 13.53% in ZnO NPs-supplemented ram lambs as compared to control which were insignificantly differed. Moreover, both periods and its interaction with treatment had no significant effects on IgG concentration. The same trend was observed in ewes and their lambs (**Mohamed *et al.*, 2017**) and in Qianbei-pockmarked goats **(Song *et al*., 2020**) which were supplemented with Zn NPs. This index enhancement may reflex the effect of Zn NPs on immune system.

3.3. Total antioxidants (TAC) and catalase (CAT)

Serum TAC and CAT levels in control and Zn NPs–treated ram lambs are listed in **Table 3.** Serum TAC levels were noted to be higher ($p \leq 0.05$) in all groups of Zn NPs–treated ram lambs than control group where the average serum TAC was 1.081±0.020, 1.0834±0.024, 1.1105±0.021, and 1.165±0.012 mM/l for control and the gradual three ZnO NPs supplemented levels 10, 20, and 30 mg/kg DM, respectively. TAC of the third treated group (30 mg/kg DM) was significantly ($p \leq 0.05$) higher than other groups. Regardless treatment, periods were significantly differed among themself where 0 and 90 days were significantly higher than other periods (30 and 60 d). Regarding interaction effect, it has been shown that significantly fluctuations occurred among different items where Zn20 at 0 d and Zn30 at 60 d have the highest levels of serum TAC while control at 60 d, Zn10 at 30, Zn10 at 60 d and Zn20 at 30 d were the least means of serum TAC concentration. The obtained results supported that of **Alimohamady *et al*. (2019)**, who pointed out that supplementation of Zn in growing lambs improved growth performance, blood antioxidants. Furthermore, **Kumar *et al.* (2013)** stated that Zn and Selenium (Se) supplementation can improve the anti-oxidative status and hormone levels by increasing the Zn and Se level in seminal plasma and serum, respectively. Concerning serum CAT, It has been indicated that serum CAT concentrations insignificantly increased with the increase of Zn-NPs supplementation levels by 3.79, 4.95 and 7.24% of control since the average serum CAT were 186.95 ±15.47, 194.04 ±16.99, 196.21 ±17.11 and 200.48 ±13.67 U/l in control, 10, 20, and 30 mg/kg DM Zn NPs groups respectively. Period effect, regardless treatment, was significantly ($p \leq 0.05$) differed among them where the highest value was for 30 d followed by 60, 90, and 0 d, respectively. Interaction also has a significantly fluctuation where Zn20 at 60 d has the highest CAT level, while con at 0 d has the lowest CAT concentration. These results were agreed with that of **Alavi-shoushtari *et al*. (2009)** who outlined that Zn supplementation increases CAT in seminal plasma in buffalo. In addition, **Kumar *et al*. (2013)** stated an increase of CAT and other antioxidants after 60d of treating with Zn and Se.

Table 3. Serum anti-oxidants (total anti-oxidants;TAC and catalase;CAT) status as affected by supplementation of ZnO nanoparticles in Ossimi ram-lambs

Item	Period (d)	G1 (Control)	G2 (Zn 10)	G3 (Zn 20)	G4 (Zn 30)	Overall mean
TAC (mM/l)	0	1.141±0.041 abc	1.174±0.003 abc	1.201±0.003 a	1.152±0.037 abc	**1.167±0.014 A**
	30	1.077±0.023 bcd	1.0165±0.022 d	1.017±0.028 d	1.146±0.035 abc	**1.064±0.018 B**
	60	1.004±0.032 d	1.0338±0.069 d	1.069±0.031 cd	1.187±0.004 a	**1.073±0.026 B**
	90	1.104±0.036 abcd	1.149±0.009 abc	1.156±0.007 abc	1.175±0.003 ab	**1.146±0.011 A**
	Average	**1.081±0.020 b**	**1.0834±0.024 b**	**1.1105±0.021 b**	**1.165±0.012 a**	**1.110±0.010**
CAT (U/l)	0	98.99±11.09 h	118.52±2.65 gh	122.67 ±3.90 fgh	124.70±7.62 efgh	**116.22±4.13 D**
	30	245.79±1.44 abc	276.10±8.42 ab	285.52±27.90 a	234.34±14.02 bc	**260.44±9.34 A**
	60	233.33±2.06 bc	234.07±15.10 bc	208.08±4.36 cd	232.32±16.91 bc	**226.95±5.90 B**
	90	169.70±1.43 de	147.48 ±7.87 efg	168.69±16.59 def	210.58±23.02 cd	**174.11±8.85 C**
	Average	**186.95±15.47**	**194.04±16.99**	**196.21±17.11**	**200.48±13.67**	**194.42±7.77**

Means having the different superscripts (capital letters) in the same column or small letters in same average row are significantly differed ($p \leq 0.05$), while that of interaction compared to all.

3.4. Serum hormones

3.4.1. Growth hormone (GH)

As shown in **Table 4**, GH levels increased with increasing ZnO NPs supplementation levels where G4 (30 mg) had the highest ($p \leq 0.05$) level compared to control group that had the least one. The average serum GH concentrations of control and Zn NPs treated ram lambs were 0.067±0.005, 0.070±0.004, 0.078±0.008, and 0.088±0.009 ng/ml for control, 10, 20 and 30 mg/kg DM-treated lambs, respectively. Regardless treatment, periods of 30, 60, and 90 d were significantly unchanged with each other however, 0 d was significantly lower than 60 d only. No significant changes were detected for the interaction (treatment × period). The enhanced growth and GH levels with the supplementation of ZnO NPs may be interpreted through the increased level of IGF-1 and IGFBP-3, in short children with zn deficiency, after supplementing them with Zn as noted by **(Hamza *et al*., 2012)**. Moreover, pituitary gland contains a higher level of Zn than other organs and Zn improved pituitary hormone function (**Henkin, 1976**). **Berrie *et al.* (1995**) recorded a similar result, pointing out that Increases in growth resulting from the Zn Met complex might be related to an increase in GH after feeding, as observed in lambs. Moreover, **Neve (1992)** recorded a relationship between Zn and GH.

Table 4. Growth hormone (GH) and testosterone (T) concentration of control and zinc oxide nanoparticles supplemented Ossimi ram-lambs

Item	Period (d)	G1 (Control)	G2 (Zn 10)	G3 (Zn 20)	G4 (Zn 30)	Overall mean
GH (ng/ml)	0	0.064±0.005	0.057±0.008	0.063±0.013	0.068±0.015	**0.063±0.005 B**
	30	0.057±0.001	0.072±0.009	0.105±0.011	0.083±0.012	**0.079±0.006 AB**
	60	0.079±0.015	0.072±0.007	0.087±0.018	0.114±0.015	**0.088±0.007 A**
	90	0.068±0.012	0.079±0.010	0.057±0.007	0.089±0.024	**0.073±0.007 AB**
	Average	**0.067±0.005 b**	**0.070±0.004 ab**	**0.078±0.008 ab**	**0.088±0.009 a**	**0.076±0.003**
T (pg/ml)	0	50.80±13.24	49.70±12.79	62.07±18.75	78.03±17.37	**60.15 ±7.65**
	30	73.50±14.60	69.87±13.47	62.07±18.75	105.75±6.04	**77.80±7.59**
	60	63.17±18.49	77.59±8.29	47.65±21.88	47.00±16.26	**58.85±8.26**
	90	60.02±6.96	62.79±5.65	61.59±9.93	64.63±9.54	**62.26±4.02**
	Average	**50.80±13.24**	**49.70±12.79**	**62.07±18.75**	**78.03±17.37**	**60.15±7.65**

Means having the different superscripts (capital letters) in the same column and small letters in the row are significantly differed ($p \leq 0.05$).

3.4.2. Serum total testosterone (T)

Regarding serum T concentrations as shown in **Table 4**, treating ram lambs with ZnO NPs insignificantly elevated serum T in all supplemented groups compared to the control group.

Furthermore, insignificant influences were observed according to periods and/or their interactions with treatment as affected by ZnO NPs administration. The averages of serum total T were 60.02 ±6.96, 62.79 ±5.65, 61.59 ±9.93 and 64.63 ±9.54 pg/ml for control, 10, 20 and 30 mg ZnO NPs treated groups, respectively. The increased levels of T might be due to the effect of Zn on pituitary and gonads level as described by **Joshi *et al*. (2014)** and **Omu *et al*. (2015**) who detected a suppression of T and FSH synthesis, as well as inhibition of spermatogenesis in zinc deficiency or could be interpreted through the suggestion of (**Bedwal and Bahuguna, 1994**) that Zn plays an essential role in testicular steroidogenesis, androgen metabolism and interaction with steroid receptors. Moreover, **Te, *et al.* (2023)** indicated that in male, Zn is involved in various biological processes, an important function of which is as a balancer of hormones such as T hormone where they concluded that Zn deficiency reduces T levels and Zn supplementation improves T levels where serum Zn was positively correlated with total T, and moderate supplementation plays an important role in improving androgen. Additionally, testicular concentrations of Zn and T hormone were lower in the Zn-deficient animals than in all Zn-treated groups **(Martin *et al*., 1994)**.

3.5. Growth or morphometric measurements

3.5.1. Body weight (BW) and body score

Means of body weights and body condition score (BCS) are presented in **Table 5**. It has been shown that ZnO NPs-treated ram lambs have higher (BW) than control one where this increase was 2.00, 7.24, and 6.81% for 10, 20, and 30 mg ZnO NPs/ kg DM compared to control group. These increases were statistically unchanged. BW significantly elevated ($p \leq 0.05$) with the progression of periods regardless treatment where the highest periods were the last two periods 75 and 90 d. The interaction has no significant changes.

Concerning body condition score (BCS), it has been detected that BCS was improved with the advance of increasing ZnO NPs levels for treated lambs comparing to control group, where the average BCS of lambs were 3.38, 3.75, 4.00, and 4.25 for control, 10, 20 and 30 mg ZnO NPs/kg DM groups, respectively. These increases reached 10.59, 18.34, and 25.74% for those groups as compared to control group.

Table 5. Effect of nano zinc oxide on body weight, condition score, and measurements in Ossimi ram-lambs

Item	G1 (Control)	G2 (Zn 10)	G3 (Zn 20)	G4 (Zn 30)	Overall mean
BW (kg)	37.58±1.59	38.33±1.37	40.30±1.73	40.14±1.55	**39.09±0.78**
BCS	3.38 ±0.24	3.75 ± 0.43	4.00 ±0.20	4.25 ±0.14	**3.84 ±0.15**
WBL (cm)	79.64±1.71	81.39 ±1.42	82.54 ± 1.63	81.71±1.47	**81.32 ±0.78**
SBL (cm)	60.39 ±1.13 ^{b}	61.29±0.80 ^{ab}	63.32 ±0.88 ^{a}	61.93±1.13 ^{ab}	**61.73 ±0.50**
BH (cm)	67.82±0.60 ^{b}	67.79 ±0.83 ^{b}	69.46 ±0.80 ^{a}	69.32 ±079 ^{a}	**68.59 ±0.38**
C.C (cm)	77.71 ±0.92 ^{b}	80.18 ±1.12 ^{a}	82.18 ±1.27 ^{a}	80.68±1.11 ^{a}	**80.19 ±0.57**
Abd.C (cm)	84.68 ±1.04 ^{c}	85.46±1.29 ^{bc}	89.89 ±1.63 ^{a}	87.61±1.57 ^{ab}	**86.91 ±0.72**

Means having the different superscripts in same row are significantly differed ($p \leq 0.05$). BW: body wight, SBL:body condition score, WBL: whole body length, SBL: shoulder body length, and BH:body height, C.C: chest circumference, and Abd.C: abdominal circumference.

3.5.2. Growth measurements (lengths, height, and circumferences)

Regarding body measurements of ram lambs (whole body length; WBL, shoulder body length; SBL, body height; BH, chest circumference; C.C, and abdominal circumference; Abd.C) as affected by oral supplementation of ZnO NPs were illustrated in **Tables 5**. Treating lambs with different doses of Zn NPs (10, 20, and 30 mg/kg DM) significantly increased (P≤0.05) SBL but insignificantly for WBL where the averages were 60.39±1.13, 61.29±0.80, 63.32±0.88, and 61.93±1.13 cm for SBL and 79.64±1.71, 81.39±1.42, 82.54±1.63, and 81.71±1.47 cm for WBL of control and treated lambs, respectively. Only G3 (20 mg ZnO NPs) has a higher (p≤0.05) SBL than control.

According to body height BH, G3 and G4 (20 and 30 mg Zn) were significantly higher (p≤0.05) than G2 (10 mg) and control group. The overall means of BH were 67.82±0.60, 67.79±0.83, 69.46±0.80 and 69.32±079 cm for control and Zn supplemented lambs.

Concerning body circumferences as affected by Zno NPs administration, both C.C and Abd.C of lambs in all treated groups showed a significantly increase except for Abd.C of 20 mg compared to control. The average means of CC were 77.71±0.92, 80.18±1.12, 82.18±1.27, and 80.68±1.11 cm while that of Abd.C were 84.68±1.04, 85.46±1.29, 89.89±1.63 and 87.61 ±1.57 cm for control, 10, 20 and 30 mg ZnO NPs treated groups, respectively.

It has been seemed that adding ZnO NPs improved growth performance through various growth traits such as body length, height and circumferences which might be due the enhancement of GH and /or thyroid hormones (under publishing data for the author) which in turn have their potent to support lamb growth directly or indirectly through metabolism (**Alimohamady *et al*., 2019)**. These increases and enhancements in most of body measurements might be due the anabolic effect of insulin–like growth factor 1 (IGF-1) on osteoblasts which is enhanced by Zn **(Wang *et al*., 2002)** and suggested that longitudinal growth might be very effective with Zn supplementation.

3.5.3. Scrotum circumference (SC) and testicular length (TL)

As shown in **Table 6**, supplementation of ZnO NPs enhanced both testicular measurements (scrotum circumference; SC and testicular length; TL) although these increases were not significant. SC increased by 0.48, 4.59 and 8.70% for G2 (Zn 10), G3 (Zn 20) and G4 (Zn 30), respectively as comparing with control group (G1). TL also showed increases of 9.65, 8.59% for groups G3 and G4 while it decreased by – 0.71 in G2 (Zn 10) as compared to control group. These moderate increases in testicular measurements might be due to the increased levels of both GH and testosterone concentrations along with Zn metalloenzymes which may affect cell proliferation and metabolism in Zn-supplemented lambs. Additionally, the improvement in testicular measurements might be due to the effect of zinc on seminiferous tubules and epithelial cells as noted by **Kheirandish *et al*. (2014)**, who pointed out that zinc supplementation for 56 days prevented the shrinkage of seminiferous tubules and epithelial degeneration in mice exposed to copper intoxication.

Table 6. Testicular measurements, scrotum circumference; SC and testicular length; TL in control and ZnO nanoparticles treated Ossimi ram-lambs

Item	Period (d)	G1 (Control)	G2 (Zn 10)	G3 (Zn 20)	G4 (Zn 30)	Overall mean
SC (cm)	**0**	21.00±1.79	20.50±2.07	21.00±2.13	21.50±1.49	**21.00±0.85** ^{C}
	15	21.25±1.89	20.75±2.10	21.25±2.25	21.75±1.44	**21.25±0.87** ^{BC}
	30	21.50±1.94	20.75±2.09	21.25±2.25	22.50±1.19	**21.50±0.87** ^{BC}
	45	21.50±1.93	20.75±2.10	21.25±2.25	23.75±1.03	**21.81±0.90** ^{BC}
	60	23.75±1.31	23.50±1.94	25.00±1.87	25.75±0.75	**24.50±0.73** ^{AB}
	75	24.25±1.65	27.25±1.31	26.75±2.36	27.25±0.85	**26.38±0.80** ^{A}
	90	25.25±1.84	25.75±2.02	29.25±2.56	29.75±0.95	**27.50±1.01** ^{A}
	Average	**22.64±0.67**	**22.75±0.82**	**23.68±0.96**	**24.61±0.67**	**23.42±0.40**
TL (cm)	**0**	10.75±1.76	10.50±1.02	10.50±1.66	10.50±0.20	**10.56±0.59** ^{C}
	15	11.00±1.68	10.75±0.95	10.50±1.65	10.88±0.13	**10.78±0.57** ^{C}
	30	11.00±1.68	10.75±0.94	11.10±1.52	11.45±0.29	**11.08±0.56** ^{BC}
	45	11.00±1.68	10.75±0.95	12.35±1.49	12.20±0.27	**11.58±0.58** ^{ABC}
	60	11.75±1.38	11.25±0.48	12.93±1.38	12.85±0.25	**12.19±0.19** ^{ABC}
	75	12.25±1.65	12.25±0.47	14.15±1.17	13.55±0.21	**13.05±0.51** ^{AB}
	90	11.25±0.95	12.25±0.85	15.10±0.97	14.40±0.24	**13.25±0.54** ^{A}
	Average	**11.29±0.53**	**11.21±0.31**	**12.38±0.57**	**12.26±0.27**	**11.78±0.22**

Means having the different superscripts (capital letters) in the same column or small letters in same row are significantly differed ($p \leq 0.05$).

4. CONCLUSIONS

In conclusion, oral supplementation of ZnO NPs particles increased serum levels of zinc, GH ($p \leq 0.05$) and testosterone along with immune indicator (IgG) and anti-oxidants levels ($p \leq 0.05$) consequently improved ($p \leq 0.05$) most of body and testicular measurements. Some of limitations in this study might be due to lake of experimental animal numbers and the way by which ZnO NPs affects lambs growth or its mode of action strongly need further investigations.

Ethical approval for animal research

All practices and experimental procedures in this study were approved by the Fayoum University Institutional Animal Care and Use Committee (FU-IACUC), with research proposal code: AEC 2355, may 2024.

Conflicts of Interest: The authors declare no conflict of interest in this work.

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