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## Effect of Inclusion Inorganic, Organic or Nano Selenium Forms in Broiler Diets On: 1-Growth Performance, Carcass and Meat Characteristics

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**Abstract:** This study was conducted to evaluate the efficiency of different selenium (Se) sources and levels in corn-soybean meal broiler diets. For that, 400 day-old unsexed Arbor Acres broiler chicks were allocated to 10 experimental treatments in a 5 (sources) x 2 (levels) factorial arrangement. Five Se sources were tested; (1) sodium selenite (NaSe) as inorganic form; (2) selenomethionine (Se-Yeast) as organic form; (3) Zinc-L-selenomethionine (Zn-Se-Meth) as more recent organic form; (4) powder form of Nano Se form (P-Nano Se) and (5) Liquid form of Nano Se (L-Nano Se). Also two inclusion of Se levels in diets; 0.15 and 0.30 ppm, were examined. The inorganic and organic forms of examined Se were obtained from commercial suppliers while both powder and liquid forms of Nano Se were prepared immediately before starting feeding phases of the experiment. The prepared 80 nm Se nano-particles were synthesized by chemical reduction method and characterized by Transmission Electron Microscope, X-ray diffraction and spectrophotometry. Three phases (1-10, 11-24 and 25-40 d) of feeding were applied and all birds were kept under similar management conditions. Parameters of growth performance, carcass characteristics and concentration of Se in both liver and thigh muscles were investigated. Also assay of Malnodialdehyde (MDA) was carried out in frozen (6 months at -20°C) thigh muscles to investigate the oxidation status of broiler meat. The obtained results showed significant improvement of growth performance and Se concentration in liver and thigh tissues either due to using organic or nano forms of Se, or by increasing the inclusion Se level from 0.15 to 0.30 ppm in broiler diets. While carcass abdominal fat%, giblets% and MDA content in thigh muscles did not affected due to Se sources or levels. Liver showed grater Se concentration than thigh muscles. The overall experimental results showed that using Se-Yeast or Zn-Se-Meth as organic forms of Se, or L-Nano Se as nano form of Se at level of 0.30 ppm in broiler diets or its equivalent in drinking water, respectively, is more effective to get better growth performance and quality of broiler meat. But further studies about the safety of using nano form of selenium as feed additives are needed.

**Key words:** Broiler, inorganic selenium, organic selenium, nano selenium, performance, carcass

### INTRODUCTION

Selenium is an essential trace element that has a large number of biological functions in poultry. Feed ingredients only contain Se in the organic form, mainly as selenomethionine (SeMet). The SeMet form is not synthesized by animals but by plants (Schrauzer, 2000). Organic and inorganic forms of Se may be used as feed supplements. Sodium selenite and sodium selenate are two inorganic forms of Se commonly used in the feed industry, while organic Se can be derived from *Saccharomyces cerevisiae*, a yeast, grown in a media containing high levels of Se. Selenium concentrations in feed ingredients vary greatly depending on both the plant species and in particular the Se status of the soil. Therefore, poultry diets require supplemental Se in order to provide a margin of safety against deficiency and to maintain productive performance (Deniz *et al.*, 2005). However the inorganic form (sodium selenite) is common used in broiler diets. This salt is very toxic and

needs to be soluble in its ionic form in order to be absorbed in gastrointestinal tract. In addition, the electric charges of this ionic form may interact with other diet components (minerals, proteins and carbohydrates), rendering them partially unavailable to animals (Saad *et al.*, 2013). This has inspired researchers to study using organic selenium to improve its bioavailability. Many researchers examined the effects of organic selenium on growth performance, carcass yield and carcass characteristics of broiler chicks (Mahan, 1996; Downs *et al.*, 2000; Naylor *et al.*, 2000; Sevcikova *et al.*, 2006; Kralik *et al.*, 2012, 2013).

While most researches on using organic selenium used SeMet form, there was Zn-L-selenomethionine (Zn-Se-Meth) which was more recent product of organic selenium. Zinc-L-selenomethionine is designed to be highly soluble and increase bioavailability of Se (Ward, 2003). By complex the zinc ion in that product it became more soluble, better absorbable and more protected

than SeMeth as mentioned by the producers which supposed to be more effective on poultry performance. Until now there are no available references about evaluation of Zn-Se-Meth in broiler feeding. However, Reis *et al.* (2009) indicated that the supplementation of Zn-L-selenomethionine in layer diets led to a higher deposition of Se in egg contents when compared with sodium selenite, this attributed to a faster transference of Se from Zn-L-selenomethionine source into egg proteins.

With the recent development of nanotechnology, nano selenium (nano Se) has attracted widespread attention because nanometer particulates exhibit novel characteristics such as a large surface area, high surface activity, high catalytic efficiency, strong adsorbing ability and low toxicity (Wang *et al.*, 2007; Zhang *et al.*, 2008). It has been reported that nano Se possesses comparable efficiency to selenite and Se-methylselenocysteine in up regulating selenoenzymes but with dramatically decreased toxicity (Zhang *et al.*, 2008). So far the effect of using nano Se in broiler feed on growth performance and produced broiler meat is under investigation. Also the safety of using this form of Se element is still not recommended globally yet.

There is global trend to increase consumption of poultry meat, which can be explained with the fact that broiler meat is of satisfactory nutritive quality and acceptable to consumers with respect to price and organoleptic traits. Intensive researches are directed towards improvement of the nutritive quality of broiler meat as a functional product. Broiler meat shall be enriched with certain functional ingredients, such as selenium (Kralik *et al.*, 2013).

The aim of this study was to study the effect of using different forms of Se at levels of 0.15 or 0.30 ppm in broiler diets on growth performance, carcass characteristics and quality of frozen broiler meat.

## MATERIALS AND METHODS

This study has done in Animal Production Research Institute (APRI) and with collaboration with Nanotechnology and Advanced Materials Central Lab (NAMCL), Agricultural Research Center, Giza, Egypt.

**Selenium nanoparticles (SeNPs) synthesis:** The SeNPs were prepared according to Zhang *et al.* (2004) with little modifications. In brief, 100 ml of 1 mM sodium selenite heated under stirring condition until boiling then 2.5 mL of 1% Ascorbic Acid was added drop wise until the color change to the characteristic yellowish orange and left to cool with stirring for 30 min, then proceeds for characterization. All used chemicals were obtained from Sigma-Aldrich and used as purchased without any modification. The prepared SeNPs was tested for concentration determination by inductively coupled plasma (ICP).

**Characterization of SeNPs:** Synthesis and characterization of the synthesized SeNPs was done at NAMCL. UV-Vis spectrophotometer, (Cary 5000, Varian, UK) monitoring SeNPs from 400 to 800 nm with a path-length of 10 mm at 12 nm/s scanning speed and 1 nm bandwidth. High Resolution Transmission electron microscopy (HR-TEM, Tecnia, G20, 200 Kv, FEI, Netherland). Sample was prepared by placing a droplet of the colloidal solution onto a carbon-coated grid and were allowed to dry for 45 min. Bright field imaging mode at electron accelerating voltage 200 kV using lanthanum hexaboride (LaB6) electron source gun was performed. Eagle CCD camera with (2k\*2k) image resolution was used to acquire and collect transmitted electron images. TEM Imaging software was used. Dynamic Light Scattering (ZS-nano, Malvern, UK) used for SeNPs particle size distribution determination. For phase analysis, X-ray diffraction (XRD) patterns of SeNPs were obtained using Panlytical X'pert Pro X-ray diffractometer using Cu Ka (1.54059 Å) radiation with the X-ray generator operating at 45 kV and 30 mA. The total Se concentration was measured by Inductively coupled plasma (ICP) technique (optical emission spectrometer optima 2000 DV, Perkin elmer).

**Mixing SeNPs in diet:** After complete the characterization of the synthesized SeNPs solution, the suggested examined levels of Se were 0.15 and 0.30 ppm in broiler diet. So there was a necessary to suggest more applicable and accurate method to use SeNPs solution in diets. Three carriers of SeNPs solution were examined (colloidal silica, skimmed milk and wheat bran) and the best one was wheat bran. The SeNPs was added to experimental diet at levels 0.15 ppm or 0.30 ppm in dry form by mixing the selected concentrations of SeNPs to wheat bran and mixed well for homogeneity before drying at 60°C overnight (P-Nano Se). Also the prepared SeNPs solution was added as it was to drinking water on equivalent levels of those added to diet (L-Nano Se).

**Growth trail:** Four hundred one day-old unsexed Arbor Acres broiler chicks were obtained from a commercial hatchery individually weighed and assigned to 10 dietary treatments (4 replicates/treatment of 10 chicks each) to study the effect of different sources and levels of supplemental Se. Five Se sources were tested; (1) sodium selenite, NaSe; (2) selenomethionine, Se-Yeast; (3) Zinc-L-selenomethionine, Zn-Se-Meth; (4) powder form of SeNP, P-Nano Se and (5) Liquid form of SeNP, L-Nano Se and two levels of Se supplementation; 0.15 and 0.30 ppm in broiler diets, were examined. The liquid form of SeNP was added in drinking water daily at equivalent doses using feed consumption of chicks in control group for the previous day as reference. The experimental diets were prepared as mash and

formulated to meet the nutrient requirements of Arbor Acres broiler chicks excluding Se. Chicks fed on corn-soybean meal basal diets during starting (1-10 d), growing (11-24 d) and finishing (25-40 d) periods (Table 1). The experimental design is shown in Table 2. All birds were kept under similar management conditions. The environmental temperature and humidity surrounding birds were recorded daily during the experimental period. Individual live body weights (BW) and feed intake (FI) of chicks were recorded at 10, 24 and 40 days of age, then live body weight gains (BWG) and feed conversion ratios (FCR) were calculated for each period and the overall experimental period.

**Carcass characteristics and tissues examinations:** At 40 d of age, 40 birds (4 birds per treatment which were around the average body weight) were slaughtered and carcass characteristics including dressing, liver, giblets, abdominal fat as percentage from live body weight were recorded. After slaughtering one thigh of each carcass was frozen (6 months at -20°C) then carried out assay of Malnodialdehyde (MDA). Concentrations of Se in liver and thigh tissues of each carcass were determined after samples were kept at -20°C for few days. The tissues samples were weighted and digested using mixture of concentrated Nitric acid and Perchloric acid (ratio 3:1). The digested samples were diluted with deionized water then introduced for measurement using the inductively coupled argon plasma spectroscopy (ICP) to evaluate the Se concentrations (in ppm) in the corresponding tissues according to the method of Cottenie *et al.* (1982).

**Statistical analysis:** Data of experimental treatments were analyzed by using two way analysis of variance to detect the effect of selenium source and supplemental level. Also data of all experimental treatments were analyzed by using one way analysis of variance to detect the best treatment between them. Variables showed significant differences at F-test ( $p \leq 0.05$ ) were compared to each other's using Duncan's Multiple Range Test (Duncan, 1955). The statistical procedures were computed using SAS (1999).

**RESULTS**

**Characterization of SeNPs**

**A-UV-Vis spectrophotometer:** The optical properties of the synthesized SeNPs have been characterized by their Plasmon absorbance band in the visible region of the electromagnetic wave particles have peaks at 265 nm (Fig. 1) with Gaussian distribution indicating formation of spherical GNPs with no aggregation of size that indicated uniformity and excellent dispersion of colloidal Se nanoparticles.

**B-transmission electron microscopy (TEM):** The high resolution-TEM (HR-TEM) image of SeNPs was

Table 1: Composition and calculated analysis of basal diet

Composition (per 100 kg)	Starter (1-10 day)	Grower (11-24 day)	Finisher (25-40 day)
Yellow corn	52.28	59.05	63.19
Soybean meal (44%CP)	34.00	26.70	22.5
Corn gluten (60% CP)	6.00	7.00	6.30
Soy bean oil	3.00	3.00	4.00
Di-calcium phosphate	1.84	1.67	1.59
Lime stone	1.43	1.20	1.10
L-lysine HCl	0.32	0.31	0.28
DL-methionine	0.26	0.20	0.17
Sodium chloride	0.24	0.24	0.24
Sodium bicarbonate	0.23	0.23	0.23
Vitamins premix *	0.10	0.10	0.10
Minerals premix**	0.30	0.30	0.30
Total	100.00	100.00	100.00
<b>Calculated analysis (%)</b>			
Crude protein	23.17	21.25	19.04
Metabolizable energy (Kcal/kg)	3100	3110	3207
Ether extract	5.63	5.08	6.88
Crude fiber	3.80	3.45	3.22
Calcium	1.04	0.90	0.84
Av. Phosphorus	0.50	0.45	0.43
Lysine	1.44	1.24	1.09
Methionine	0.68	0.60	0.54
Methionine+cystine	1.06	0.95	0.86
Sodium	0.15	0.16	0.17

\* Supplied per kg of diet: Vit. A, 11000 IU; Vit. D3, 5000 IU; Vit. E, 50 mg; Vit K3, 3 mg; Vit. B1, 2 mg; Vit. B2 6 mg; B6 3 mg; B12, 14 mcg; Nicotinic acid 60 mg; Folic acid 1.75 mg, Pantothenic acid 13 mg and Biotin 120 mcg

\*\*Supplied per kg of diet: Choline 600 mg; Copper 16 mg; Iron 40 mg; Manganese. 120 mg; Zinc 100 mg and Iodine 1.25 mg

Table 2: Experimental design

	Supplementation of Se sources and levels to basal diet in ppm (1-40 d of age)									
	1	2	3	4	5	6	7	8	9	10
NaSe	0.15	0.30								
Se-yeast <sup>1</sup>			0.15	0.30						
Zn-Se-Meth <sup>2</sup>					0.15	0.30				
P-Nano Se <sup>3</sup>							0.15	0.30		
L-Nano Se <sup>4</sup>									0.15	0.30

1: Sel-plex®; 2: Availa-Se®; 3: Prepared in NAMCL and added in diet; 4: Prepared in NAMCL and added to drinking water

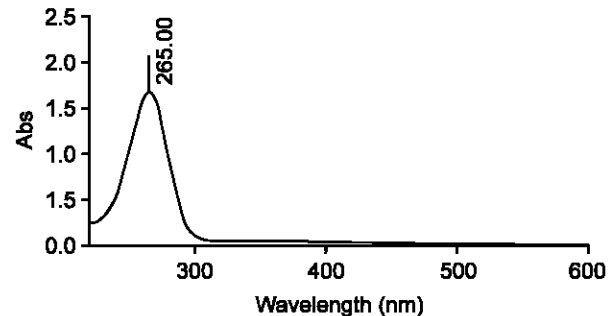


Fig. 1: Shows the UV-Vis spectrum of SeNPs with absorption peak at 265 nm

synthesized by ascorbic reduction method with monodisperse spherical shape with average size 80 nm. SeNPs was clearly evident that particles were spherical and uniform in size demonstrating homogeneity and monodispersity (Fig. 2).

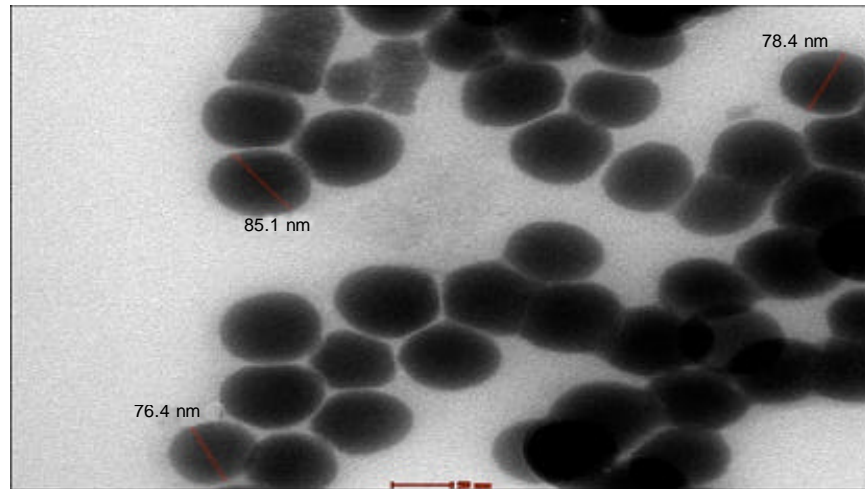


Fig. 2: HR-TEM image of SeNPs with average size 80 nm

**C-particles size distribution:** The particle size distribution of SeNPs was measured by dynamic light scattering (DLS) where the average measured size was 80 nm with perfect Poly Dispersity Index (PDI) 0.077 which confirmed the result obtained by TEM imaging and the corresponding characterized absorption peak (Fig. 3). XRD measurement were employed to investigate the phase and structure of the synthesized sample. Figure (4) shows the XRD pattern of the as prepared PEG-SeNPs shows a broad peak at  $2\theta = 29.682^\circ$  corresponding to the (101) reflection of selenium. Broad suggesting that the sample in nano size which confirm the results obtained from TEM and DLS. This all the new diffraction peaks of the PEG-SeNPs sample matched well with the data from the JCPDS card (1-086-2246) for SeNPs (the diffraction angles at  $2\theta$ ):  $23.499^\circ$ ,  $41.305^\circ$ ,  $43.618^\circ$ ,  $45.337^\circ$ ,  $51.684^\circ$  and  $65.197^\circ$  can be assigned to (100), (110), (012), (111), (201) and (210) of the crystal planes of SeNPs.

**Growth performance:** During the experimental period (1 to 40 d of age) environmental temperature and relative humidity% surrounding chicks were recorded daily and there values were ranged between  $36-41^\circ\text{C}$  and 30-55%, respectively. The recorded and calculated values of growth performance parameters of broiler chicks during experimental period are presented in Table 3. Means of initial BW of chicks in the different treatments ranged from 52.2 to 53.5 g. During starting phase there was no significant difference ( $p>0.05$ ) in BW, BWG, FI or FCR due to main factors or their interactions, then the significant differences were detected from growing phase in the same trend of results of overall experimental period. The BW, BWG and FCR of chicks during grower, finisher and over all experimental period

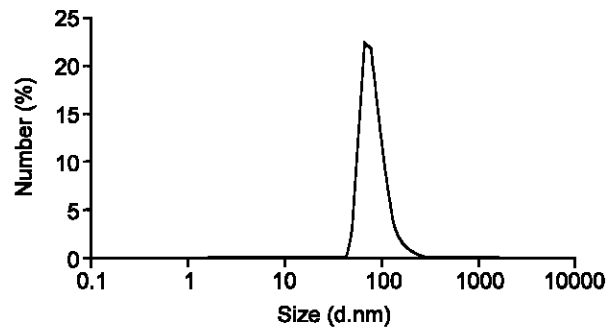


Fig. 3: particle size distribution of SeNPs

were significantly affected by supplemental Se source, inclusion Se level in broiler diets and their interactions. While the recorded values of FI did not affected by either the main studied factors or their interactions during any of the experimental periods. During growing period, using Se-Yeast could improve BW, BWG and FCR significantly than using inorganic form of Se, while using Zn-Se-Meth improve FCR only during the same period. By increasing the age of chicks and continuing feeding on the different Se sources, using organic or nano form of Se showed clear significant improvement of BW, BWG and FCR. The presented results clearly showed that among supplemental Se sources using organic or nano products of Se as a source of supplemental Se for broiler chicks improved BW, BWG and FCR than using inorganic NaSe which recorded the worst values (1950, 1896 and 1.63, respectively). Although inclusion 0.30 ppm of Se in broiler diets improved BW, BWG and FCR significantly during grower, finisher and over all experimental periods compared with adding 0.15 ppm, values of FI did not affected during the same periods. The improvement of BW, BWG and FCR were 2.4, 2.6

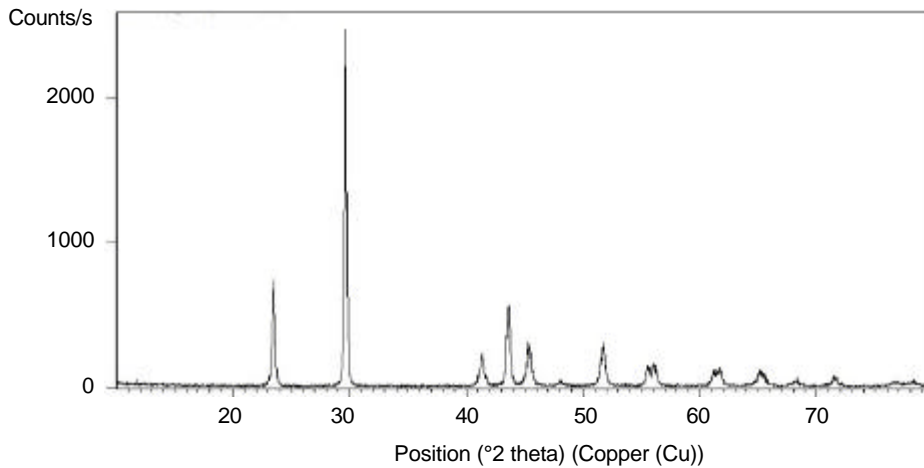


Fig. 4: XRD pattern of SeNPs

and 3.1%, respectively during the experimental period. Among all interactions, chicks in most treatments of organic or nano forms of Se recorded better BWG and FCR during the overall experimental period compared with values recorded by using inorganic NaSe-0.15 group.

**Carcass characteristics:** Effects of different Se sources and levels on carcass characteristics are shown in Table 4. The results of different Se sources showed significant increase in dressing% recorded by chicks fed on L-Nano Se (72.05%) compared with other Se sources. Using L-Nano Se caused 2.8% improvement of dressing% compared with using inorganic form of Se (NaSe). The same trend recorded by increasing Se supplementation from 0.30 to 0.15 ppm. Among experimental treatments only using L-Nano Se-0.30 could improve dressing% (73.78%) than other groups which meaning 5.4% improvement than that of NaSe-0.15 group. On the other side neither values of giblets% nor abdominal fat% were affected by supplemental Se source, Se level or their interactions. Although the obtained results of liver% showed significant reduction due to using organic or nano forms of Se compared to using inorganic NaSe form, increasing Se level from 0.15 to 0.30 ppm could not cause any significant effect on liver%. The interactions between Se sources and Se levels were significant and the highest liver% recorded by using NaSe-0.30 (2.84%) followed by NaSe-0.15 (2.58%) compared with the other of experimental treatments (ranged from 2.30 to 2.51%).

**Tissue examinations:** The presented results of Se concentrations in liver and thigh samples and MDA of

thigh muscles in Table 4, showed significant increase of Se concentration in both liver and thigh tissues by using organic or nano forms of Se comparing with sodium selenite inorganic form or by elevating Se supplementation level from 0.15 to 0.30 ppm. The highest Se concentration of liver recorded by using Zn-Se-Meth and L-Nano Se in broiler diets, while Zn-Se-Meth caused the highest Se concentration in thigh muscles followed by L-Nano Se. On the other side using NaSe as a source of Se resulted in the lowest Se concentration in both liver and thigh tissues. Among interactions between main factors Zn-Se-Meth-0.30 and L-Nano Se-0.30 showed the superior values of Se concentration. Generally, liver samples showed higher Se concentrations than thigh muscle samples. The determined values of MDA concentrations in thigh muscles did not respond supplemental Se sources, Se levels or their interactions. However, Zn-Se-Meth-0.30 and L-Nano Se-0.30 showed the lowest numerical values compared with that determined by NaSe-0.15 samples (2.58, 2.43 vs 2.94 nmol/ml, respectively).

## DISCUSSION

The recorded non significant differences in growth performance parameters during starting period was in match with that reported previously by Srimongkol *et al.* (2004) who studied the effect of adding 0.2 mg inorganic or organic Se/kg broiler diets and could not detect differences in broiler performance during first two weeks of age while adding the organic form of Se enhanced performance parameters during growing, finishing and overall periods. Furthermore, many workers reported improvement of growth performance parameters of broiler chicks as BW, BWG and FCR when fed on Se-

Table 3: Effect of different Se sources and levels on growth performance parameters of broiler chicks at 40 days of age

Treatments	Starter	Grower	Finisher	Overall	Starter	Grower	Finisher	Overall	Starter	Grower	Finisher	Overall	Starter	Grower	Finisher	Overall	
<b>Effect of selenium source</b>																	
NaSe	211	872 <sup>a</sup>	1950 <sup>b</sup>	1078 <sup>b</sup>	1896 <sup>b</sup>	217	1017	1864	138	154 <sup>a</sup>	173 <sup>a</sup>	1.63 <sup>a</sup>	1.38	1.48 <sup>a</sup>	1.65 <sup>a</sup>	1.57 <sup>b</sup>	
Se-Yeast	216	911 <sup>a</sup>	2040 <sup>a</sup>	1129 <sup>a</sup>	1987 <sup>a</sup>	228	1033	1868	140	148 <sup>b</sup>	1.65 <sup>b</sup>	1.58 <sup>b</sup>	1.40	1.48 <sup>b</sup>	1.65 <sup>b</sup>	1.57 <sup>b</sup>	
Zn-Se-Meth	215	895 <sup>ab</sup>	2039 <sup>a</sup>	1144 <sup>a</sup>	1985 <sup>a</sup>	220	1004	1911	137	147 <sup>b</sup>	1.67 <sup>b</sup>	1.59 <sup>b</sup>	1.37	1.47 <sup>b</sup>	1.67 <sup>b</sup>	1.58 <sup>b</sup>	
P-Nano Se	210	882 <sup>b</sup>	2012 <sup>a</sup>	1126 <sup>a</sup>	1959 <sup>a</sup>	218	1015	1899	139	151 <sup>ab</sup>	1.68 <sup>b</sup>	1.59 <sup>b</sup>	1.39	1.51 <sup>ab</sup>	1.68 <sup>b</sup>	1.59 <sup>b</sup>	
L-Nano Se	208	887 <sup>b</sup>	2022 <sup>a</sup>	1135 <sup>a</sup>	1969 <sup>a</sup>	222	1018	1921	142	150 <sup>ab</sup>	1.69 <sup>b</sup>	1.60 <sup>b</sup>	1.42	1.50 <sup>ab</sup>	1.69 <sup>b</sup>	1.60 <sup>b</sup>	
SEM	±1.25	±4.38	±10.83	±8.25	±10.84	±1.75	±3.70	±12.47	±0.012	±0.007	±0.009	±0.007	±0.012	±0.007	±0.009	±0.007	
Probability	N.S	0.01	0.01	0.04	0.01	N.S	N.S	N.S	N.S	0.01	0.03	0.002	N.S	0.01	0.03	0.001	0.002
<b>Effect of selenium level (ppm)</b>																	
0.15	211	881 <sup>b</sup>	1988 <sup>b</sup>	1107 <sup>b</sup>	1935 <sup>b</sup>	225	1016	1892	142	152 <sup>a</sup>	1.71 <sup>a</sup>	1.62 <sup>a</sup>	1.36	1.48 <sup>b</sup>	1.66 <sup>b</sup>	1.57 <sup>b</sup>	
0.3	213	898 <sup>a</sup>	2035 <sup>a</sup>	1136 <sup>a</sup>	1983 <sup>a</sup>	218	1019	1890	136	148 <sup>b</sup>	1.66 <sup>b</sup>	1.57 <sup>b</sup>	1.36	1.48 <sup>b</sup>	1.66 <sup>b</sup>	1.57 <sup>b</sup>	
SEM	±1.25	±4.38	±10.83	±8.25	±10.84	±1.75	±3.70	±12.47	±0.012	±0.007	±0.009	±0.007	±0.012	±0.007	±0.009	±0.007	
Probability	N.S	0.02	0.01	0.04	0.01	N.S	N.S	N.S	N.S	0.01	0.01	0.001	N.S	0.01	0.01	0.001	0.001
<b>Interaction between selenium source and level</b>																	
NaSe-0.15	207	862 <sup>b</sup>	1904 <sup>b</sup>	1042 <sup>b</sup>	1850 <sup>b</sup>	223	1019	1858	145	156 <sup>a</sup>	1.78 <sup>a</sup>	1.68 <sup>a</sup>	1.45	1.56 <sup>a</sup>	1.78 <sup>a</sup>	1.68 <sup>a</sup>	
NaSe-0.30	215	882 <sup>bc</sup>	1996 <sup>ab</sup>	1114 <sup>a</sup>	1943 <sup>ab</sup>	213	1013	1871	132	152 <sup>bc</sup>	1.68 <sup>bc</sup>	1.59 <sup>bc,d</sup>	1.45	1.52 <sup>bc</sup>	1.68 <sup>bc</sup>	1.59 <sup>bc,d</sup>	
Se-Yeast-0.15	213	893 <sup>bc</sup>	1999 <sup>ab</sup>	1106 <sup>b</sup>	1946 <sup>ab</sup>	234	1031	1865	145	151 <sup>abc</sup>	1.69 <sup>bc</sup>	1.61 <sup>bc</sup>	1.45	1.51 <sup>abc</sup>	1.69 <sup>bc</sup>	1.61 <sup>bc</sup>	
Se-Yeast-0.30	217	928 <sup>a</sup>	2080 <sup>a</sup>	1152 <sup>a</sup>	2027 <sup>a</sup>	222	1036	1883	135	145 <sup>d</sup>	1.63 <sup>d</sup>	1.55 <sup>d</sup>	1.35	1.45 <sup>d</sup>	1.63 <sup>d</sup>	1.55 <sup>d</sup>	
Zn-Se-Meth-0.15	216	886 <sup>b</sup>	2018 <sup>a</sup>	1133 <sup>a</sup>	1965 <sup>ab</sup>	225	1000	1912	138	150 <sup>cd</sup>	1.69 <sup>bc</sup>	1.60 <sup>bc</sup>	1.38	1.50 <sup>cd</sup>	1.69 <sup>bc</sup>	1.60 <sup>bc</sup>	
Zn-Se-Meth-0.30	214	904 <sup>ab</sup>	2060 <sup>ab</sup>	1155 <sup>a</sup>	2006 <sup>ab</sup>	219	1008	1910	136	146 <sup>d</sup>	1.65 <sup>cd</sup>	1.56 <sup>cd</sup>	1.36	1.46 <sup>d</sup>	1.65 <sup>cd</sup>	1.56 <sup>cd</sup>	
P-Nano Se-0.15	212	895 <sup>bc</sup>	2042 <sup>ab</sup>	1148 <sup>a</sup>	1989 <sup>ab</sup>	222	1016	1934	140	149 <sup>cd</sup>	1.68 <sup>bc</sup>	1.59 <sup>bc,d</sup>	1.40	1.49 <sup>cd</sup>	1.68 <sup>bc</sup>	1.59 <sup>bc,d</sup>	
P-Nano Se-0.30	209	870 <sup>c</sup>	1982 <sup>b</sup>	1104 <sup>ab</sup>	1930 <sup>b</sup>	214	1015	1865	137	154 <sup>ab</sup>	1.69 <sup>bc</sup>	1.60 <sup>bc</sup>	1.37	1.54 <sup>ab</sup>	1.69 <sup>bc</sup>	1.60 <sup>bc</sup>	
L-Nano Se-0.15	207	867 <sup>c</sup>	1975 <sup>bc</sup>	1108 <sup>ab</sup>	1922 <sup>c</sup>	220	1015	1914	143	154 <sup>ab</sup>	1.72 <sup>ab</sup>	1.63 <sup>ab</sup>	1.43	1.54 <sup>ab</sup>	1.72 <sup>ab</sup>	1.63 <sup>ab</sup>	
L-Nano Se-0.30	210	907 <sup>ab</sup>	2069 <sup>a</sup>	1162 <sup>a</sup>	2016 <sup>a</sup>	224	1020	1929	142	147 <sup>cd</sup>	1.66 <sup>cd</sup>	1.57 <sup>cd</sup>	1.42	1.47 <sup>cd</sup>	1.66 <sup>cd</sup>	1.57 <sup>cd</sup>	
SEM	±1.25	±4.38	±10.83	±8.25	±10.84	±1.75	±3.70	±12.47	±0.012	±0.007	±0.009	±0.007	±0.012	±0.007	±0.009	±0.007	
Probability	N.S	0.01	0.001	0.02	0.001	N.S	N.S	N.S	N.S	0.003	0.01	0.0001	N.S	0.003	0.01	0.0001	0.0001

a, b, c, d: Means in the same column with different superscripts, differ significantly (p<0.05); N.S: Not Significant (p>0.05); SEM: Standard Error of Means

Table 4: Effect of different Se sources and levels on carcass characteristics (% live body weight) and Se concentration and MDA of some broiler tissues

Treatments	----- Carcass characteristics (% of live body weight) -----				-- Se Concentration (ppm) --		
	Dressing	Giblets	Liver	Abdominal fat	Liver	Thigh muscle	MDA of thigh muscle (nmol/ml)
<b>Effect of selenium source</b>							
NaSe	70.10 <sup>b</sup>	4.85	2.71 <sup>a</sup>	1.54	4.535 <sup>d</sup>	2.081 <sup>a</sup>	2.868
Se-Yeast	70.61 <sup>b</sup>	4.69	2.42 <sup>b</sup>	1.75	5.791 <sup>e</sup>	2.416 <sup>b</sup>	2.760
Zn-Se-Meth	70.39 <sup>b</sup>	4.73	2.43 <sup>b</sup>	1.63	7.068 <sup>a</sup>	3.630 <sup>a</sup>	2.603
P-Nano Se	70.46 <sup>b</sup>	4.91	2.46 <sup>b</sup>	1.59	6.385 <sup>b</sup>	2.983 <sup>b</sup>	2.688
L-Nano Se	72.05 <sup>a</sup>	4.87	2.40 <sup>b</sup>	1.68	7.196 <sup>a</sup>	3.373 <sup>b</sup>	2.610
SEM	±0.221	±0.049	±0.035	±0.044	±0.292	±0.162	±0.059
Probability	0.01	N.S	0.02	N.S	< 0.0001	< 0.0001	N.S
<b>Effect of selenium level (ppm)</b>							
0.15	70.34 <sup>b</sup>	4.78	2.47	1.58	5.280 <sup>b</sup>	2.314 <sup>b</sup>	2.780
0.3	71.12 <sup>a</sup>	4.84	2.50	1.70	7.110 <sup>a</sup>	3.479 <sup>a</sup>	2.631
SEM	±0.221	±0.049	±0.035	±0.044	±0.292	±0.162	±0.059
Probability	0.02	N.S	N.S	N.S	< 0.0001	< 0.0001	N.S
<b>Interaction between selenium source and level</b>							
NaSe-0.15	70.00 <sup>b</sup>	4.66	2.58 <sup>ab</sup>	1.49	4.46 <sup>f</sup>	1.84 <sup>f</sup>	2.940
NaSe-0.30	70.20 <sup>b</sup>	5.04	2.84 <sup>a</sup>	1.59	4.61 <sup>ef</sup>	2.316 <sup>b</sup>	2.796
Se-Yeast -0.15	70.45 <sup>b</sup>	4.61	2.32 <sup>b</sup>	1.77	4.51 <sup>f</sup>	1.92 <sup>f</sup>	2.826
Se-Yeast -0.30	70.78 <sup>a</sup>	4.77	2.50 <sup>b</sup>	1.74	7.06 <sup>bc</sup>	2.910 <sup>a</sup>	2.693
Zn-Se-Meth-0.15	70.24 <sup>b</sup>	4.75	2.46 <sup>b</sup>	1.43	6.91 <sup>e</sup>	2.846 <sup>b</sup>	2.626
Zn-Se-Meth-0.30	70.55 <sup>b</sup>	4.72	2.41 <sup>b</sup>	1.83	7.22 <sup>b</sup>	4.413 <sup>a</sup>	2.580
P-Nano Se-0.15	70.70 <sup>a</sup>	5.00	2.49 <sup>b</sup>	1.76	5.65 <sup>d</sup>	2.613 <sup>d</sup>	2.726
P-Nano Se-0.30	70.22 <sup>b</sup>	4.82	2.44 <sup>b</sup>	1.46	7.11 <sup>bc</sup>	3.353 <sup>b</sup>	2.650
L-Nano Se-0.15	70.32 <sup>b</sup>	4.88	2.51 <sup>b</sup>	1.42	4.86 <sup>f</sup>	2.343 <sup>b</sup>	2.783
L-Nano Se-0.30	73.78 <sup>a</sup>	4.83	2.30 <sup>b</sup>	1.89	9.53 <sup>a</sup>	4.403 <sup>a</sup>	2.436
SEM	±0.221	±0.049	±0.035	±0.044	±0.292	±0.162	±0.059
Probability	0.001	N.S	0.04	N.S	< 0.0001	< 0.0001	N.S

a,b,c,d,e,f: Means in the same column with different superscripts, differ significantly (p<0.05); N.S: Not Significant (p>0.05); SEM: Standard Error of Means

yeast as organic form of Se or when used nano Se, as obtained in this study (Sevcikova *et al.*, 2006; Dlouha *et al.*, 2008; Upton *et al.*, 2008; Fu-xiang *et al.*, 2008; Zhou and Wang, 2011). Upton *et al.* (2008) added organic Se at level of 0.2 mg/kg of broiler diet and recorded improvement of growth performance parameters of chicks at 40 days of age. The same trend during feeding phases recorded by Zhou and Wang (2011) when investigated the effect of adding different levels of nano Se (0, 0.1, 0.3 and 0.5 mg/kg diet) on growth performance of Chinese chicken strain and recorded improved BWG and FCR of overall experimental period. They concluded that the best supplementing level of nano Se was 0.3 mg/kg diet. However Cai *et al.* (2012), Choct *et al.* (2004), Mei-Sheng (2005), Payne and Southern (2005) and Niu *et al.* (2009) could not record improvement of growth performance of broiler chicks when used organic or nano form of Se in broiler diets. These results might be associated with the high dietary levels of Se in control diet which masked the effect of supplemental Se (Zhou and Wang, 2011). The presented results in this study showed 2.36 and 3.1% improvement in final body weight and FCR by increasing level of supplemental Se from 0.15 to 0.30 ppm, respectively and these results in match with those reported previously by Naylor *et al.* (2000), Stolic *et al.* (2002), Fu-xiang *et al.* (2008) and Zhou and Wang (2011) when added organic or nano form of Se to broiler diet.

In addition the obtained results of dressing% of broilers at the end of experiment showed clear increase (2.1-2.8%) by using L-Nano Se compared with other Se sources and this confirmed the reported results by Fu-xiang *et al.* (2008) who added nano Se, while organic forms of Se failed to improve dressing% as Edens (2001) and Naylor *et al.* (2000).

The reported significant increase of Se concentration in thigh muscles and liver by either increasing supplemental level from 0.15 to 0.30 ppm, or by using organic or nano form of Se was in the same side of results of Zhou and Wang (2011), Hu *et al.* (2012) and Cai *et al.* (2012). Where using nano Se compared with NaSe in broiler diets. Surai (2006) reported that indeed increase of Se concentration in breast muscles of broilers when organic Se was at level of 0.20 ppm compared with the same dose of NaSe according to the results of Kricova *et al.* (2003). The same trend of Se concentration in chicken liver or broiler muscles was reported by Payne and Southern (2005) and Pan *et al.* (2007) when adding Se-yeast to broiler diets. Sevcikova *et al.* (2006) stated that increased content of Se in broiler diets significantly increased its content in thigh muscle tissue, which is in accordance with our results. Kralik *et al.* (2012, 2013) reported that increased amount of selenium in broiler feed affected its better deposition in muscle tissue of breasts and thigh.

The higher determined Se concentration of liver than that of thigh muscles was explained previously by Zhou and



Wang (2011) who suggested that there is a limit for chicken muscle deposition of Se within a certain range of added nano Se. At a certain level of nano Se, selenoenzymes have already been fully saturated and cells may alter to control the Se deposition. However the accumulation of Se in liver of Chinese chicken was dose dependent.

On the same trend of the results of this study, Kralik *et al.* (2013) investigated the effect of using organic Se in broiler diets (up to 0.5 ppm) on the Se content and lipid oxidation of thigh muscle tissue. They reported significant increase of Se content of thigh tissue from 0.511 to 1.071 ppm of dry matter, by increasing supplemental organic Se in feed. While shows that the increase of Se content in broiler feed did not have effects on the intensity of lipid oxidation in thigh muscle tissue ( $p>0.05$ ).

The overall experimental results were in harmony, where obtained results of growth performance parameters and dressing% were in agreement and confirmed by the determined Se concentrations in liver and thigh tissues. The overall results showed higher ability of organic and nano forms of Se than sodium selenite (inorganic form) in improving growth performance and carcass characteristics. Early explanation by Huang *et al.* (2003) showed that nano Se has a size-dependent effect. With more details, Wang *et al.* (2007) and Zhang *et al.* (2008) explain this improvement due to using nano form of Se by the novel characteristics such as a large surface area, high surface activity, high catalytic efficiency, strong adsorbing ability, high bioavailability and low toxicity. Recently, Wang and Fu (2012) studied the transport and uptake of sodium selenite, selenomethionine and nano Se by broiler intestine cells and found that the transportation of selenomethionine and nano Se from intestine cells were higher than that of sodium selenite and the highest uptake efficiency ( $p<0.05$ ) was observed in cells treated with nano Se.

Both of Se-Yeast and zinc-L-selenomethionine which mainly contain Se in the form of selenomethionine are organic forms of Se (Schrauzer, 2000). Absorption of selenomethionine is accelerated by the specific amino acid active transport mechanisms in the gut mucosa. Sodium selenite is absorbed more slowly, possibly by simple diffusion through the intestinal mucosa, than the amino acid-bound selenium compounds (Reasbeck *et al.* 1985). Following the absorption, selenomethionine is readily incorporated into tissue proteins in a non-specific and unregulated manner. While sodium selenite is inorganic form of Se incorporated into a body pool which is used for functional forms of Se and appears to be under homeostatic regulation (Thomson, 1998). Sodium selenite have a quite limited impact on the Se content in animal products, because Se can be retained only by incorporated into the selenocysteine protein (Olivera *et al.*, 2005).

The overall experimental results showed that using Se-Yeast or Zn-Se-Meth as organic forms of Se, or L-Nano Se as nano form of Se at level of 0.30 ppm in broiler diets or its equivalent in drinking water, respectively, is more effective to get better growth performance and quality of broiler meat. But further studies about the safety of using nano form of selenium as feed additives are needed.

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