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IMPACT OF NANO-ZINC OXIDE SUPPLEMENTATION ON PRODUCTIVE PERFORMANCE AND SOME BIOCHEMICAL PARAMETERS OF EWES AND OFFSPRING

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ABSTRACT

This study conducted to evaluate the effect of zinc element, either in Large particles (LP-Zn) or Nano particles (NP-Zn)] forms, as a feed supplement in rations of Ossimi ewes on feed intake, digestibility, feeding value, milk yield and offspring performance. Fifteen ewes used before lambing. They averaged 39.37±0.64 kg live body weight and aged 3-4 years. Animal allotted randomly into three similar groups (5 animals each) to receive one of the experimental diets. The control ration (CR) consisted of concentrate feed mixture (CFM), Berseem hegazi hay (BHH) and rice straw (RS). The two tested groups fed the same control ration supplemented either with 10 mg large size particles of zinc (LP-Zn) or 5 mg nano particles of zinc (NP-Zn) per kg CFM, G2 and G3 respectively.

Results indicated that digestibility of most nutrients (DM, OM, CF and NFE) were significantly the highest with NP-Zn ration in comparison with other treatments. Similar trends observed with TDN and DCP values among treatments. Percentage of body weight changes during experimental period increased ($P<0.05$) by 4.96 and 12.54 % for ewes treated by LP-Zn and NP-Zn, respectively. In addition, the feed intake calculated as DMI, TDN and DCP were significantly higher with NP-Zn ration than other ones. Actual daily milk yield (ADMY) and 6% fat corrected milk yield (FCMY) were higher for both tested rations (LP-Zn & NP-Zn) than control, with superiority of NP-Zn ration. Data indicated that supplementation of diet with NP-Zn affected positively ($P<0.01$) the total antioxidant capacity (TAC), glutathione-S-transferase (GST) activity, prolactin, triiodothyronine (T3), and thyroxin (T4) concentrations at 15, 45 and 75 days post-lambing in comparison to control group for ewes and their lambs. Also, concentration of immunoglobulin (IgG) in serum samples of ewes and their lambs recorded the highest values with NP-Zn group. Serum total protein, albumin, globulin and glucose values in NP-Zn group recorded the highest values, followed by LP-Zn group, while control group had the lowest, either for ewes or suckling lambs. Serum AST, ALT and urea measurements were within the normal range for healthy sheep.

It conclude that small amount of zinc in nano size, could replace zinc in normal size without any side effects on animal performance. In addition, zinc supplementation could improve nutrients digestibility, feed conversion, milk production and some related serum biochemical indicators.

Keywords: *Nano, zinc, digestibility, milk yield, ewes, performance, biochemical.*

INTRODUCTION

Zinc (Zn) is the second most important trace element in the animal body. It cannot be stored in the body (Mandal *et al.*, 2007), therefore has to be regularly supplied to the diet to meet the physiological needs. It considers a component for synthesizing numerous enzymes and hormones, Zn is necessary for the proper physiological functioning (Miyamoto *et al.*, 1991). These include alcohol dehydrogenase, alkaline phosphatase (ALP), aldolase, lactate dehydrogenase (LDH), RNA and DNA polymerases, reverse transcriptase, carboxypeptidase A, B, G and superoxide dismutase (SOD). Zn is essential for proper physiological functions in the body, like, growth (Keçeci and Keskin,2002), reproduction (Berrie *et al.*, 1995), DNA synthesis, cell division and gene expression (Kincaid *et al.*, 1997), photochemical processes of vision, wound healing (Koracevic *et al.*, 2001), ossification (Walsh *et al.*,1994), augmenting the immune system of the body (Mandal *et al.*, 2007) through energy production, protein synthesis, protection of membranes from bacterial endotoxins and lymphocyte replication and antibody production (Zhao *et al.*, 2014). Role of Zn on livestock reproduction was emphasized by Cunningham-Rundles *et al.* (1991) who reported that sterility in heifers was attributed to insufficient Zn. It has a synergistic effect on the reproductive performance of animals.

Absorption of Zn in the body is very low and differs with the age of animal and the sites in the gastrointestinal tract. The net absorption of Zn administered daily was differed in mature cows (12%), 5 to 12 months calves (20%) and also in week-old calves (55%). Zn can incorporate in the diet as inorganic salts like ZnO and $ZnSO_4$ and as organic chelates such as Zn propionate and Zn acetate. Even though, the bio-availability of Zn in organic sources is higher than that of inorganic Zn salts. The use of organic Zn chelates in animal' diet is limited due to its high cost (Zhao *et al.*, 2014). High levels of Zn excreted from animals supplemented with it have raised concerns pertaining to environmental pollution (Fadayifar *et al.*, 2012). Nanoparticle of Zn has produced positive responses when fed as alternative to the conventional mineral sources. If we talk about Zn as an example, feeding NP-Zn to livestock and poultry has produced encouraging responses on growth, immunity, and reproduction. Nano Zn enhance growth and improve feed efficiency in piglets (Yang and Sun, 2006), poultry (Lina *et al.*, 2009; Mishra *et al.*, 2014), rabbit (Fatma *et al.*, 2016) and sheep (Mohamed *et al.*, 2015).

Thus, this problem opens a window for better bio-available Zn sources and if possible, to reduce the supplemental dose of Zn to the animal food. Among all the probable approaches, use of nanotechnology to produce nano sized Zn called as nano Zn (NP-Zn) is a potential alternative to both organic and inorganic Zn sources. Thus, the objective of this study was to compare the effect of introducing normal metal particles of Zn or nano-Zn as feed supplement to ewe rations and identifying their effect on feed intake, nutrient digestion, feeding values, milk yield, some blood parameters and offspring performance.

MATERIALES AND METHODS

The present study was carried out at the Experimental Station belong to Faculty of Agricultural, Minia University, Minia Governorate during 2015 and 2016. The influence of zinc element in both forms (normal and nano particles) on productive performance and some biochemical parameters of Ossimi ewes and their offspring were studied throughout feeding and digestibility trials. Fifteen ewes averaged 39.37±0.64 kg live body weight and aged 3-4 years were chosen, before lambing and allotted at random into three similar groups (5 animals each). Each received one of the experimental diets. The control group (CR) received the control ration, composed of concentrate feed mixture (CFM), Berseem Hegazi hay (BHH) and rice straw (RS), while the two tested groups fed the control ration supplemented with 10 mg/kg CFM the normal size particles of zinc or 5 mg/kg CFM nano particles of zinc for the second (LP-Zn) and third (NP-Zn) rations, respectively. Nutritional requirements for experimental ewes calculated according to NRC (2007) by using total mixed ration (TMR) as feeding system over the experimental period. Composite samples of feedstuff analyzed according to AOAC (1995). Change of body weight recorded biweekly before feeding in the morning. Compositions of feedstuffs and TMR presented in Table (1). Three digestibility trials were conducted for animals of the feeding trial when the ewes became dry, to determine nutrient digestion coefficients and feeding values of experimental rations. Animals were fed twice daily at 8:00 am and 4:00 pm and feed intake and refusal were recorded every day. Daily feces were weighed, then 10% sample collected for analysis. Fresh water was available and animals were under veterinary care.

Feed conversion was calculated and expressed in terms of DM (g), TDN (g) and DCP (g) required for producing one kg of milk. Biweekly, representative milk samples were individually collected post-lambing for 90 days. Milk samples preserved immediately after milking by adding three drops of potassium dichromate (5 ppm). Milk constituents such as fat, protein, lactose, total solids and solids-not fat were determined using Milko-Scan® analyzer (USA), Bently 150, USA. Milk yield was corrected to 6% fat using Economides and Louca

(1981) equation where, FCM in kg (6% fat for sheep) = daily milk yield × (0.428+0.095×fat %).

Table (1): Proximate analysis of feed ingredients and calculated composition of experimental basal ration, DM basis (%).

Items	DM	OM	CP	EE	CF	NFE	Ash
CFM	90.64	91.72	14.69	4.53	15.82	55.17	8.28
RS	93.50	85.40	2.39	1.56	38.42	43.03	14.60
BHH	81.38	84.53	26.26	1.24	22.72	34.31	15.47
TMR	89.36	89.02	15.45	3.28	21.72	48.57	10.98

****CFM**=Concentrate feed mixture; **RS**=Rice straw; **BHH**=*Berseem Hegazi hay; ***TMR**= Total mixed ration.*

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Blood samples were collected from three animals of each group, before feeding, for 3 times at 15, 45 and 75 days post-lambing, in the morning during the experimental period. Blood serum samples separated by centrifugation at 3500 rpm for 15 minutes and then frozen at -20 °C until analysis. Commercial kits were used to determine serum Total protein (TP) according to Gornal *et al.* (1949), Albumin (AL) was determined according to Doumas *et al.* (1971). Meanwhile globulin calculated by the difference between TP and AL. Serum Aspartate transferees (AST), Alanine transferees (ALT) were determined according to Reitman *et al.* (1957). Prolactin hormone was determined by a radioimmunoassay procedure according to Downing (1994) and Downing *et al.* (1995), kits purchased from diagnostic products corporation, United States. Also, Tri-iodothyronine (T_3) and Thyroxin (T_4) were determined by radioimmunoassay procedures according to Chopra *et al.* (1971), and Irvin and Standeven (1968), respectively. Kits purchased from diagnostic products corporation, United States. Levels of immunoglobulin (IgG) determined three times (at 12, 24 and 48 hours post-lambing) by bovine IgG ELISA kits according to the procedure outlined by manufacturer (Alpha Diagnostic international, Texas, USA and Kamiya Biomedical Company, Seattle, Washington, USA, respectively). Total antioxidant capacity and Glutathione-S-transferase were determined in serum according to the procedure of Koracevic *et al.* (2001) and Habig *et al.* (1974), using Biodiagnostic reagent kits.

Data statistically analyzed using the general linear model procedure (SAS, 2002). The differences among means tested using Duncan's Multiple-rang test (Duncan, 1955).

RESULTS AND DISCUSSIN

Physical structure of ZnNps used:

Images of synthesized zinc nanoparticles, shown at two different magnifications, indicate that the zinc exhibits agglomerated flower shape for zinc nanoparticles, as shown in (Fig.1).

An improvement of digestion coefficients for DM, CP, and CF nutrients with LP-Zn recorded as 6.34; 4.14; and 5.5%, versus 12.52; 8.2 and 10.3% with NP-Zn group, respectively compared with control group. Meanwhile, there were no significant differences between the dietary treatments concerning digestion coefficients of EE. These agglomerates consisted of several cube nanoparticles with size ranging between 6-11 nm.

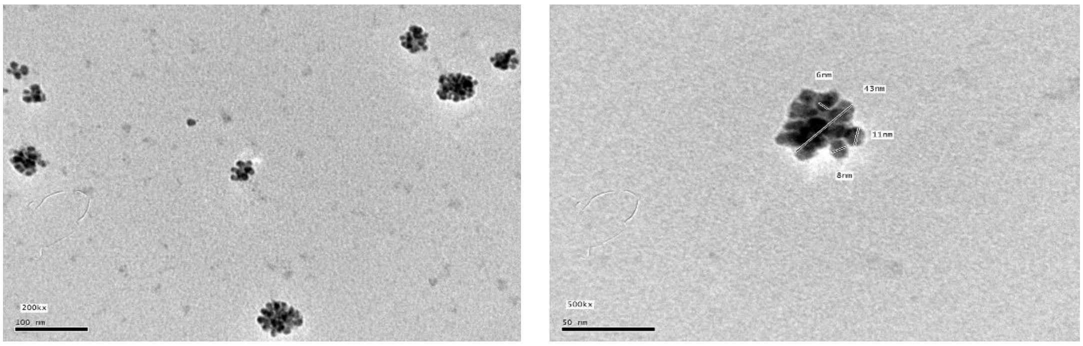

Figure (1): Depicts TEM images of synthesized zinc nanoparticles at two different magnifications.

Table (2): Digestibility coefficients and feeding values as affected by different forms of zinc.

Traits	Treatments			P. value
	CR	LP-Zn	NP-Zn	
Digestion coefficients, %				
DM	62.00^{c}	65.93^{b}	69.76^{a}	0.001
OM	66.25^{c}	69.58^{b}	73.32^{a}	0.001
CP	69.82^{c}	72.71^{b}	75.55^{a}	0.001
EE	80.34	80.46	80.52	0.731
CF	62.12^{b}	65.51^{a}	68.52^{a}	0.003
NFE	74.85^{b}	77.01^{a}	79.31^{a}	0.001
Feeding values, %				
TDN	65.36^{b}	67.80^{a}	69.96^{a}	0.001
DCP	10.50^{b}	11.20^{a}	11.64^{a}	0.001

$^{a,\ b\ and\ c}$ **:** Means within each row with different superscripts are significantly differ (P<0.05).
*CR: Control ration, LP-Zn: Experimental ration containing large particles Zinc, NP-Zn: Experimental ration containing Nano particles Zinc.

Digestibility coefficients and feeding values:

Digestion coefficients and feeding values for the experimental rations presented in Table (2). Digestibility of DM, OM, CP, CF and NFE nutrients for LP- Zn & NP-Zn rations (containing large-Zn and Nano-Zn) were significantly ($P<0.001$) higher compared with control ration (CR).

The feeding values expressed as TDN and DCP was a reflection for nutrient digestibility improvement. The feeding values (TDN & DCP) for LP-Zn and NP-Zn rations were significantly ($P<0.001$) improved compared to control one. The feeding values for NP-Zn ration recorded the highest values followed by (LP-Zn) group, compared to the control one. These results are in agreement with those obtained by Mohamed *et al.*, (2015) who reported that supplementing of NP-Zn to lambs' ration improved the digestibility and feeding value of NP-Zn ration.

These results indicate that nano form of Zinc improved the digestion coefficients of nutrients and feeding values better than the normal particles of LP-Zn, which may be related to the more activity of biological processes and the great specific surface area, high surface activity and strong adsorbing ability of elements for nano form (Zhang *et al.*, 2008; Wang *et al.*, 2007). Additionally, it improved the soluble fiber in case of high water holding capacity, readily forms gel, increased ruminal viscosity, and therefore it easily degraded by micro-flora in the large bowel. On the contrary, insoluble fiber had little water holding capacity, decreased transit time, only partially degraded by micro-flora, and increased fecal bulk (Swanson *et al.*, 2001). The present results are in agreement with those obtained by Wang *et al.* (2011) who reported that supplementation of nano-zinc oxide can improve the growth of ruminal microorganisms, increase the ruminal microbial protein synthesis, and raise the energy utilization efficiency which will reflect on improvement of digestive processes and hence nutritional value.

Meanwhile, Bunglavan *et al.* (2014) observed that the particle size of minerals, as feed additives, in nanoparticle form is claimed to be smaller than 100 nanometer, so they can pass through the stomach wall and into body cells more quickly than ordinary ones with larger particle size. Nano-additives can also incorporated in micelles or capsules of protein or another natural food/feed ingredient. Development of suitable carriers remains a challenge because bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and because their susceptibility to gastrointestinal degradation by digestive enzymes. Manipulation of matter at the Nano level also opens possibilities for improving the functionality of food/feed molecules to the benefit of product quality.

Milk yield and composition

Data of daily milk yield (DMY), Total milk yield (TMY) and 6% fat corrected milk (FCM) and milk composition presented in Table (3). Results showed that ewes fed large particles or Nano-Zn rations (LP-Zn & NP-Zn) yielded significant higher DMY, TMY and 6%-FCM than those of control group (CR). On the other hand, NP-Zn group reached to peak of milk yield earlier (5^{th} week) compared with the other groups. Also, the DMY for animals fed ration with N-Zn recorded the highest value for milk yield at the end of lactation period (10^{th} week) compare with other dietary groups. The improvement of productive performance of ewes, as DMY and TMY, were increased by 44.12 - 61.8 and 40.8 - 60.6% for LP-Zn & NP-Zn groups, respectively than the control group during the whole lactation period.

Table (3): Milk yield and composition of Ossimi ewes as affected by both zinc forms supplementation.

Items	Treatments			P. value
	CR	LP-Zn	NP-Zn	
Average daily milk yield, kg/d	0.34 ± 17.62^{b}	0.49 ± 36.45^{a}	0.55 ± 35.44^{a}	0.001
Total milk yield, kg	21.41 ± 1.11^{b}	$30.15 \pm 2.30a^{a}$	34.38 ± 2.23^{a}	0.001
Daily 6% FCM yield, kg/d	0.35 ± 0.02^{c}	0.52 ± 0.05^{b}	0.59 ± 0.05^{a}	0.002
Milk composition, %				
Fat	6.57±0.08	6.97±0.04	6.88±0.005	0.001
Protein	4.51 ± 0.11^{b}	5.39 ± 0.17^{a}	5.21 ± 0.11^{a}	0.001
Lactose	4.51±0.07	5.07±0.08	4.90±0.08	0.001
SNF	9.71 ± 0.17^{b}	11.28 ± 0.18^{a}	10.84 ± 0.16^{a}	0.001
Total solids	15.28 ± 0.16^{b}	18.25 ± 0.17^{a}	17.52 ± 0.17^{a}	0.001
Ash	0.69±0.02	0.82±0.02	0.73±0.05	0.001

[a, b and c] **:** Means within each row with different superscripts are significantly differ ($P<0.05$).

The superiority of NP-Zn ration for DMY & TMY values may reflect the increase of nutrients digestibility and feeding value of this ration. It is clear that, nutrients digestibility and the feeding values of both zinc rations (LP-Zn & NP-ZN) were positively reflect on the 6% FCM produced by ewes fed those rations, which had estimated more by 48.57% and 68.57%, respectively than control one.

These results are in agreement with those obtained by Arguello *et al.* (2008) and Hassan *et al.* (2011) who reported that Nano-Zn supplementation improved significantly DMY compare with control group. These significant differences in milk production among dietary treatments may be due to increasing both body weight and condition score of ewes (Table, 4) and/or increasing of prolactin level (Table, 9). In raw cow's milk, a correlative dependency was determined between; zinc and proteins contents of milk (r_{xy} 0.30), urea in milk (r_{xy} 0.44) (Trávníček *et al.*, 2004). For an instance, a reduction in somatic cell count in subclinical mastitis cow and an increase in milk production observed due to supplementation of nano Zn-O (Rajendran, 2013). Regarding milk composition, results presented in Table (3) showed that milk constituents (protein, SNF and Total solids %) were significantly higher (P<0.01) in tested rations (larg-Zn and Nano-Zn) than those of control one. But, the differences among the experimental rations were not significant in respect of the percentage of fat, lactose and ash of produced milk.

The superiority of experimental groups in milk protein content might be due to increasing the level of total protein in blood of these groups compared to control one (Table 10) or due to the improvement of prolactin levels (Table 9), where it is a primary hormone responsible for regulating milk protein synthesis and maintaining lactation (Neville, 1990).

Milk lactose percentage was insignificantly higher in LP-Zn and NP-Zn groups compared to control group. The insignificant increase in milk lactose is probably due to the increase of serum glucose in treated ewes compared to untreated ewes, as shown in Table (10). It known that, glucose and galactose are precursors for milk lactose **(Cronje *et al.*, 1991)**. Generally, these results are in agreement with the results of **Hassan *et al.* (2011)** who reported that Zinc supplement had positive influence on ewe' milk production, composition and fat corrected milk.

Body weight changes of Ossimi ewes

Data in Table (4) show the changes in ewes' body weight pre- and at-lambing and at weaning of offspring. There were no significant (P<0.01) differences among treatments in initial weight at pre-lambing, while body weight at lambing was significantly heavier with NP-Zn ration control while LP-Zn ration intermediate others Body weight at weaning s significantly increased with the two experimental rations (two forms of zinc) than control one. Likewise, it was significantly higher with NP-Zn ration than LP-Zn one. The increased body weight by different forms of Zinc might due to increase in digestion coefficients and feeding values, and/or improvement in secretion of thyroid hormones (Table 9). The values of T3 and T4 proportionally correlated with the live body weight, being lower in lighter control group and higher in heavier ones (NP-Zn).

Table (4): Body weight changes of Ossimi ewes as affected by normal or Nano particles zinc

Items	Treatments			P. value
	CR	**LP-Zn**	**NP-Zn**	
Initial body weight pre-lambing, kg	39.20±0.49	39.08±.68	39.83±0.75	0.686
Body weight at lambing, kg	36.42±0.79^{b}	37.42±.59^{ab}	39.08±0.59^{a}	0.033
Body weight at weaning, kg	37.32±0.49^{c}	39.17±0.47^{b}	42.00±0.64^{a}	0.001
% Change	----	4.96	12.54	

$^{a\ and\ b}$ **:** Means within each row with different superscripts are significantly differ (P<0.05).

Feed intake and feed conversion

Values for feed intake as DM or feed units by different groups presented in Table (5). Comparisons among groups indicated that intakes of CFM, BHH, RS, Total dry matter (TDM), total digestible nutrients (TDN) and digestible crude protein (DCP) for NP-Zn group were almost insignificantly higher than those of LP-Zn ration while significant higher (P<0.05) than those of control one. These results are in agreement with Mohamed *et al.* (2015) who reported that dry matter intake (DMI) of experimental rations was not significantly affected with large particles zinc (LP-Zn) or large particles Selenium (Se). Meanwhile, the DMI were significantly higher (P<0.05) with adding Nano particles Zinc (NP-Zn) or Nano particles selenium (NP-Se) to the ration compared with control group.

Table (5): Feed intake and feed conversion of Ossimi ewes as affected by both forms of zinc supplementation.

Items	Treatments			P. value
	CR	Zn	N-Zn	
Daily DM intake (g/h):				
CFM	784.04±9.28^{b}	806.77±9.93^{ab}	838.36±13.05^{a}	0.008
BHH	253.31±3.00^{b}	260.65±3.21^{ab}	270.85±4.21^{a}	0.008
RS	168.87±2.00^{b}	173.77±2.14^{ab}	180.57±2.81^{a}	0.008
Total DM intake	1206.22±14.27^{b}	1241.19±15.27^{ab}	1289.78±20.07^{a}	0.008
Daily feed unit intake(g/h)				
TDN	751.72±8.90^{c}	840.28±10.34^{b}	952.63±14.82^{a}	0.001
DCP	124.48±1.47^{c}	139.01±1.71^{b}	150.13±2.34^{a}	0.001
Daily milk yield (g)	339.81±34.89^{b}	494.97±32.60^{a}	545.68±35.44^{a}	0.001
Feed conversion ratio(FCR):				
g DMI/g milk	3.55±0.13^{a}	2.51±0.18^{b}	2.36±0.15^{b}	0.001
g TDN/g milk	2.21±0.08^{a}	1.70±0.12^{b}	1.75±0.11^{b}	0.007
g DCP /g milk	0.37±0.01^{a}	0.28±0.02^{b}	0.28±0.02^{b}	0.003

$^{a,\ b\ and\ c}$ **:** Means within each row with different superscripts are significantly differ (P<0.05).

The obtained results may be a reflection to more degradability of DM and CP of these rations (Mohamed *et al.*, 2015). Feed conversion calculated as grams of DM, TDN and DCP consumed per one-gram milk yield significantly improved with both forms of Zn supplementation compared with control group. The NP-Zn group showed the best-feed utilization efficiency among the experimental dietary treatments. Data show no significant differences between LP-Zn & NP-Zn concerning feed conversion efficiency. These results are in agreement with those obtained by Vignola *et al.* (2009) and Wenjuan *et al.* (2012). Meanwhile, Zhao *et al.* (2014) reported that improvement accompanied Nano-zinc supplementation might be due to the shape and small size of molecules, which helped increasing its spread and reach to all cells. The improved in both dry matter intake and feed conversion observed in this study following Zn supplementation are in agreement with the previous findings of Garg *et al.* (2008) and Fadayifar *et al.* (2012).

Suckling lambs' performance

Data of growth performance of suckling lambs until weaning presented in Table (6). Results of birth weight, weaning weight, total gain and daily gain of lambs during suckling period were significantly (P<0.05) higher with NP-Zn ration than those of control one (CR).

Otherwise, most differences between LP-Zn ration and control one, in respect of the mentioned traits, were not significant. The enhancement in daily gain may be attributed to increase of serum

Table (6): Suckling lambs performance as affected by the experimental supplementation

Items	Treatments			P. value
	CR	LP- Zn	NP-Zn	
Birth weight, kg	3.18±0.11^{b}	3.34±0.13^{b}	3.90±0.13^{a}	0.002
Weaning weight, kg	18.49±0.51^{b}	21.20±0.60^{a}	23.67±1.28^{a}	0.004
Total gain, kg	15.31±0.56^{b}	17.86±0.61^{ab}	19.77±1.18^{a}	0.008
Average daily gain, g	204.18±7.50^{b}	238.13±8.13^{ab}	263.56±15.78^{a}	0.008
% Change	----	16.63	29.08	
Survival rate (%)	100	100	100	

$^{a\ and\ b}$ **:** Means within each row with different superscripts are significantly differ (P<0.05).

immunoglobulin for lambs and their mothers (Table, 8). This indicate an improvement in the immune status of lambs and hence their performance. One of the most important immune variables is the immunoglobulin concentration (mainly IgG and IgM). However, other immune variables that directly affect lamb immune status, such as chitotriosidase (ChT), play an important role on animal productivity. **Arguello *et al.* (2008)**, reported that ChT is an important component of innate immunity against chitin-containing pathogens. The maternal immune-globulins acquired through the colostrum play a significant role in the defense mechanism of lamb against neonatal diseases until its own immune system is primed and produces significant amount of antibodies **(Khan and Ahmad, 1997).** Nano particles of Zn has significant positive responses respecting growth, immunity and reproductive performance of livestock when fed as alternative to the conversional mineral sources. Nano Zn found to improve growth and feed efficiency in rabbit (Fatma *et al.*, 2016) and sheep (Mohamed *et al.*, 2015).

Activity of antioxidant enzymes

Results related to the effect of different dietary forms of zinc on total antioxidant capacity (TAC) and glutathione-S-transferase (GST) at 15, 45 and 75 days post-lambing for ewes and their lambs presented in Table (7).

Table (7): Total antioxidant capacity and glutathione-S-transferase levels in Ossimi ewes and their lambs after 15, 45 and 75 days post-lambing.

Items	Days post-lambing	Treatments			P. value
		CR	LP-Zn	NP-Zn	
Ewes:					
	15	1.22±0.10^{c}	1.50±0.07^{b}	1.94±0.05^{a}	0.001
TAC (g/dl)	45	1.50±0.25^{b}	2.13±0.04^{ab}	2.58±0.34^{a}	0.035
	75	2.01±0.26^{b}	2.35±0.12^{b}	3.22±0.10^{a}	0.003
	15	98.63±5.75	117.42±3.32	122.11±14.48	0.216
GST (U/l)	45	87.76±7.09^{c}	126.20±6.12^{b}	147.70±3.68^{a}	0.001
	75	84.54±11.50^{c}	121.96±2.64^{b}	160.14±9.09^{a}	0.001
Lambs:					
	15	1.31±0.12^{c}	1.88±0.17^{b}	±2.33±0.07^{a}	0.001
TAC (g/dl)	45	2.02±0.35^{b}	2.36±0.15^{b}	3.21±0.09^{a}	0.013
	75	2.51±0.06^{c}	2.95±0.18^{b}	3.55±0.06^{a}	0.001
	15	33.73±2.59^{b}	104.66±3.72^{a}	120.30±7.84^{a}	0.001
GST (U/l)	45	68.32±5.91^{c}	99.11±5.76^{b}	147.99±3.38^{a}	0.001
	75	74.46±6.42^{c}	103.44±3.20^{b}	154.66±3.19^{a}	0.001

a, b and c **:** Means within each row with different superscripts are significantly differ (P<0.05).

Results indicated that supplementing diet of ewes with Nano-Zn (NP-Zn ration) significantly (P<0.01) raised TAC and GST values in comparison with control group during different periods. The highest values was at 75 day post-lambing for ewes fed NP-Zn ration (3.22 & 3.55 g/dl of TAC and 160.14 & 154.66 U/l of GST) and the lowest ones (2.01 & 2.51 g/dl of TAC and 84.54 & 74.46 U/l of GST) for ewes fed (CR) ration and their lambs, respectively.

These results disagree with the findings of Berg and Shi (1996) and Walsh *et al.* (1994) who reported an inverse relationship between malondialdehyde (MDA) value and zinc oxide Nano particles. On the other hand, the present results are in agreement with those obtained by Fatma *et al.* (2016) who showed that rabbit fed diet supplemented with Nano-Se or NP-Zn increased significantly (P<0.05) the total antioxidant capacity. Also, Ahmadi *et al.* (2014) reported that increasing the level of zinc oxide nanoparticles from 60 to 90 mg/kg basal diet improved antioxidant status and serum enzymes of broiler chickens. Burmana *et al.* (2013) reported that zinc plays a central role in stability of biomembranes and protein as it helps in balancing reactive oxygen species (ROS) production and scavenging because of its presence in superoxide dismutase (SOD).

Zinc is a cofactor and a component of more than 240 enzymes and can influence oxidative processes. Cunningham-Rundles *et al.* (1990) showed that Zn acts as an antioxidant to reduce cell membrane damage due to free radicals. TAC in the body contributes to the dynamic balance of active oxygen, where TAC is an integrative parameter reflecting the status of all the antioxidants in serum and body fluids. Moreover, they reported that Zn is an essential component in Cu-Zn-SOD, and that dietary Zn levels positively correlate with Cu-Zn-SOD activity. It could be shown that Cu-Zn-SOD involved in the cellular scavenging of free radicals and ROS (Prasad, 2008; Ozturk and Gumuslu, 2004). NP-Zn has a significant effect on Cu-Zn-SOD activity in serum, while higher concentrations of NP-Zn were not associated with the significant growth in Cu-Zn-SOD activity in serum, suggesting that excess of Nano-Zn does not contribute to biological function (Zhao *et al.*, 2014). These findings are consistent with those of previous reports (Prasad 2009; Zhao *et al.*, 2014; Fathi *et al.*, 2016) who found that appropriate concentrations of NP-Zn may stimulate Cu-Zn-SOD activity, and the resulted enhance of Cu-Zn-SOD will suppress the generation of ROS.

Immune response

Data presented in Table (8) show that serum IgG values for ewes and their lambs, fed ration supplemented with (NP-Zn), recorded the highest concentration ($P<0.05$) compared to LP-Zn and CR. Results show also that the concentration of immunoglobulin in serum samples of ewes and their lambs with NP-Zn ration was significantly the highest, followed by LP-Zn ration, while the control had the lowest concentration ($P < 0.05$).

Table (8): Serum IgG levels in Ossimi ewes and their lambs during the period from 12- 48 hr after suckling.

Items	Treatments			P. value
	CR	LP-Zn	NP-Zn	
<u>Ewes</u>				
After 12hr	7.37 ± 0.11^c	8.56 ± 0.04^b	9.35 ± 0.07^a	0.001
After 24hr	8.78 ± 0.22^c	9.90 ± 0.07^b	11.44 ± 0.16^a	0.001
After 48hr	8.34 ± 0.17^c	9.53 ± 0.11^b	10.55 ± 0.16^a	0.001
<u>Lambs</u>				
After 12hr	8.25 ± 0.11^c	9.21 ± 0.09^b	9.85 ± 0.09^a	0.001
After 24hr	9.77 ± 0.26^c	10.75 ± 0.11^b	11.85 ± 0.27^a	0.001
After 48hr	9.47 ± 0.21^c	10.42 ± 0.14^b	11.18 ± 0.17^a	0.001

a, b and c : Means within each row with different superscripts are significantly differ ($P<0.05$).

These results might related to the composition of colostrum. In general, after suckling colostrum, the lamb's serum immunoglobulin showed the highest concentration within the first 48 hours of age. This strongly approved by Stott *et al.* (1979). They indicated that newborn derives little immunity from their dam and that born essentially devoid of protection from indigenous microbial challenges. Colostrum is the only source of immunoglobulin (Ig) for newborn animals, since placenta does not allow transport of Ig from maternal to the foetal circulation (Quigley *et al*., 1998).

These absorbed proteins can be transported via a tubule system to the basal cell and released into lymphatic and hence to the vascular system. This absorption provides temporary immunity to lamb. Absorption essentially stoped by 24 hours and IgG is no longer released into the lymphatic system.

This process is not abrupt done but, begins shortly after birth and then proceeds most rapidly, from 12 to 24 hours post-lambing. The ability of lamb to absorb antibodies decreases rapidly after birth. Between 12 to 24 hours, ability to absorb antibodies dropped to 50 % of the capability just after birth (Kruse, 1970).

Data presented in Table (8) showed that the concentration of immunoglobulin IgG in serum samples of ewes in NP-Zn group had the highest values followed by LP-Zn then CR group was the lowest. Zinc (Zn) deficiency reduces immune responses (Chesters, 1997) and impairs the antioxidant defense system (Miller *et al.,* 1993). Zinc has a major role in immune responsiveness (Keen and Gershwin, 1990) and the dietary deficiency of Zn has been associated with increased morbidity and mortality (Kincaid *et al.,* 1997).

Hormonal measurements

Prolactin and thyroid gland hormones concentrations presented in Table (9). All hormones estimated in ewes and their lambs after 15, 45 and 75 days post-lambing. Prolactin and thyroid hormones as Triiodothyronine (T3) and Thyroxin (T4) were almost significantly ($P<0.01$) increased in blood serum by zinc supplementation to rations. Prolactin and T3 concentrations in ewes serum was not influenced by dietary treatments at 15 days of lactation but they were significantly ($P<0.01$) increased in ewes serum when animals fed NP-Zn ration compared with control one at 75 days post lambing. Prolactin concentration had the highest values (4.64, 4.92 and 5.23 ng/dl) in the first 15 days of lactation and lowered with decreasing milk production recording (1.12, 1.92 and 2.39 ng/dl) at 75 days of lactation for ewes consumed CR, LP-Zn and NP-Zn rations, respectively.

Also, T3 and T4 (Table, 9) are correlated with milk yield and constituents being higher with high yielders zinc groups, (large or Nano particles) and lower in low yielder (control) as shown in Table (3). Likewise data of suckling lambs indicated that T3 and T4 concentrations were significantly ($P<0.01$) higher in lambs' serum of NP-Zn ration than those of the other treatments over all times. Results here showed that NP-Zn ration had the highest values (3.70 nmol/ L and 78.65 nmol/ L) of T3 and T4 in lambs serum, respectively, at 75 days post-lambing, while control group had the lowest values (2.28 nmol/L and 61.30 nmol/L, respectively).

These results are in agreement with those obtained by Gueorguiev (1999) that concentrations of T3 and T4 increased in the high producing cows. Collier *et al.,* (1984) reported that the pituitary thyroid axis is an important physiological factor controlling metabolic processes and milk secretion. Thyroid hormones (Thyroxin, T4) and Triiodothyronine (T3) synergize with other hormones to promote growth, develop the mammary gland and maintain lactation. Prolactin is the primary hormone responsible of regulating milk protein synthesis and maintaining lactation (Neville, 1990).

These results also are in contrast with those recorded by Brandao-Neto *et al.* (1989) that zinc was shown to inhibit prolactin secretion from the anterior pituitary. They studied the response of plasma prolactin to oral zinc administration in healthy male and female adults and observed that prolactin concentration significantly decreased, below basal levels, in response to the increase of plasma Zinc levels. The present results are in agreement with those reported by Burger (1980) who indicated that stimulation of metabolic processes in Zinc supplemented sheep was corresponding by marked increase in plasma T3 as well. The observation that plasma T_4 remained essentially constant is consistent with the theory that T4 is the storage form of thyroid hormones (Burger, 1980). Deficiency or excessive level of zinc has observed to adversely affect the endocrine system. It has reported that serum or plasma levels of thyroid hormones were reduced by high Zn intake (Nishiyama *et al.,* 1994).

Some blood serum parameters

Blood parameters are important index of physiological, pathological and nutritional status of the organism. Changes in constituents of blood, compared to their normal values, could use to interpret the metabolic status of animal

Table (9): Prolactin, T_3 and T_4 concentration in Ossimi ewes and their lambs after 15, 45 and 75 days post-lambing.

Items	Days post-lambing	Treatments			P. value
		CR	LP-Zn	NP-Zn	
Ewes:					
	15	4.64±0.23	4.92±0.24	5.23±0.14	0.185
Prolactin (ng/dl)	45	2.12±0.23^{b}	2.79±0.08^{a}	3.18±0.12^{a}	0.003
	75	1.12±0.06^{b}	1.92±0.24^{a}	2.39±0.13^{a}	0.001
	15	1.69±0.18	1.97±0.11	2.10±0.07	0.133
T_3 (nmol/L)	45	1.98±0.07^{b}	2.24±0.17^{b}	2.74±0.03^{a}	0.002
	75	2.28±0.13^{b}	2.51±0.18^{b}	3.02±0.03^{a}	0.008
	15	22.65±1.45^{c}	29.72±0.92^{b}	37.59±1.46^{a}	0.001
T_4 (nmol/L)	45	39.57±3.22^{c}	51.88±2.32^{b}	60.09±1.54^{a}	0.001
	75	54.63±2.16^{b}	59.22±2.13^{b}	72.85±0.96^{a}	0.001
Lambs:					
	15	1.87±0.07^{c}	2.11±0.06^{b}	2.70±0.06^{a}	0.001
T_3 (nmol/L)	45	1.93±0.05^{c}	2.76±0.06^{b}	3.01±0.06^{a}	0.001
	75	2.28±0.13^{b}	2.51±0.18^{b}	3.70±0.06^{a}	0.001
	15	53.67±3.79^{b}	67.14±2.43^{a}	72.85±0.96^{a}	0.002
T_4 (nmol/L)	45	58.60±4.51^{b}	67.22±1.15^{b}	73.42±0.94^{a}	0.013
	75	61.30±1.60^{c}	69.22±2.30^{b}	78.65±2.48^{a}	0.001

[a, b and c] : Means within each row with different superscripts are significantly differ (P<0.05).

and perhaps nutrient adequacy of consumed feeds (Nworgu *et al.*, 2007).

The data referring to concentrations of blood-serum total proteins, albumin and globulin in the three different groups of ewes and their lambs after 15, 45 and 75 days post-lambing are shown in Table (10). Almost, serum TP, Alb and Glob values in NP-Zn group recorded the highest values, followed by LP-Zn group, while control group recorded the lowest ones either for ewes or suckling lambs over the three periods post lambing. This superiority in NP-Zn and LP-Zn groups, compared to control group, either for ewes or suckling lambs might due to increase of feed intake (Table 5), metabolic rate and thyroid hormones, which was reflected on the blood metabolites (Table 10). These results are in agreement with Mohamed *et al.* (2015) who indicated that serum total protein and albumin with NP-Zn was increased compared with control group.

Serum total protein and its fractions considered a biological index reflecting health and productive performance of animals (Gabbedy, 1971). Data indicated the healthy status of the liver since the liver is the main organ for albumin synthesis. Serum Glucose (Glu) and cholesterol values shown in Table (10). It could be shown that the present data of Glu among different experimental groups followed the same trend as TP, Alb and Glob, either with ewes or suckling lambs, where superiority was to NP-Zn and LP-Zn compared to control. These results may be attributed to the increases of voluntary feed intake (Table, 5), rumen fermentation, enzymes activities and high secretion of thyroid gland (Table, 10).

Concerning the items of serum AST, ALT and urea, their values obtained in the present study are within the normal range for healthy sheep (Saleh and Saleh, 2003). The AST and ALT enzymes are most important indicator for liver cells activity. While urea and creatinine enzymes are the most important indicator for kidney cells activity. The effects of dietary treatments on concentrations of these items did not have obvious trends among the experimental treatments, but generally there were a favorable effect due to the NP-Zn treatment in relation to the other ones.

In harmony, with the present results, NP-Zn had no significant effects on ALT and AST activities in serum of broilers (Ahmadi *et al.*, 2014) and sheep (Mohamed *et al.*, 2015). Wang

Table (10): Some blood serum parameters of Ossimi ewes and their lambs as affected by ZnNps supplementation.

Items	Treatments						
	15 days post-lambing			45 days post-lambing			
	CR	Zn	ZnNps	CR	Zn	ZnNps	CR
Ewes:							
TP (g/dl)	5.14±0.32^{b}	7.47±0.37^{a}	7.91±0.30^{a}	6.52±0.50	5.85±0.30	6.26±0.49	6.79±
Alb (g/dl)	3.33±0.24^{b}	3.36±0.20^{b}	5.12±0.26^{a}	3.47±0.25	3.48±0.02	4.27±0.47	3.92±
Glob (g/dl)	1.80±0.38^{c}	4.11±0.37^{a}	2.79±0.04^{b}	3.05±0.25^{a}	2.37±0.29^{ab}	1.99±0.02^{b}	2.87±
Glu (mg/dl)	65.91±4.64^{c}	81.82±5.57^{b}	99.70±2.78^{a}	61.36±0.93^{b}	86.36±5.57^{a}	100.00±9.28^{a}	81.82
Chol (mg/dl)	75.22±4.37	77.88±5.05	83.19±1.82	72.75±6.40	83.85±2.57	82.61±3.55	86.96
AST (U/L)	24.00±4.08	24.50±1.84	34.67±3.66	22.00±0.41^{b}	33.67±2.05^{a}	32.33±2.87^{a}	44.50
ALT (U/L)	23.00±0.82^{a}	13.83±1.85^{b}	15.00±0.41^{b}	26.00±0.41^{a}	25.50±2.25^{a}	16.50±1.43^{b}	18.00
Urea (mg/dl)	46.67±2.36^{b}	51.67±3.01^{a}	60.33±2.25^{a}	52.67±2.62^{a}	59.89±3.71^{a}	45.18±2.36^{b}	37.43
Lambs:							
TP (g/dl)	6.63±0.13^{b}	6.83±0.14^{ab}	7.15±0.13^{a}	6.01±0.26^{b}	6.88±0.20^{a}	6.96±0.20^{a}	5.77±
Alb (g/dl)	3.21±0.09	3.33±0.18	3.48±0.13	3.19±0.33	3.53±0.17	3.52±0.02	3.37±
Glob (g/dl)	3.41±0.09^{b}	3.50±0.07^{ab}	3.67±0.06^{a}	3.16±0.25	3.01±0.56	3.44±0.21	2.06±
Glu (mg/dl)	83.44±5.13^{b}	108.33±3.54^{a}	105.76±3.72^{a}	70.76±5.74^{b}	103.79±3.54^{a}	110.45±3.71^{a}	82.73
Chol (mg/dl)	87.99±4.20	84.06±2.05	90.58±5.06	76.93±4.35^{b}	93.77±3.83^{a}	95.06±3.66^{a}	73.91
AST (U/L)	32.33±1.31	33.00±0.82	33.50±1.02	42.50±1.34^{a}	38.00±2.45^{ab}	33.00±0.95^{b}	35.00
ALT (U/L)	25.00±4.26^{a}	15.67±1.65^{b}	17.50±0.61^{ab}	18.83±2.99^{a}	12.50±0.61^{b}	13.00±0.94^{b}	20.00
Urea (mg/dl)	40.71±3.44^{ab}	50.15±4.17^{a}	35.98±2.94^{b}	47.62±12.74	66.67±6.80	48.87±6.18	40.53

$^{a,\ b\ and\ c}$ **:** Means within each row with different superscripts are significantly differ (P<0.05). TP= Total protein; Alb= Albumin; Glob= Globulin; Glu= Glucose; Chol= Cholesterol *et al.* (2006) used powder of Zn in the diet of rats at level 5 g/kg body weight as micro-particles M-Zn) and nanoparticles (N-Zn) and measured activity of some enzymes in plasma and liver. The results showed that the effect of micro-particles on hepatocellular damage was more severe than that of nanoparticles.

One reason to explain these differences may be related to the doses and time in which animal exposed to the ZnNpsO. It has reported that levels above 50 mg/kg of ZnNpsO induce oxidative stress and increase the plasma level of ALT and AST (Sharma *et al.*, 2009).

CONCLUSION

The present study showed that normal or nano zinc supplementation to pregnant and lactating ewes had useful impacts on feed intake, nutrients digestibility, feed conversion, milk production and some related serum biochemical indicators. Finally, it can conclude that LP-Zn additive could replace by NP-Zn in ewes' rations with favorable effects on animal performance.

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تأثير أكسيد الزنك فى صورة جسيمات النانو كإضافات غذائية على أداء النعاج ونتاجها وبعض القياسات الكيموحيوية.

علاء الدين حسن محمد ، محمود يسن محمد ، خميس ابراهيم ، فاطمة طلعت فرج عبد الغنى،عبد المنعم سيد محجوب

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تهدف هذه الدراسة الى تقييم اضافة أكسيد الزنك كما هو أو فى صورة جسيمات النانو وتأثيرة على أداء النعاج ونتاجها وبعض القياسات الكيموحيوية. استخدمت 15 نعجة أوسيمى متوسط وزنها 39،37كجم وعمرها 3-4 سنوات ، حيث قسمت الحيوانات الى ثلاث مجاميع متماثلة. المجموعة الأولى تم تغذيتها على علف مركز و برسيم حجازى وقش أرز كمجموعة مقارنة. المجموعتين الأخرتين تم تغذيتهما على عليقة المقارنة باضافة 10ملجم من أكسيد الزنك فى صورتها الطبيعية (جزئيات كبيرة) أو 5 ملجم من الزنك أكسيد فى صورة جسيمات النانو / كجم من العلف المركز وذلك للمعاملات م1 ، ،م2 على التوالى.

ولقد أوضحت النتائج ان معاملات هضم المادة الجافة ، والمادة العضوية والبروتين الخام والألياف الخام كانت الأعلى معنويا مع اضافة الزنك فى صورة جسيمات النانو مقارنة بالمعاملات الأخرى. وفى نفس الاتجاه كانت القيمة الغذائية كمركبات مهضومة كلية وبروتين خام مهضوم هى الأعلى معنويا مع اضافة الزنك فى صورة جسيمات النانو مقارنة بالمعاملات الأخرى وفى نفس الوقت لم توجد فروق معنوية بين مجموعة المقارنة ومجموعتى إضافة الزنك الطبيعية (جزئيات كبيرة) أو فى صورة جسيمات النانو بالنسبة لمعامل هضم مستخلص الأثير. كما أوضحت النتائج ان اضافة الزنك فى صورة جسيمات النانو كان لها تأثير ايجابى على المأكول من المادة الجافة مقارنة بمجموعة الكونترول ومجموعة الزنك فى صورته الطبيعية .

كما أظهرت النتائج ان اضافة الزنك فى صورة جسيمات النانو وكذلك اضافته فى صورته الطبيعية قد أدتا الى زيادة معنوية لكل من متوسط انتاج اللبن اليومى و انتاج اللبن الكلي ، مع ملاحظة ان التفوق فى محصول اللبن اليومى قد تحقق مع إضافة الزنك فى صورة جسيمات النانو. كذلك أدت اضافة الزنك فى صورة جسيمات النانو الى زيادة متوسط وزن النعاج من الولادة حتى الفطام. و زيادة total antioxidant capacity ونشاط GST، وتركيز البرولاكتين ، ترايدوثيرونين و الثيروكسين فى سيرم النعاج ونتاجها. كذلك زاد تركيز الأميونو جلوبيولين جاما فى سيرم النعاج ونتاجها. كما سجل تركيز البروتين الكلى والألبيومين فى سيرم الدم أعلى قيمه مع المجموعة م2، وتلاه مجموعة الزنك الطبيعى ثم مجموعه المقارنة. ولم توجد فروق معنوية بين المجاميع المختبرة ومجموعه المقارنة بالنسبة لباقى قياسات سيرم الدم. ويمكن تفسير الاختلاف بين مجموعتى اضافة الزنك فى صورته الطبيعية (جزئيات كبيرة) ومجموعة اضافة الزنك فى صورة جسيمات النانو الى ان صغر حجم جسيمات النانو يساعد على زيادة معدل انتشارها .كما تتميز جسيمات النانو زنك بانها أكثر توزيعا وانتشارا.

الخلاصة ان عنصر الزنك يمكن ان يتم اضافته بكميات أقل فى صورة جزيئات النانو بدون أى أثار سلبية على أداء الحيوان .