Int.J.Curr.Microbiol.App.Sci (2017) *6*(5): 340-347

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 5 (2017) pp. 340-347**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.605.039

Influence of Dietary Nanoselenium Supplementation on the Meat Characteristics of Broiler Chickens

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Keywords

Chicken, Nanoselenium, Meat characteristics, pH, Drip loss, Lipid peroxidation

Article Info

Accepted:
04 April 2017
Available Online:
10 May 2017

A B S T R A C T

Dietary selenium supplementation in the poultry has been regularly practiced using the inorganic and organic forms to enhance the growth and antioxidant defence system. 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 for purity, morphology and size by XRD analysis, transmission electron microscopy and UV spectrophotometry. To investigate the role of selenium forms and levels on the meat characteristics of broiler chickens, a biological trial was conducted in one hundred and eighty day old straight run chickens, divided into six treatment groups each having three replicates. The treatment groups were supplemented with 0.3 mg sodium selenite /kg (T2), 0.3 mg organic selenium /kg (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 birds were slaughtered at the end of 42nd day and breast meat characteristics - pH, drip loss and lipid peroxidation were estimated. The results of the study indicated that the nanoselenium supplemented chickens had significant ($p<0.05$) reduction in breast muscle drip loss and lipid peroxidation as compared with the control. The selenium levels and forms did not influence the pH of breast muscle both at 24 and 48 hrs. Thus nanoselenium (0.3 – 0.6mg /kg diet) can be fed to the broiler chickens to reduce the drip loss and lipid peroxidation and thereby enhance the meat properties.

Introduction

Selenium is a dietary essential trace mineral having plethora of biological functions in the living system (NRC, 1994). Se research has attracted tremendous interest because of its important role in antioxidant selenoproteins for protection against oxidative stress initiated by excess reactive oxygen species (ROS) and reactive nitrogen species (NOS). Animals obtain selenium directly by ingestion of the plants or indirectly via intake of selenium containing dietary components of plants or animal origin or by dietary supplementation (Whanger, 2002). Due to uneven uptake by plants and loss during feed processing and storage selenium needs to be supplemented in the poultry and livestock feeds. Food and

Drug Administration, USA (FDA, 1987) approved the use of selenium as sodium selenate or selenite in poultry feed at levels of 0.3 mg/kg. Hence, using inorganic selenium has significant limitations that include potential toxicity, poor absorption, interaction with other minerals and dietary components, storage loss, low efficiency of transfer to meat and eggs, inability to supply and maintain selenium reserve in the body. Thus, the use of sodium selenite is recently being debated (Surai, 2000; Pehrson, 1993).

To overcome these limitations of inorganic selenium, in the past decade, 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). Selenomethionine is more toxic during long term consumption, owing to non-specific retention in proteins. The presence of excess selenium analogs of sulphur containing enzymes and structural proteins play a role in avian teratogenesis (Spallholz and Hoffman, 2002). Also the supplementation of selenomethionine is too expensive to be used in animal feeds (Sager, 2006).

Currently, nano elemental selenium (nanoSe) is used in nutritional supplements and has been advocated for applications in medical therapy (Zhang *et al.,* 2001 and Gao *et al.*, 2002).

Nano-elemental Se has attracted widespread attention due to its high bioavailability and low toxicity because nanometer particulates exhibit novel characteristics, such as great specific surface area, high surface activity, a lot of surface active centers, high catalytic efficiency and strong adsorbing ability and low toxicity of routine Se0 (Wang *et al.*, 2007; Zhang *et al.,* 2008). Since surface area-to-volume ratio increases with decreasing particle size, selenium nanoparticles have high biological activity (Zhang *et al.,* 2005), including anti-hydroxyl radical property (Gao *et al.,* 2002) and a protective action against the oxidation of DNA (Huang *et al.,* 2003). Furthermore, Zhang *et al.,* (2005) reported that nanoSe possessed higher efficiency than selenite, selenomethionine, and methylselenocysteine (Zhang *et al.,* 2008; Wang *et al.,* 2007) in upregulating selenoenzymes in mice and rats and exhibited lesser toxicity (Zhang *et al.,* 2001).

The antioxidant systems in the body contain numerous antioxidant enzymes, such as superoxide dismutase (SOD), thioredoxin peroxidase (TPx) and glutathione peroxidase (GSH-Px), GPX and numerous nonenzymatic substances to protect the body from oxidative stress (Flohé, 2010).Selenium forms an intregral part of nearly 30 selenoproteins in the body. Selenium prevents the cellular damage caused by oxidative stress by being a component of important antioxidant enzymes found in most of the body tissues such as glutathione peroxidase and thioredoxin peroxidase. Se improves the meat quality through its antioxidant ability to protect against deteriorative reactions during lipid peroxidation.

Drip-loss and lipid peroxidation are the major cause for economic problem in the broiler industry, especially for companies marketing pieces of chicken and processed products. Northcutt *et al.*, (1994) estimated that drip loss can account for more than 3 % of the total yield of cut-up chicken. Mahan 1996 suggested that excessive cellular damage resulting from oxidation may be the cause of

drip loss. The supplementation of selenium especially organic selenium improves the meat quality and shelf life of poultry meat (Sevcikova *et al.,* 2006). Increasing the dietary selenium improved the selenium status and retention of the muscle and oxidative stability of chicken meat during refrigerated storage (Yoon *et al.,* 2007; Smet *et al.,* 2008) Surai (2002) reported that GSH-Px contributes significantly to the overall antioxidant defence of muscle in broilers: moreover, organic selenium supplementation of the diet could achieve to decrease tissue susceptibility to lipid peroxidation and increase oxidative stability of skeletal muscle.

Even though studies have conducted earlier to assess the effect of selenium on the meat characteristics in poultry, there is only little research done to study the influence of nanoselenium on broiler chicken meat characteristics. Consequently, the aim of the present study was to determine the effects of dietary supplementation of nanoselenium on carcass drip-loss and lipid peroxidation in 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.,* (2004) 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 managemental practices up to 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 15-40nm as characterised by X Ray diffraction 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.

Treatment groups	**Diets**
T1(control)	Standard diet with no selenium supplementation
T2	Standard diet + 0.3mg sodium selenite/kg feed
T3	Standard diet + 0.3mg organic selenium (Selplex™)/kg feed
T4	Standard diet + 0.15mg nanoselenium/kg feed
T5	Standard diet + 0.3mg nanoselenium/kg feed
T6	Standard diet + 0.6mg nanoselenium/kg feed

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 (42^{nd} day), six birds per treatment group were randomly selected and slaughtered. The breast muscle samples were collected and stored at -20°C to study the meat characteristics.

Assessment of meat characteristics

pH

The pH value of the meat was measured by using pH meter at 24 and 48 h after slaughter (AOAC, 1975). Briefly, ten grams of meat sample was blended with 90 ml of distilled water in a blender for 2 min, filtered and then pH of the filtrate was determined by digital pH-meter (Systronics, India).

Drip loss

Drip loss in the breast meat was measured according to the method of Rasmussen and Andersson (1996). Briefly, breast muscle was trimmed to 3 × 3 × 3 cm size, blotted to remove the surface water and the initial weight was taken using weighing balance. Samples were then placed in a plastic bag filled with air, fastened to avoid evaporation and kept at 4°C. The final weight was determined at 24 and 48 h after slaughter. Percentage of drip loss was calculated using the formula

$$\text{Drip Loss (\%)} = \frac{\text{(Initial weight of the meat sample – Final weight of the meat sample)}}{\text{Initial weight of the meat sample}} \times 100$$

Lipid peroxidation

Thiobarbituric acid (TBA) value as a measure of lipid peroxidation was estimated by extraction method described by Witte *et al.*, (1970). Briefly, to 2 g of meat sample, 10 ml of chilled 20 per cent TCA was added and homogenized in vortex mixer for 2 min and filtered. 3 ml of filterate was taken in test tubes and 3 ml of 0.1 per cent TBA reagent was added and placed in boiling water bath for 35 min. Then the tubes were allowed to cool and optical density was read at 530 nm in spectrophotometer. The TBA value was expressed as mg malondialdehyde / kg of meat. Standards were prepared by using 1, 1, 3, 3, tetra-ethoxy propane (TEP) (Merck, India); for this 0.3055 g of 1,1,3,3, TEP was dissolved in 100 ml of 95 per cent alcohol. This solution contained 1 mg malondialdehyde per ml. Working standard solution ranged from 0.2 to 1.0 µg malondialdehyde per ml.

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

Meat characteristics

pH and drip loss

The effect of inorganic, organic and nanoselenium supplementation on the meat characteristics of broiler chickens is presented in table 1.

The mean pH at both 24 and 48 h did not differ significantly between any of the treatments, although lower pH values were recorded in meat of control group than the selenium treated birds. The pH showed an increasing trend at 48 h irrespective of the selenium source and dose. Similar results

were reported by Yang *et al*., (2012) who observed that different forms of dietary selenium did not affect pH values of breast meat.

The mean drip loss (%) of breast muscle at 48 h was 2.86, 1.81, 1.42, 1.37 and 1.24 in the treatment groups (T2, T3, T4, T5 and T6) as compared to 2.98 in the control.

The mean drip loss (%) in the breast muscle of organic (T3) and all nanoselenium supplemented groups (T4, T5 and T6) decreased significantly ($p<0.05$) than the control and inorganic selenium supplemented groups at both 24 and 48 h. The lowest drip loss (%) at both 24 and 48 h was recorded in T6 group. These results were in agreement with Zhou and Wang (2011) and Cai *et al*., (2012) who observed lesser drip loss in nanoselenium supplemented chicken (0.3 - 0.6 mg/kg) than the control. Similarly, Wang *et al*., (2011) reported that compared with the control group, 0.15 mg/kg Se supplemented group had the significantly lesser ($p<0.05$) drip loss in the breast muscle at 24 and 48 h after slaughter but the effects were more noticeable ($p<0.05$) in the 0.15 mg/kg SeMet group when compared to the sodium selenite supplemented group. Downs *et al*., (2000) recorded that drip loss of chicken breast muscle was reduced by 17 per cent when sodium selenite was replaced by organic selenium to supply between 0.1 and 0.3 ppm selenium. Both Edens (2001) and Naylor *et al*., (2000) reported that birds receiving dietary organic Se had significantly lesser drip loss ($p<0.01$) than those receiving inorganic selenium.

Thus, it could be inferred from the results that birds supplemented with the nanoselenium and organic selenium showed higher glutathione peroxidase activities and total antioxidant capacity in the serum and tissues compared to the inorganic selenium supplemented groups and control and as a result, drip loss was decreased in the birds fed organic and nanoselenium. The improved antioxidant status promoted the maintenance of cell membrane integrity (Cheah *et al*., 1995) which might have resulted in reduced drip loss.

Table.1 Mean (±SE) meat characteristics in broiler chickens fed inorganic, organic and nanoselenium

Treatment groups	pH		Drip loss (%)		MDA (g/kg)
	24 h	48 h	24 h	48 h	24 h
T1 - standard diet	5.72± 0.04	5.81± 0.03	2.47^{b}± 0.12	2.98^{b}± 0.06	1.33^{d}± 0.05
T2 - standard diet + 0.3mg inorganic Se/kg	5.73± 0.09	5.82± 0.11	2.37^{b}± 0.19	2.86^{b}± 0.16	1.31^{d}± 0.04
T3 - standard diet + 0.3mg organic Se /kg	5.77± 0.10	5.85± 0.11	1.61^{a}± 0.10	1.81^{a}± 0.09	1.17^{c}± 0.03
T4 - standard diet + 0.15mg nanoSe /kg	5.92± 0.10	6.00± 0.08	1.19^{a}± 0.17	1.42^{a}±0.18	1.15^{c}± 0.03
T5 - standard diet + 0.3mg nanoSe /kg	6.00± 0.08	6.05± 0.08	1.15^{a}± 0.22	1.37^{a}±0.15	1.01^{b}± 0.02
T6 - standard diet + 0.6 mg nanoSe /kg	6.05± 0.04	6.10± 0.03	1.03^{a}± 0.14	1.24^{a}± 0.16	0.95^{a}± 0.01

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

Lipid peroxidation

The lipid peroxidation, as measured by malonaldehyde formation (mg/kg) is presented in table 1. The inorganic selenium supplementation did not cause any reduction in the lipid peroxidation over the control in the breast muscle. The lipid peroxidation was significantly ($p<0.05$) decreased in meat samples in the organic selenium and nanoselenium supplemented groups as compared to the control and sodium selenite supplemented group.

The results concurred with the observations of Azar *et al*., (2010) who found decreased lipid peroxidation in breast meat when sodium selenite was replaced with selenium enriched yeast. Similarly, lipid peroxidation was decreased by chlorella enriched yeast supplementation in broiler chicken meat after 0, 3 and 5 days in cold storage (Sevcikova *et al.,* 2006).

In conclusion selenium is an important component of the selenoprotein enzyme GSH-Px in animal tissues (Arthur, 2000). The GSH-Px family of enzymes is a crucial player in the integrated antioxidant system, neutralizing potential threats to the integrity of cellular macromolecules by eliminating hydrogen peroxide and detoxifying lipid hydroperoxides.

Meat oxidation could decrease the sensitivity to hydrolysis, weaken protein degradation, and reduce water reserves among the myofibrils, thus which would increase the juice loss of the meat (Huff-Lonergan and Lonergan, 2005). Thus, factors such as GSH-Px activity, which affect the oxidation state of myofibrillar protein, would reduce the drip loss by improving the integrity of cell membranes (Wang *et al.,* 2009). The drip loss and lipid peroxidation data obtained in the present study suggests that Nano-Se might be associated with an antioxidative process that reduced postmortem deteriorating changes in the compromised cell membranes and breast meat as a whole.

In general, nanoselenium (0.3-0.6mg /kg) supplemented chickens had significant reduction in breast muscle drip loss and lipid peroxidation as compared with the control and other forms selenium. These results also suggest that nanoselenium is of the greater value to improve the meat quality and extend the shelf life of fresh meat than sodium selenite and Se-Met in broiler chickens.

Acknowledgement

The authors wish to thank the Dean, Veterinary College and Research Institute, Namakkal and Tamilnadu Veterinary and Animal Sciences University for providing necessary funds and research facilities to carry out the study.

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How to cite this article:

Visha, P., K. Nanjappan, P. Selvaraj, S. Jayachandran and Thavasiappan, V. 2017. Influence of Dietary Nanoselenium Supplementation on the Meat Characteristics of Broiler Chickens. *Int.J.Curr.Microbiol.App.Sci.* 6(5): 340-347. doi: http://dx.doi.org/10.20546/ijcmas.2017.605.039