J.Animal and Poultry Prod., Mansoura Univ., Vol.6 (11): 693 - 703, ,2015

INFLUENCE OF SOME TRACE MINERALS IN FORM OF NANO PARTICLES AS FEED ADDITIVES ON LAMBS PERFORMANCE.

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ABSTRACT

This study was carried out to investigate the effect of large particles (zinc Zn or selenium SE) or nano-Zn and nano-Se particles as feed supplement in lambs rations on feed intake, degradability, growth performance and some blood parameters. Twenty five growing lambs aging about 5-6 months with average body weight of 24.5kg, were used. Lambs were divided into five similar groups and fed randomly on one of the following rations. The first group received the basal ration, composed of concentrate feed mixture (CFM) to cover 50% of protein and energy allowances recommended by NRC (1989) and clover hay (CH) without feed additives which served as the control group (R1). The other tested groups were randomly fed the control ration supplemented with 10 mg large particles zinc (L-Zn), 0.04 mg large particles selenium (L-Se), 5 mg nano particles zinc (N-Zn) and 0.02 mg nano particles selenium (N-Se) / kg CFM for rations R2,R3, R4 and R5, respectively. Two canulated sheep were used to measure in sacco disappearance of DM, OM and CP of tested rations.

Results showed that N-Zn & N-Se particles groups improved DM, OM and CP degradability compared with control group. But there were no significant differences among large particles groups and control group. Ration contained N-Zn had a significant effect on DM intake, but there were no significant differences among the large particles zinc (L-Zn) or selenium (L-Se) groups and control group among DMI. On the meantime, results showed that R4 & R5 groups increased ($P<0.05$) average daily gain and total weight gain compared to the control group. Also, feed conversation as DMI/ kg gain for R4 & R5 groups were improved by 16.93 and 18.25%, as compared with control group. Feed cost /kg gain was decreased for R4 and R5 groups as compared with control group. Serum total protein and albumin for R4 was increased compared with control group. While no significant differences between control group and other groups were found among AST and ALT values.

In conclusion, large size particles of Zn or Se could be replaced with smaller amount of nano particles Zn or Se without any side effects on the animal performance.

Keywords:Nano particales, zink, selenium,degradability, lambs performance, serum parameters.

INTRODUCTION

Nano is a word derived from the Latin *nanus*, (meaning dwarf). Nanoparticles are of different types based on their ability to arry different ingredients and react to different environmental conditions. Surej *et al.,* (2014) stated that nanotechnology will play a major role in the future areas of research in animal nutrition. The tremendous development of science and technology that occurred in the second half of the twentieth century has resulted in not only new, bold ideas, but it has also created tools that enable

to perceive atoms in surrounding matter. Recently achievements, especially those made at the end of the twentieth century, have occurred as a result of the obvious progress of nanotechnology. Nanotechnology creation and utilization of materials, devices and systems through the control of properties and structure of matter of nanometric scale. Nanotechnology is interesting and attractive field of research, in particular into molecular biology and cell biology, " Nano" refers to a size scale between 1 nanometer (nm) and 100 nm.

Zinc (Zn) is one of the most essential trace minerals required for the metabolic activity of about 300 of the body enzymes and is considered essential for cell division and the synthesis of DNA and protein. It is also critical to tissue growth, wound healing, taste acuity, connective tissue growth and maintenance, immune system function, prostaglandin production, bone minerlization, proper thyroid function. Thus the presence of Zn in the proper concentration in the diet of the animals is of immense importance not only for the well-being of the animals but also for optimizing the overall performance of the animals and enhance their production potential (MacDonald, 2000).

Selenium (Se) is a component of selenoproteins, it displays metabolic function in preventing oxidative damage to body tissue (Neve, 2002 and Abd El-Ghany and Tortora-Perez, 2010). Its deficiency has resulted in impaired heats, cystic ovaries, birth of unthrifty kids with poor immunity and white muscle disease (Hartikainen, 2005). If Se deficiency occur in grazing and forage feed, it should be supplemented for ruminant ration. Selenium supplements could increase live weight gains, wool production and growth rate (Gabbedy,1971; Whelan *et al.,* 1994; and Mahima *et al.,* 2006). Selenium (Se), in the form of selenocysteine, is the central structural component of a number of specific enzymes, and especially catalase, glutathione peroxidase, which allows the host defense against oxidative stress. An adequate intake of selenium is needed to reduce the risk of myopathy, immunodeficiency, cardiovascular disease and cancer (Hartikainen, 2005). For animals and especially lambs, selenium deficiency is associated with white muscle disease. Selenium from food is mainly derived from plants, which are adopted from the soil selenium in inorganic form, and synthesize most of the seleno-methionine (Mahima**,** *et al.*,2006).

Also, selenium is an essential micronutrient in sheep and the deficiency of it can limit lamb growth and survival (Abd El-Ghany and Tortora-Perez 2010). Moreover, Se is a component of selenoproteins and is involved in immune and neuropsychological function in the nutrition of animals (Neve, 2002). The soils contain inorganic selenites and selenates that plants accumulate and convert them to organic forms, mostly selenocysteine and selenomethionine and their methylated derivatives. Since little is known about influence of nano- Zn and nano-Se on ruminant nutrition

Thus, the objective of this study was to investigate the effects of supplemental normal particles of Se & Zn or nano-Se & nano-Zn as feed

supplements in lambs rations on feed intake, degradability in the rumen, growth performance and some blood parameters.

MATERIALES AND METHODS

The present study was carried out at El-Emaan farms (private farm) Minia Governerate. The influence of the two trace elements zink (Zn) or selenium (Se) of both forms (large and Nano particles) on degradability of DM, OM and CP for different rations without or with supplementation was studied by the in sacco artificial fiber bags technique (Meherez and Ørskov 1977). Two cumulated male sheep were used to determine degradability of different rations without or with supplementation fed commercial concentrate feed mixture + clover hay. Two polyester bags with poor size of 45um were used for each incubation time for each of the 5 treatments (incubation divided into two phases). Approximately 5g of air dried ration (concentrate feed mixture + clover hay in proportion as consumed) were placed in each bag. All bags were incubated in the rumen of each animal, for 3,6,12,24,48 and 72h, then they were rinsed in tab water until the water became clear, then they were squeezed gently. Microorganisms attached to the residual samples were eliminated by freezing at -20C^0 (Kamel *et al.,*1995). The degradability constants were calculated by fitting the disappearance values of the experimental rations to the equation illustrated by Ørskov and McDonlad (1979), being:

$$P = a + b\,(1-e^{-ct})$$

Where: P represents the disappearance after time t.

(a) soluble fraction.

(b) slowly degraded fraction .

(c) the rate of degradation.

To identify the particle size and morphology of the synthesized materials (nano particles), transmission electron microscope (TEM) type "JEOL JEM-1230 operating at 120 kV attached to a CCD camera", was used.

A feeding trial was conducted using twenty five growing male lambs, aged 5-6 months and averaged 24.5 $\pm$1.3kg live body weight. Animals were divided into five similar groups (5 animals each) according to body weight. The feeding trial lasted 100 days and animals were fed according to the allowances of growing lambs recommended by NRC (1990).

The first group received basal ration, composed of commercial concentrate feed mixture (CFM) (consists of (included soybean meal corn, wheat bran, minerals mixture and sodium chloride) to cover 50% of protein and energy allowances recommended by NRC (1990) and clover hay (CH) ad-lib without feed additives which served as the control group (R1). The other tested groups were randomly fed the control ration supplemented with 10 mg large size particles zinc (L-Zn), 0.4 mg large size particles selenium (L-Se) according to NRC (1990), 5 mg nano particles zinc (N-Zn) and 0.2 mg nano particles selenium (N-Se)/kg CFM for rations R2,R3, R4 and R5,respectively.

Change of body weight were recorded twice monthly before morning feeding. Feed conversion values were calculated and expressed in terms of dry matter intake (DMI in kg) to produce one kg weight gain. Chemical composition of the ingredients and control ration were determined as described by A.O.A.C.,(1995). Blood samples were collected from three animals in each group, post morning feeding for 3 times (At the beginning and in the middle and at the end of the experimental period) during the experimental period. Blood serum samples were separated by centrifugation at 4000 rpm for 10 minutes, then frozen at -200C until analysis. Commercial kits were used to determine serum Total protein, Albumin, globulin and activities of AST and ALT.

Data were statistically analyzed according to SAS (1996) and the differences between means were tested using Duncan's new multiple range test Duncan, (1955).

RESULTS AND DISCUSSION

The chemical analysis of ingredients and calculated nutrients content for the control ration are presented in Table (1). The concentrations of both Zn and Se are less than recommended by NRC (1990).

Table (1): Chemical analysis of ingredients and experimental rations (% on DM basis).

Item	Chemical analysis							
	DM	OM	CP	NDF	ADF	Ash	L-Zn	L-Se
CFM	90.78	91.08	16.56	25.63	17.24	8.92	$6.7x10^{-3}$	$2.2x10^{-4}$
CH	88.42	88.52	12.76	39.42	27.36	11.48	$1.7x10^{-3}$	-
R1	89.68	89.83	14.62	31.82	21.67	10.17	$4.7x10^{-3}$	$1.32x10^{-4}$

***CFM = Concentrate Feed mixture BH = Clover hay R1= Control ration L-Zn = large Zink, L-Se =large selenium**

Nano particles identification

Figure (1) depicts TEM images of synthesized selenium (1), and zinc Fig (2) nanoparticles at two different magnifications.

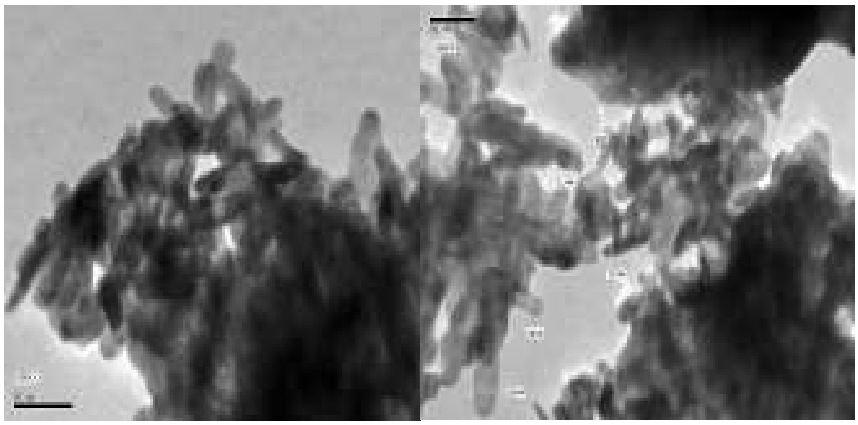

Fig (1)

It is indicated that the selenium exhibits agglomerated rod-like particles with homogeneous particles having length of about 50nm and width ranging between 8 and 26 nm.. Their sizes are ranging between 25 and 90 nm. On the other hand, agglomerated flower shape of zinc nanoparticles are detected (Fig. 2). These agglomerates consist of several cube nanoparticles with size ranging between 6 and 11 nm.

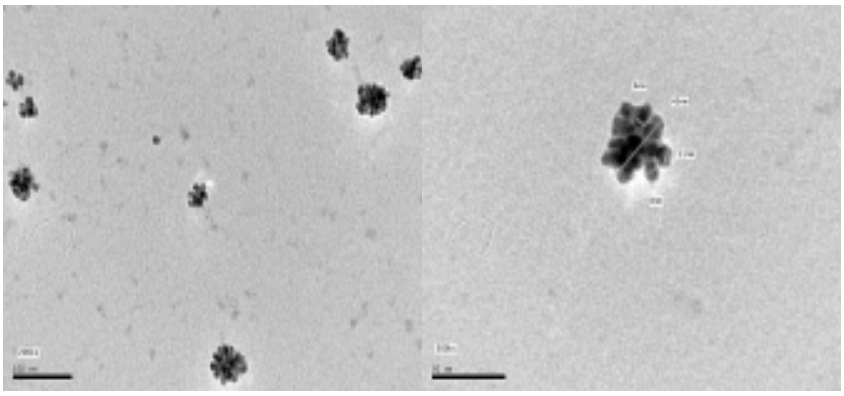

Fig (2)

Degradation kinetics (In sacoo):

The effect of the large size (L-Zn) or (L-Se) and nano particles (N-Zn) or (N-Se) as feed additives on ruminal degradation constants (a,b and c) of rates DM, OM and CP disappearance of ration are presented in Table (2). The washing loss fraction (a) for DM, had no significant differences among metal minerals additives R2 & R3 groups and control group.

Table (2): Degradation kinetics of DM, OM and CP for experimental rations.

	Experimental Rations				
		Metal minerals		Nano particles	
DM	**R1**	**R2**	**R3**	**R4**	**R5**
a	26.70^{B}	26.92^{B}	27.34^{AB}	27.93^{B}	27.74^{A}
b	54.84^{C}	55.78^{BC}	56.65^{B}	58.54^{A}	57.48^{AB}
c	0.044	0.047	0.046	0.049	0.048
OM					
a	27.20^{B}	27.34^{B}	27.68^{B}	28.17^{A}	27.93^{AB}
b	55.62^{C}	57.52^{B}	56.17^{BC}	59.46^{A}	58.29^{AB}
c	0.046	0.048	0.047	0.050	0.049
CP					
a	24.43^{B}	24.78^{AB}	25.16^{AB}	25.82^{A}	25.63^{A}
b	59.32^{C}	62.82^{BC}	62.27^{BC}	64.28^{A}	63.82^{AB}
c	0.048	0.050	0.049	0.053	0.051

R1 = control ration without any additives
R2 = control ration with large particles zinc additives
R3 = control ration with large particles selenium additives
R4 = control ration with nano particles zinc particles additives
R4 = control ration with nano particles selenium particles additives
A,B and C Means in the same row with different superscripts diifer significantly at ($P<0.^{05)}$.

On the other hand, the washing loss fraction (a) of DM was higher ($P<0.05$) for nano particles additives groups (R4 and R5) as compared with the control group (R1). While, no significant differences between the two nano- particles groups (R4 & R5) was found for their rate of degradation (c). Mean while, washing loss fraction (a) of OM was higher ($P<0.05$) for nano Zinc additive group (R4) as compared with the control group (R1). On the other hand, there were no significant differences between tested groups with additives (R2, R3 & R5) and the control group among their washing loss fraction (a) of OM. The degradable fraction (b) of OM was higher ($P<0.05$) for nano particles additives (R4 and R5) groups as compared with the control groups R1. While, there were no significant difference among metal zinc additives (R2) group and control group about degradable fraction (b) of OM. On the mean time, there were no significant differences among additives groups and control group for the rate of degradation (c). No significant differences among metal minerals additives groups (R2 &R3) and control group for the washing loss fraction (a) and rate of degradation (c) of CP.

Although, the degradable fraction (b) of CP was higher ($P<0.05$) for both zinc and selenium nano particles groups compared with control group, no significant differences among zinc and selenium metalized additives and control group for degradable fraction (b) of CP were detected.

These results agree with the findings of Vignola *et al.,* (2009) and Wenjuan Xun *et al.,* (2012) who reported that nano-Se increased degradability of DM, organic matter OM, and crude protein CP. The

significant differences between large size particles and nano particles (Zn or Se) are mainly due to the surface effects, where the atoms of nano materials are less stable than those of larger structures since the energy required to join adjacent atoms is less. As a consequence of this the fusion point of a given element changes (Ruzic-Muslic *et al.,* 2014).

***In vivo* trial**

Lambs performance

The effect of the normal size particles Zn (no -Zn) & Se (no -Se) and nano particles –Znic (N-Zn) & nano particles –Selenium (N-Se) as feed additives on dry matter intake (DMI), average daily gain, feed and economic efficiency are shown in Table (3).

Table (3): Effect of different mineral supplementation on average feed intake, average daily gain, feed conversion and economic evaluation.

Items	Experimental groups				
	G1	Normal size particles		Nano particles	
		G2 L-Zn	G3 L-Se	G4 N-Zn	G5 N-SE
No. of animal	5	5	5	5	5
Days of trial	100	100	100	100	100
Initial weight kg	25.7	24.5	24.7	24.70	24.3
Finial weight kg	40.7^{b}	41.2^{ab}	41.7^{ab}	44.2^{a}	43.4^{a}
Total gain kg	15.0^{c}	16.6^{bc}	17.0^{b}	19.5^{a}	19.1^{a}
Av. daily gain gm	150^{c}	166^{bc}	170^{b}	195^{a}	191^{a}
Average feed intake (kg / day)					
CFM	0.598	0.598	0.598	0.598	0.598
CH	0.652^{b}	0.652^{b}	0.672^{ab}	0.752^{a}	0.702^{a}
Av. feed intake as DM kg/ head	1.25^{b}	1.25^{b}	1.27^{b}	1.35^{a}	1.30^{ab}
Feed efficiency					
DM kg / kg gain	8.33	7.53	7.47	6.92	6.81
Economical evaluation					
Feed cost for kg weight gain, LE	15.67	14.0	14.12	13.00	13.10
Total feed cost LE	282	285.6	285.6	298.8	292.8
Price of TBWG*	450	498	510	585	573
Economic return**	168	212	225	286	280
Economic Efficiency	1.6	1.74	1.79	1.97	1.96
Economic Efficiency improved	--	8.75	11.9	23.1	22.5

***TBWG=Total body weight gain**

**** Economic return =Price of TBWG –total feed cost**

a,b,c Means in the same row with different superscripts diifer significantly at (P<0.05).

Dry matter intake (DMI) for experimental groups (R2 &R3) were not significantly affected with large size particles Znic (L-Zn) or large size particles Selenium (L-Se). While, the DMI values were significantly higher

(P<0.05) with adding N-Zn or N-Se to the ration as compared with control group.

Average daily gain and total gain for lambs were significantly (P<0.05) improved by adding L-Ze or L-Se to the rations. However, R4, group showed the best results of average daily gain and total gain. The superiority of R4 ration than the other rations, may reflect the obtained results of degradability values of DM and CP of this ration. Also, the calculated feed efficiency as DM kg/ kg gain was improved 9.6, 10.32, 16.93 and 18.25% as compared with the control group. The nano particles groups (Zn or Se) showed the best feed utilization efficiency (R4 &R5) versus to control group. These results are in agreement with results obtained by Vignola et al., (2009) and Wenjuan *et al.,* (2012).

Meanwhile, Ružić-Muslić *et al .,*(2014) reported that the clear improvement with nano-zinc supplement could be as results that form nano particles as well as the small size, which helped to spread and access to all cells.

Feed cost per head (LE/day) of minerals additives rations (large particles or Nano particles) were higher than the control one (Table 3). The lowest feed cost per kg gain (LE) recorded with R4 was reflected the obtained results of feed utilization efficiency. The Economic return above feeding cost was higher with additives groups than the control group. When comparing between the additives groups, it was observed that R4 indicated the best economic return followed by R5 and R3.

Blood serum parameters .

Some blood serum parameters as affected by feeding the experimental rations are shown in Table (4). Data in Table (4) showed that the highest mean values of serum total protein (TP) was that of R4 compared to R1. But there were no significant differences among (R2 and R3) and control group.

Table (4): Effect of experimental rations on some blood serum parameters.

Experimental rations	T.P (g/Di)	Albu. (g/Di)	Globu. (g/Di)	A/G Ratio	AST (u/l)	ALT (u/l)
R1	8.53^{b}	4.35^{b}	4.18	0.90	27.88^{ab}	14.80^{a}
R2	8.80^{b}	4.43^{b}	4.37	.93	28.00^{a}	13.73^{ab}
R3	8.63^{b}	4.40^{b}	4.23	.92	26.50^{b}	13.88^{ab}
R4	9.52^{a}	4.81^{a}	4.71	1.07	28.88^{a}	12.85^{b}
R5	9.06^{ab}	4.69^{ab}	4.47	1.03	27.52^{ab}	14.73^{a}
SE±	0.47	0.32	0.27	0.16	1.42	1.83

T.P. = Total protein Albu = Albumen Globu= Globulin
AST = Aspartate Aminotransferase ALT = Alanine Aminotransferase

Vignola *et al.,* (2009) noticed that the increase in digestibility of CP may be the reason for the increase in each of serum total protein and albumin concentration. Values of serum protein fraction indicated better utilization of dietary protein and ruminal nitrogen through digestive tract.

Mean values of albumin and globulin concentration appeared to follow the same toward of the serum total protein with significantly differences with globulin of serum of R4, which may reflect the improvement in protein digestibility in the gut intestinal tract. Data indicated healthy status of the liver since the liver is the main orgain of albumin synthesis. Serum otal protein and its fractions are considered as a biological index reflecting health and performance of animal (Gabbedy, 1971). The values of serum A/G ratio ranged from 1.14 (R1) to 1.19 (R2). It is important to note that all values of A/G ratios were higher than 1.0, which indicates that animal did not suffer from any health problems which might affect the performance of the experimental animals. Significant increases ($P<0.05$) were found with AST in blood serum of lambs fed non organic minerals containing rations (R2 &R3) ratio. This result came in line with protein digestibility of lambs fed R4 & R5 compared to R1, but serum ALT activity values were insignificantly. These results may be due to high body weight recorded for this group. Vignola *et al.,* (2009) found that there is a positive correlated between body weights and AST. The AST and ALT enzymes are most important indicator for liver cells activity.

The values obtained herein were within the normal range for healthy lambs (Rock et al ., 2001).

From this study , it can be concluded that large mineral additives of both Zn & Se could be replaced with smaller amount of nano particles Zn or Se for lambs rations with good economic efficiency and without any adverse effects on animal performance which reflect on feeding cost and economical efficiency.

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تأثير بعض العناصر النادرة فى صورة جسيمات النانو كاضافات غذائية على أداء الحملان.

علاء الدين حسن محمد ، محمود ياسين محمد ، فاطمة طلعت فرج عبد الغنى , عبد المنعم على سيد محجوب و خميس ابراهيم
معهد بحوث الانتاج الحيوانى- مركز البحوث الزراعية . دقى . جيزة. مصر

استخدمت بعض التخمرات المعملية لدراسة تأثير جسيمات النانو للزنك أو السلينيم مقارنة باضافة جزئيات للزنك أو السلينيم فى صورتها الطبيعية (جزئيات كبيرة) على معدلات تحلل المادة الجافة والعضوية والبروتين للعليقة والمكونه من(علف مركز ودريس برسيم وتبن قمح) باستخدام طريقة Nylon bags وكانت الاضافة بمعدل ٠، ١٠ أو ٤و ملجم من الزنك أو السيلينيم فى حجمها الطبيعي (جزئيات كبيرة) أو ٥ أو ٢و ملجم من الزنك أو السيلينيم فى صورة جسيمات النانو / كجم علف وذلك للمعاملات م١،م٢، م٣،م٤ ،م٥ على التوالى.

ولقد أوضحت النتائج ان اضافة الزنك أو السلينيم فى صورة جسيمات النانو قد أدى الى تحسين معدل تكسير كل من المادة الجافة ،والمادة العضوية والبروتين الخام تحسنا معنويا مقارنة بمجموعة المقارنة . وفى نفس الوقت لم توجد فروق معنوية بين مجموعة المقارنة ومجموعتى الاضافة الزنك أو السيلينيم فى صورتها الطبيعية (جزئيات كبيرة) بالنسبة لمعدل تكسير كل من المادة الجافة ،والمادة العضوية والبروتين الخام . كما أوضحت النتائج ان اضافة الزنك فى صورة جسيمات النانو كانت لها تأثير ايجابى على المأكول من المادة الجافة مقارنة بمجموعة الكونترول. كذلك لم توجد فروق معنوية بين مجموعه المقارنة ومجموعتى الاضافة الزنك أو السيلينيم فى صورتها الطبيعية (جزئيات كبيرة) بالنسبة المأكول من المادة الجافة مقارنة بمجموعة الكونترول.

كما أظهرت النتائج ان اضافة الزنك أو السلينيم فى صورة جسيمات النانو قد أدى الى زيادة معنوية لكل من متوسط الزيادة اليوميه والزيادة الكلية فى الوزن للمجموعتين مقارنة بمجموعة المقارنة. بينما لم توجد فروق معنوية بين مجموعتى الاضافة الزنك أو السيلينيم فى صورتها الطبيعية (جزئيات كبيرة) ومجموعه المقارنه بالنسبة لمتوسط الزيادة اليوميه والزيادة الكلية فى الوزن. كذلك تكاليف التغذية /كجم زيادة فى الوزن قد انخفضت مع المجموعين م٤، م٥ مقارنه بمجموعه المقارنة. ويمكن تفسير الاختلاف بين مجموعتى الاضافة الزنك أو السيلينيم فى صورتها الطبيعية (جزئيات كبيرة) ومجموعتى اضافة الزنك أو السلينيم فى صورة جسيمات النانو الى ان صغر حجم جسيمات النانو يساعد على زيادة معدل انتشارها .كما تتميز جسيمات النانو للزنك بانها أكثر توزيعا وانتشارا من جسيمات السلينيم. كما زاد تركيز البروتين الكلى واالألبيومين فى سيرم الدم بالنسبة للمجموعة م٤ مقارنة بمجموعه المقارنة . ولم توجد فروق معنوية بين المجاميع المختبرة ومجموعه المقارنة بالنسبة لباقى قياسات سيرم الدم .

الخلاصة : ان كل من الزنك و السلينيم يمكن ان يتم اضافتهما بكميات أقل فى صورة جزيئات النانو بدون أى أثار جانبية على أداء الحيوان.