

Original Research Article

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Nanoselenium as a Growth Promoting Feed Supplement in Broiler Chickens

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ABSTRACT

Selenium is an essential nutritional trace element having vital role in immunoprotection, antioxidant defence system, muscle metabolism, growth and reproduction. Hence dietary selenium supplementation in the poultry has been regularly practiced using the inorganic and organic forms. These forms have the limitations of having narrow margin of safety and non specific binding to tissue proteins, hence an alternate form of selenium i.e. nano selenium having greater potential as poultry and livestock feed supplement with higher bioavailability, higher margin of safety and seven fold lower acute toxicity was prepared using starch, ascorbic acid and bovine serum albumin. The nanoselenium (15-40 nm) synthesized were characterized by XRD analysis, transmission electron microscopy and UV spectrophotometry. This nanoselenium was used for the biological trial in broiler chickens owing to simplicity and easy adaptability for large scale production. The treatment groups were supplemented with 0.3 mg/kg sodium selenite (T2), 0.3 mg/kg organic selenium (T3), nanoselenium at three levels viz.0.15 (T4), and 0.3 (T5) and 0.6 mg/kg (T6) and T1 group was the control, fed with the basal diet alone. The weekly body weight of individual bird was recorded upto the end of sixth week. The mean body weight gain (g) recorded at the end of 6th week were 2050.35, 2108.40, 2117.58, 2136.50 and 2141.65 for the groups T2 to T6, respectively when compared to 2030.60 of T1 group. The mean body weight gain at the end of 6th week was higher (p<0.05) in the organic and nanoselenium supplemented groups compared to control. All the nanoselenium supplemented groups (T4, T5 and T6) showed significantly higher (p<0.05) body weight gain than T1 from 4th week onwards. The T5 and T6 groups supplemented with nanoselenium at 0.3 mg/kg and 0.6 mg/kg showed significantly (p<0.05) increased body weight gain than the groups T1, T2, T3 and T4. The mean body weight gain of T3 did not differ from T4. Hence supplementing the nanoselenium (0.3 – 0.6mg/kg diet) resulted in increased body weight in broiler chickens with minimal risk of toxicity and better bioavailability.

Keywords

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Introduction

Intensive rearing of broiler chickens faces challenges such as high metabolic demand, disease susceptibility, physical stress and inadequate micronutrients and trace minerals. These stressors are responsible for causing immunosuppression. Incompetency of the immune system increases susceptibility to various infectious diseases resulting in decreased productive performance of the birds. These stress factors disrupt the delicate critical balance existing between antioxidant defense system and repair mechanisms in tissues, thus generating free radicals leading to tissue damage. Higher level of free radical accumulation in tissues damages the cell membrane and DNA and thereby depresses growth rate (Morrissey and O' Brien, 1998).

Glutathione peroxidase, thioredoxin reductase, reduced glutathione and tissue thiols are endogenous antioxidant systems which provide defense against free radicals in tissues. Owing to the faster growth rate in broiler chickens, production of endogenous antioxidant systems is insufficient to scavenge the free radicals. Hence, various synthetic and natural antioxidants are being added in the poultry diet (Godin and Wohaiieb, 1988).

Trace minerals like selenium are added to the diet of broiler chickens to enhance the antioxidant, immune status and overall growth performance.

Selenium has a wide range of biological functions in the body (NRC, 1994). Dietary antioxidants such as selenium is important in protecting against the free radical generation, oxidative stress and tissue damage as it is a vital component of selenoenzymes such as glutathione peroxidase, phospholipid peroxidase and thioredoxin reductase (Arner and Holmgren, 2000; Surai, 2000).

Currently, sodium selenite (Na_2SeO_3) and selenate (Na_2SeO_4) as inorganic form, and selenium enriched yeast (SY) as organic form are being used as commercial selenium supplements in animal and poultry diets (FDA, 2002).

Compared to organic form of selenium, the inorganic selenium salts have inherent toxicity and low bioavailability. Because of these properties, inorganic selenium salts cannot be added in the diet more than the recommended level, though more selenium is required during stress and disease conditions. Hence, the organic selenium in the form of SeMet and selenium enriched yeast is used in nutritional supplements due to their excellent bioavailability and lower acute toxicity among various selenium forms (Schrauzer, 2003). But as SeMet can be nonspecifically incorporated into proteins in place of methionine, concerns have been raised that SeMet could potentially cause accumulation of selenium in tissues to toxic levels (Waschulewski and Sunde, 1988). Thus, exploring a cost effective selenium source with high bioavailability and low toxicity is necessary.

In this regard, nanotechnological approaches in trace mineral nutrition allows intervention in the absorption process in biological system as well as enhances bioavailability (Hilty *et al.*, 2010). Nanoparticles have higher surface activity, high catalytic efficacy, strong adsorbing ability and have extensive domino effect (Xu *et al.*, 2003). Nanoselenium exhibits novel properties different from other selenium sources and possess equal efficacy compared to organic form of selenium. Similarly, the toxicity of nanoselenium is seven times lower than that of inorganic selenium and three times lower than that of organic selenium (Peng *et al.*, 2007). Currently, nano elemental selenium (nanoSe), is used in nutritional supplements and has

promising applications in medical therapy (Zhang *et al.*, 2001 and Gao *et al.*, 2002). Nanoselenium improved the growth performance, feed conversion efficiency (Zhou and Wang, 2011) and antioxidant enzyme activity (Cai *et al.*, 2012) in broiler chickens. The range between optimal and toxic dietary levels of nanoselenium was wider than that of sodium selenite, and also, nanoselenium was more efficiently absorbed in the body than sodium selenite (Hu *et al.*, 2012).

Furthermore, Zhang *et al.*, (2005) reported that nanoSe possessed higher efficiency than selenite, selenomethionine, and methyl-selenocysteine (Zhang *et al.*, 2008; Wang *et al.*, 2007) in upregulating selenoenzymes in mice and rats and exhibited lesser toxicity (Zhang *et al.*, 2001).

Even though studies have conducted earlier to assess the effect of selenium in poultry, there is only little research done to study the influence of nanoselenium on growth performance of broiler chickens. Consequently, the aim of the present study was to determine the effects of dietary supplementation of nanoselenium on production performance of broiler chickens and to compare its efficacy with the inorganic and organic forms.

Materials and Methods

Preparation and characterisation of nanoselenium

Nanored selenium particles were synthesized as per the method described by Zhang *et al.*, (2001) with slight modification using sodium selenite, starch, ascorbic acid and bovine serum albumin. The compositional analysis of the samples were studied based on the energy dispersive analysis of X-Rays using PANalytical X-Ray diffractometer (JEOL

Model JED-2300). Samples for transmission electron microscopy (TEM) analysis were prepared by drop-coating selenium nanoparticles solution on to carbon-coated copper TEM grids. Transmission electron micrographs were obtained on JEM- 2100F (JEOL Inc., Japan) instrument with an accelerating voltage of 80 kV.

A biological trial was conducted with one hundred and eighty numbers of day-old straight broiler chicks (Vencobb 400) obtained from commercial hatchery. The birds were wing banded, weighed and randomly allotted to six groups with three replicates of ten chicks each based on the body weight. The birds were reared in cages under uniform standard managerial practices upto six weeks of age.

Sodium selenite and Selplex™ (Alltech, USA) were used as inorganic and organic selenium supplement forms in the diets. The nanoselenium synthesized in the department of Veterinary Physiology, Veterinary College and research Institute Namakkal was used in the experimental diets. The size of the nanoselenium was found to lie in the range of 15-40 nm as characterised by X Ray diffractational analysis and Transmission electron microscopic studies. The diets were formulated according to the standards prescribed in Bureau of Indian Standards (BIS, 1992) and fed to the birds as per the following schedule.

Broiler prestarter, starter and finisher diets were fed *ad libitum* to the birds from 1 to 14, 15 to 28 and 29 to 42 days of age, respectively. At the end of the experiment (42nd day), six birds per treatment group were randomly selected and slaughtered. At the end of the experiment (42nd day), six birds per treatment group were randomly selected and slaughtered by halal method.

Growth performance

During the course of experiment the data on growth performance parameters viz., body weight, feed consumption and feed efficiency were collected as follows.

Body weight (Weekly Cumulative)

The body weights all the chicks were weighted individually on day one and at the end of each week. For entire six weeks of the trial the body weight gain was calculated.

Feed consumption (Weekly cumulative)

The feed consumption in each replicate was recorded weekly by subtracting the weight of residual feed from the total quantity of feed supplied during the respective week.

Feed conversion ratio

At the end of every week, feed intake was recorded and mean daily feed consumption per bird and feed conversion ratio of each group were calculated.

The feed conversion ratio (FCR) expressed as the relationship between amount of feed consumed (kg) to the body weight gain (kg) under each treatment of birds was determined. The FCR was calculated by using the following formula,

$$\text{Feed conversion ratio} = \frac{\text{Average weekly feed consumption per bird during the week(Kg)}}{\text{Average body weight gain per bird during the week (Kg)}}$$

Statistical methods

The completely randomized design method was followed for the experiment (Snedecor and Cochran, 1994) and the data collected were analysed using SPSS® 20.0 software package. Post-hoc analysis was done by Tukey honestly significance difference test.

Results and Discussion

Growth performance

Body weight gain

The effect of supplementation of different sources of selenium on the mean body weight gain (g) of broiler chicken from 1 to 6 weeks of age is presented in Table 1 and Fig. 1.

The mean body weight gain (g) recorded at the end of 6th week were 2050.35, 2108.40, 2117.58, 2136.50 and 2141.65 for the groups T2 to T6, respectively when compared to 2030.60 of T1 group.

The mean body weight gain at the end of 6th week was higher ($p < 0.05$) in the organic and nanoselenium supplemented groups compared to control. All the nanoselenium supplemented groups (T4, T5 and T6) showed significantly higher ($p < 0.05$) body weight gain than T1 from 4th week onwards. The T5 and T6 groups supplemented with nanoselenium at 0.3 mg/kg and 0.6 mg/kg showed significantly ($p < 0.05$) increased body weight gain than the groups T1, T2, T3 and T4. The mean body weight gain of T3 did not differ from T4.

These findings are in agreement with Zhou and Wang (2011) and Hu *et al.*, (2012) who observed that the final body weight and daily weight gain were significantly ($p < 0.05$) increased in broiler chickens supplemented with nanoselenium (0.3 mg to 0.6 mg/kg).

Choct *et al.*, (2004) and Upton *et al.*, (2008) also reported an improved body weight and feed conversion ratio in broiler chicken fed organic selenium (0.2 ppm) compared to broilers fed inorganic selenium (0.2 ppm) and control group (no selenium supplementation).

Table.1 Mean (\pm SE) body weight gain (g) of broiler chickens fed inorganic, organic and nanoselenium

Treatment groups	Weekly body weight gain (g)					
	I	II	III	IV	V	VI
T1- standard diet	94.37 \pm 1.80	264.99 ^a \pm 7.05	618.23 ^a \pm 12.36	958.60 ^a \pm 15.75	1500.17 ^a \pm 16.08	2030.60 ^a \pm 23.65
T2 - standard diet + 0.3mg inorganic Se/kg	94.89 \pm 1.45	273.17 ^{ab} \pm 4.19	626.82 ^a \pm 11.45	983.28 ^{ab} \pm 9.81	1518.18 ^{ab} \pm 17.77	2050.35 ^{ab} \pm 13.39
T3 - standard diet + 0.3mg organic Se /kg	95.28 \pm 1.73	276.53 ^{ab} \pm 4.95	630.08 ^a \pm 9.63	1013.56 ^{bc} \pm 8.76	1566.66 ^{ab} \pm 19.91	2108.40 ^{bc} \pm 10.95
T4 - standard diet + 0.15mg nanoSe /kg	96.19 \pm 1.22	287.94 ^{bc} \pm 5.15	656.55 ^{ab} \pm 8.19	1022.92 ^{bc} \pm 14.36	1588.58 ^b \pm 20.80	2117.58 ^{bc} \pm 17.42
T5 - standard diet + 0.3mg nanoSe /kg	98.65 \pm 1.38	292.06 ^{bc} \pm 3.96	674.48 ^b \pm 12.35	1040.17 ^c \pm 14.91	1684.47 ^c \pm 21.79	2136.50 ^c \pm 25.36
T6 - standard diet + 0.6 mg nanoSe /kg	99.24 \pm 1.34	300.18 ^c \pm 5.43	679.95 ^b \pm 9.25	1056.7 ^c \pm 14.96	1686.30 ^c \pm 19.65	2141.65 ^c \pm 17.47

Means within the same column bearing different superscripts differ significantly (p<0.05)

Table.2 Mean (\pm SE) weekly and cumulative feed consumption (g) of broiler chickens fed inorganic, organic and nanoselenium

Treatment groups	Weekly feed consumption (g)						Cumulative feed consumption (g)
	I	II	III	IV	V	VI	
T1- standard diet	91.93 \pm 1.03	265.27 \pm 1.94	474.63 ^c \pm 0.70	654.23 ^d \pm 2.28	935.40 ^c \pm 0.95	1069.00 ^c \pm 0.29	3490.47 ^d \pm 3.92
T2 - standard diet + 0.3mg inorganic Se/kg	91.90 \pm .21	260.47 \pm 4.32	469.87 ^{ab} \pm 0.84	647.83 ^d \pm 0.60	933.67 ^{bc} \pm 0.68	1059.83 ^d \pm 1.30	3463.57 ^d \pm 5.12
T3 - standard diet + 0.3mg organic Se /kg	89.97 \pm 1.13	259.80 \pm 1.74	469.80 ^{ab} \pm 1.65	626.53 ^c \pm 1.74	923.83 ^{abc} \pm 0.73	1050.00 ^c \pm 1.44	3419.93 ^c \pm 6.33
T4 - standard diet + 0.15mg nanoSe /kg	90.40 \pm 1.95	256.60 \pm 0.45	469.87 ^{ab} \pm 0.84	616.03 ^{bc} \pm 3.78	920.37 ^{ab} \pm 5.61	1039.70 ^b \pm 3.21	3392.97 ^{bc} \pm 10.2
T5 - standard diet + 0.3mg nanoSe /kg	89.53 \pm 1.16	255.40 \pm 1.80	468.47 ^a \pm 1.53	607.23 ^{ab} \pm 2.74	913.60 ^a \pm 3.95	1038.53 ^{ab} \pm 2.82	3372.77 ^{ab} \pm 7.68
T6 - standard diet + 0.6 mg nanoSe /kg	87.77 \pm 0.37	255.73 \pm 1.88	467.80 ^a \pm 1.17	605.07 ^a \pm 0.58	912.67 ^a \pm 1.86	1030.50 ^a \pm 0.29	3359.53 ^a \pm 1.12

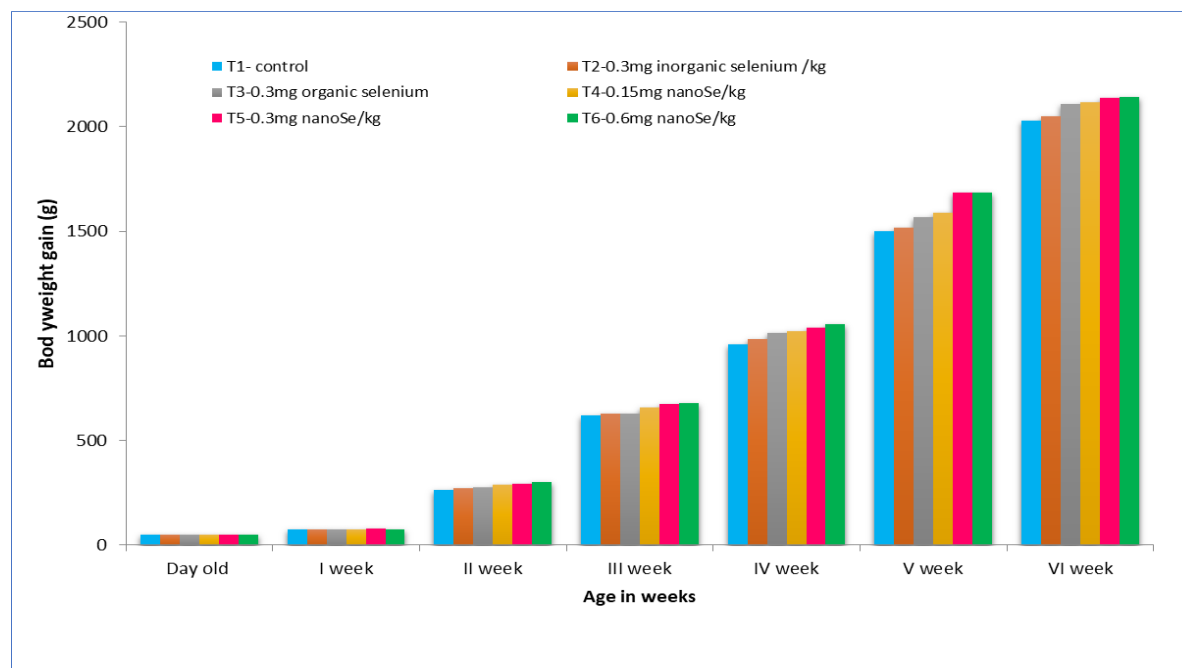
Means within the same column bearing different superscripts differ significantly (p<0.05)

Table.3 Mean (\pm SE) feed conversion ratio of broiler chickens fed inorganic, organic and nanoselenium

Treatment groups	Weekly feed conversion ratio					
	I	II	III	IV	V	VI
T1 - standard diet	1.24 \pm 0.05	1.35 ^b \pm 0.06	1.35 ^b \pm 0.04	1.55 ^c \pm 0.05	1.62 ^c \pm 0.03	1.72 ^b \pm 0.03
T2 - standard diet +0.3 mg inorganic Se/kg	1.23 \pm 0.03	1.29 ^{ab} \pm 0.02	1.31 ^{ab} \pm 0.03	1.50 ^{bc} \pm 0.03	1.58 ^{bc} \pm 0.03	1.69 ^b \pm 0.02
T3 - standard diet +0.3 mg organic Se/kg	1.20 \pm 0.03	1.27 ^{ab} \pm 0.05	1.30 ^{ab} \pm 0.02	1.43 ^{abc} \pm 0.02	1.51 ^{bc} \pm 0.04	1.62 ^{ab} \pm 0.03
T4 - standard diet + 0.15 mg nanoSe/kg	1.17 \pm 0.04	1.21 ^{ab} \pm 0.03	1.24 ^{ab} \pm 0.02	1.40 ^{ab} \pm 0.03	1.48 ^{ab} \pm 0.02	1.60 ^{ab} \pm 0.04
T5 - standard diet + 0.3mg nanoSe /kg	1.14 \pm 0.05	1.18 ^{ab} \pm 0.01	1.21 ^a \pm 0.02	1.37 ^a \pm 0.01	1.39 ^a \pm 0.04	1.58 ^{ab} \pm 0.03
T6 - standard diet +0.6 mg nanoSe/kg	1.11 \pm 0.01	1.15 ^a \pm 0.05	1.20 ^a \pm 0.03	1.34 ^a \pm 0.01	1.38 ^a \pm 0.02	1.57 ^a \pm 0.02

Means within the same column bearing different superscripts differ significantly (p<0.05)

Fig.1 Mean body weight gain (g) of broiler chickens fed inorganic, organic and nanoselenium



However, Cai *et al.*, (2012) reported that there was no significant increase in growth performance in nanoselenium supplemented (0.3 to 2.0 mg/kg) chicken. Similarly, Yoon *et al.*, (2007) and Rao *et al.*, (2013) reported no difference in body weight in comparison to control, when broiler chickens were fed diets containing 0.2 to 0.4 ppm organic selenium.

Selenium is a component of glutathione peroxidase enzyme (GSH-Px) (Rotruck *et al.*, 1973), which is an antioxidant enzyme that catalyzes the reduction of hydrogen peroxide and lipid hydroperoxides and thereby destroys the free radicals produced during normal metabolic activity. Selenium participates in various physiological functions, mostly as an integral component of a range of selenoproteins. Selenoproteins play an important role in thyroid hormone metabolism. Iodothyronine deiodinases (ID) are selenoenzymes responsible for converting the inactive thyroxine (T₄) into an active form -3,5,3'-triiodothyronine (T₃) (Arthur *et al.*, 1990). Thyroid hormones play an important role in growth and protein turnover. The level of selenium affects the growth of chickens via thyroid hormone metabolism. Dietary selenium supplementation increased plasma T₃, whereas decreased T₄. Hepatic 5' deiodinase activity was elevated by selenium (Schmidt and Reavill, 2008).

Selenium is essential for the optimum functioning of the immune system. It has beneficial effects on both the innate and adaptive (cell mediated and antibody mediated) immune responses (Brown and Arthur, 2001) and influences the immune system at several mechanistic levels. Selenium via selenoproteins influences inflammatory responses by regulating the oxidative state of immune cells. Not only does the immune system rely on the antioxidant properties of Se to control oxidative bursts by phagocytic cells, it also has specific endocrine effects that benefit

antibody-mediated immunity. Lymphocyte proliferation was enhanced by Se supplementation due to the role of Se in the modulation of the arachidonic acid metabolism (Cao *et al.*, 1992).

Thus, the significantly increased body weight in the nanoselenium supplemented broiler chickens in the present study could be related to the higher antioxidant status, higher T₃ hormone, increased T₃/T₄ activation ratio, and immune status, in these groups compared to the control.

Feed Consumption and Feed Conversion Ratio

The effect of inorganic, organic and nanoselenium supplementation on the mean weekly and cumulative feed consumption and feed efficiency of broiler chickens from 1 to 6 weeks of age are presented in Table 2 and 3.

The mean weekly feed consumed (g) recorded at the end of first two weeks did not differ significantly between the unsupplemented and selenium supplemented groups. The mean cumulative feed consumed (g) at the end of 6th week was 3463.57, 3419.93, 3392.97, 3372.77 and 3359.53 for the groups T2 to T6, respectively when compared to 3490.47 in group T1.

The mean cumulative feed consumption of nanoselenium supplemented groups (T4, T5 and T6) was significantly ($p < 0.05$) decreased in comparison to control, inorganic and organic selenium supplemented birds. Moreover, throughout the trial period the weekly feed consumption of 0.6 mg/kg nanoselenium supplemented group (T6) was significantly ($p < 0.05$) decreased when compared to all other treatment groups and control. The feed consumption of T3 and T4 decreased significantly ($p < 0.05$) from T1 and T2.

The mean feed conversion ratio at the end of 6th week was 1.69, 1.62, 1.60, 1.58 and 1.57 in the treatment groups T2 to T6, respectively as compared to 1.72 in the control birds.

The mean feed efficiency was better in 0.6 mg/kg nano Se chickens in comparison to inorganic selenium groups and control. However, the feed efficiency did not differ in the birds supplemented with either inorganic selenium or those without any selenium supplementation. Similarly, the feed efficiency did not differ in the organic and nanoselenium (0.15 and 0.3 mg/kg) supplemented chickens.

These results concurred with the findings of Zhou and Wang (2011) and Hu *et al.*, (2012) who reported that the feed efficiency was better in chickens supplemented with 0.3 mg/kg and 0.5 mg/kg of nanoselenium. Similarly, Deniz *et al.*, (2005) and Naylor and Choct (2000) observed improved ($p < 0.01$) feed conversion ratio from the 1st to the 42nd day when broiler birds were supplemented with 0.25 ppm of organic Se in comparison to sodium selenite supplemented groups and control.

On the contrary, Cai *et al.*, (2012) reported no change in the feed efficiency in the chickens supplemented with nanoselenium. Payne and Southern (2005) and Peric *et al.*, (2009) also observed insignificant effect by selenium supplementation on feed efficiency in broiler chickens.

The decrease in feed consumption and improved feed efficiency in nanoselenium and organic selenium supplemented groups could be attributed to the marked hypercellularity and increase in the intestinal villi length and width when compared to the other groups. This increase in the intestinal epithelial surface area would have aided in enhanced absorption and assimilation of nutrients in the gastrointestinal tract, thus finally contributing

to better growth performance seen in these birds.

In conclusion, selenium is pharmacologically active and optimisation of selenium nutrition of poultry will result in increased efficiency of egg and meat (Surai, 2000). The form of selenium influences the bioavailability and activity. Selenium nanoparticles show special characteristics of transport, exhibit higher absorption efficiencies and function. Thus, it could be concluded that the mean body weight gain (g) of broiler chicken supplemented with nano Se at 0.15, 0.3 and 0.6 mg/kg was significantly ($p < 0.05$) increased than the control birds. Chickens supplemented with nanoselenium at 0.15 mg/kg and 0.3 mg/kg of organic selenium had similar body weight gain. Increasing the nanoselenium supplementation from 0.3 to 0.6 mg/kg did not cause any further increase in the body weight gain. Overall, the nanoselenium supplemented in the range 0.15-0.6 mg/kg increased body weight gain of broiler chickens significantly ($p < 0.05$) when compared to the control birds.

The mean cumulative feed consumption of all the nanoselenium supplemented groups was significantly ($p < 0.05$) decreased in comparison to control and inorganic selenium supplemented birds. The mean feed efficiency was better in 0.6 mg/kg nanoSe chickens in comparison to inorganic selenium groups and control. The mean feed efficiency was better in organic and nanoselenium supplemented chickens than the control and the 0.6 mg/kg nanoselenium supplemented group showed highly efficient feed conversion.

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