Impact of biogenic nanoscale metals Fe, Cu, Zn and Se on reproductive LV chickens

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Received 5 April 2015
Accepted for publication 3 June 2015
Published 21 July 2015

Abstract
Using biogenic nanoscale metals (Fe, Cu, ZnO, Se) to supplement into diet premix of reproductive LV (a Vietnamese Luong Phuong chicken breed) chickens resulted in certain improvement of poultry farming. The experimental data obtained showed that the farming indices depend mainly on the quantity of nanocrystalline metals which replaced the inorganic mineral component in the feed premix. All four experimental groups with different quantities of the replacement nano component grew and developed normally with livability reaching 91 to 94%, hen’s bodyweight at 38 weeks of age and egg weight ranged from 2.53–2.60 kg/hen and 50.86–51.55 g/egg, respectively. All these farming indices together with laying rate, egg productivity and chick hatchability peaked at group 5 with 25% of nanoscale metals compared to the standard inorganic mineral supplement, while feed consumption was lowest. The results also confirmed that nanocrystalline metals Fe, Cu, ZnO and Se supplemented to chicken feed were able to decrease inorganic minerals in the diet premixes at least four times, allowing animals to more effectively absorb feed minerals, consequently decreasing environmental pollution risks.

Keywords: nanoscale metal powders, LV chickens, hatchability, livability

Classification numbers: 2.10, 4.04

1. Introduction

In poultry feed, there are usually some inorganic salts of metals such as iron, copper, zinc and selenium with essential concentration to sustain the development of livestock. These metals are necessary for development and growth of poultry as well as for formation of egg shell. However, absorption of these inorganic salts by animals (especially poultry) is limited, leading to excretion of these metals in poultry feces, increasing waste and contribution to environmental pollution. According to Petrovich [1], inorganic minerals in sulfate form were absorbed up to only 20%, while the ultradispersed mineral complex and nanoscale metal powder preparations were absorbed up to 80–90%.

In recent years, the dramatic development of technology for metal nanomaterials has been affecting significantly animal feed production [2–5]. These metal nanoparticles (NPs) have a large surface area to associate easily with...
various organic molecules, to form complexes such as chelates which are capable of forming very small particle size. So, these particles are easily absorbed by an animal’s digestive system. Many studies [6–9] have indicated that nanocrystalline metals assist fast growth and enhance production of animals. The synthesized nanoscale metals will presumably replace inorganic minerals (sulphates and oxides) in animal diet, although under the form of sulfate salts or chelates inorganic minerals have been applied widely [10, 11].

In Vietnam, most minerals supplemented into animal feed are in the form of inorganic salts that lead to low feeding efficiency, causing waste and environmental pollution. Recently, there have been some studies [7, 9, 11, 12] which prefer the application of nanoscale metals instead of inorganic minerals for animal feed. Presumably, introducing nanoscale metal preparations into poultry farming is essential in improving the meat and egg productivity.

This study was aimed to assess the possible effects of nanoscale metals, viz Fe, Cu, Co and Se, on growth and quality performance of chickens.

### Table 1. Experimental design.

| Farming indices                | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 |
|-------------------------------|---------|---------|---------|---------|---------|---------|
| LV chickens (heads)           | 200     | 200     | 200     | 200     | 200     | 200     |
| Replicate number              | 3       | 3       | 3       | 3       | 3       | 3       |
| Total LV chickens (heads)     | 600     | 600     | 600     | 600     | 600     | 600     |
| Microelements supplemented in to LV chickens’ diet |         |         |         |         |         |         |
| Fe (mg/kg)                    | 60      | 60      | 9       | 12      | 15      | 18      |
| Cu (mg/kg)                    | 8       | 8       | 1.2     | 1.6     | 2       | 2.4     |
| Zn (mg/kg)                    | 65      | 40      | 9.75    | 13      | 16.25   | 19.5    |
| Se (mg/kg)                    | 0.10    | 0.12    | 0.015   | 0.02    | 0.025   | 0.03    |
| ME (kcal/kg of food)          |         |         |         |         |         |         |
| Protein (%)                   |         |         |         |         |         |         |

*Group 1: microelements were supplemented using 100% of inorganic minerals according to the poultry nutritional demands of American standard (NAS Washington dc—1984), as 1st control group,
Group 2: microelements were supplemented (as organic minerals) according to the Bayer Company’s Regulations, as 2nd control group,
Group 3: the poultry diet was supplemented with nanoscale metals by 15% compared to the 1st control group,
Group 4: the poultry diet was supplemented with nanoscale metals by 20% compared to the 1st control group,
Group 5: the poultry diet was supplemented with nanoscale metals by 25% compared to the 1st control group,
Group 6: the poultry diet was supplemented with nanoscale metals by 30% compared to the 1st control group.

### Table 2. Nutrition values of the parent LV chickens’ diet.

| Farming indices                | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 |
|-------------------------------|---------|---------|---------|---------|---------|---------|
| Raw protein (%)               | 17.5    | 17.5    | 17.5    | 17.5    | 17.5    | 17.5    |
| Energy ME (kcal/kg)           | 2.750   | 2.750   | 2.750   | 2.750   | 2.750   | 2.750   |
| Calcium (%)                   | 3.20    | 3.20    | 3.20    | 3.20    | 3.20    | 3.20    |
| Phosphor TS (%)               | 0.60    | 0.60    | 0.60    | 0.60    | 0.60    | 0.60    |
| Lysine TS (%)                 | 0.8     | 0.8     | 0.8     | 0.8     | 0.8     | 0.8     |
| Methionine (%)                | 0.4     | 0.4     | 0.4     | 0.4     | 0.4     | 0.4     |

Observation of the farming indices up to 38 weeks: livability, body weight, egg weight, laying ratio (%), egg productivity/hen/38 weeks of age, feed consumption/10 eggs; biochemical, physiological and hatching indices.

2. Materials and methods

2.1. Materials

The experiment was conducted on LV chickens at the age of 24 weeks (3600 heads). Nanoscale metal powders of Fe, Cu, ZnO and aqueous solution of nano Se with average particle size of 20–90 nm were produced by chemical method [13].

2.2. Experimental methods

In the pre-laying stage, the experimental LV chickens were looked after following the farming and disease preventing veterinary procedures of Thuy Phuong Poultry Research Center with equal nutritional diets. At the end of the pre-laying period, LV chickens were divided randomly into 6 experimental groups with 3 replicates.

The experiment was arranged by using the completely randomized design method with one factor (different levels of metal NPs). Incorporation of the metal NPs onto nutritional diet premixes was conducted by spraying them with a sonicated aqueous nanoscale metals mixture in the presence of 2%...
starch as described in table 1, while table 2 presents nutrition values of the parent LV chickens’ diet.

EDAX spectrum of a chicken diet premix supplemented with nanoscale metals (Fe, Cu, ZnO and Se) (group 5) was presented in figure 1(a) in comparison with the premix without nanoscale metal supplementation (figure 1(b)). The appearance of characteristic K-peaks of iron and zinc, regardless of the absence of copper and selenium peaks due to their much smaller concentration, confirmed the presence of the supplemented microelements in the chicken diet premix.

2.3. Data processing methods

Experimental data were collected daily and processed by the statistical software Minitab version 16. The experimental results presented in the tables are mean values of 3 replicates.

3. Results and discussion

3.1. Livability

Livability is an important technico-economic index in the general livestock and in particular poultry. This index is not only the evaluation of farming process and management, but also the assessment of productivity and adaptability of each chicken line, breed [14].

Table 3 shows that the livability of parent LV chicken from 25 to 38 weeks of age for all groups ranged from 91–94%. The highest livability (94%) was found in group 5 with 25% of nanoscale metal powders used in respect of the standard diet mineral components, while the lowest livability (91%) was found in group 2 (as 2nd control group). Our results were in good agreement with those of Tran et al [15].
using organic mineral supplement, where livability of reproductive LV chickens ranged from 90.64 to 92.56%.

3.2. Body weight and egg weight

Body weight and egg weight of parent LV chickens are presented in table 4.

Egg weight of LV chickens using organic minerals peaked in group 2 (51.74 g/egg) and the lowest egg weight (50.86 g/egg) was in group 3 with 15% of nanoscale metal powders used. According to Tran et al [15], egg weight of LV chickens at 38 weeks of age ranged from 55.23 – 56.99 g.

Mature body weight of parent LV chickens at 38 weeks of age was found lowest in group 4 with 20% of nanoscale metal powders used and peaked in group 2 with organic minerals. The egg weight and body weight were not statistically different amongst the groups (P > 0.05).

3.3. Laying performance and egg production

The reproductive performance of LV chickens is presented in tables 5 and 6.

Results presented in table 5 shows that the laying ratio of LV chickens up to 38 weeks of age ranged from 59.2 – 60.05% and peaked in group 5 and equal 60.05%. For group 2 which used organic minerals the laying ratio was 59.66%, while for group 1 using inorganic minerals was a little smaller, 59.2%. Average laying rate between groups was not significantly different (P > 0.05). According to Tran et al [15], the laying percentage of LV chicken at 3rd generation ranged from 53.21 – 59.46%.

The results in table 6 show that the egg production up to 38 weeks of age peaked at 58.85 eggs/hen in group 5 and the lowest value was found in group 1 (58.01 eggs/hen). For the egg production and specific feed consumption values there is no significant difference between experimental groups (P > 0.05). Feed consumption per 10 eggs in group 5 was found the lowest (2.2 kg/10 eggs) while the highest was in group 3 (2.26 kg/10 eggs). According to Tran et al [15], the egg production/hen/38 weeks of age of LV chickens was 59.6 eggs/hen and feed consumption/10 eggs was 2.84 kg/10 eggs.

3.4. Embryonic rate and hatchability

The hatching experiments play an important role in the assessment of fertility and productivity of poultry. This depends on different factors such as quality of eggs, preservation time, incubation solution, chickens’ age and nutrition. Results are presented in table 7.

The hatching ratio presented in the table 7 indicates that, the embryonic rate peaked at in the group 2 (94%) and was the lowest in group 4 (92.1%). The hatching rate per total incubating eggs in group 6 was highest (85.91%). However, the percentage of 1st type chicks peaked at group 5, which was higher than that of group 3 by 2.83% and group 1 by 1.15%. No significant difference between the groups (P > 0.05) was found. Our results in this experiment were similar to those of Tran et al [15] for the three parent LV
### Table 5. Laying performance of LV chickens up to 38 weeks of age (%).

| Week of age | Group1 | Group2 | Group3 | Group4 | Group5 | Group6 |
|-------------|--------|--------|--------|--------|--------|--------|
| 25          | 21.81  | 25.40  | 27.37  | 26.10  | 25.22  | 21.94  |
| 26          | 41.68  | 44.75  | 45.12  | 47.41  | 45.47  | 45.50  |
| 27          | 57.99  | 61.17  | 62.67  | 59.31  | 61.73  | 62.21  |
| 28          | 64.53  | 68.43  | 69.43  | 66.13  | 68.34  | 66.31  |
| 29          | 67.49  | 70.88  | 70.17  | 70.58  | 72.35  | 69.94  |
| 30          | 67.89  | 69.40  | 66.99  | 65.95  | 67.48  | 68.19  |
| 31          | 58.40  | 61.68  | 51.97  | 55.99  | 57.09  | 56.69  |
| 32          | 60.17  | 56.76  | 55.48  | 55.12  | 60.00  | 58.21  |
| 33          | 66.20  | 62.03  | 62.78  | 64.05  | 64.25  | 65.61  |
| 34          | 68.54  | 66.74  | 66.51  | 68.46  | 69.43  | 66.09  |
| 35          | 67.93  | 67.47  | 66.22  | 69.20  | 66.66  | 66.79  |
| 36          | 64.51  | 63.14  | 60.49  | 66.48  | 66.16  | 66.33  |
| 37          | 58.57  | 56.13  | 54.20  | 58.38  | 55.60  | 57.44  |
| 38          | 63.07  | 61.23  | 57.44  | 60.94  | 60.95  | 62.98  |
| **Average** | **59.20**<sup>a</sup> | **59.66**<sup>a</sup> | **58.35**<sup>a</sup> | **59.58**<sup>a</sup> | **60.05**<sup>a</sup> | **59.59**<sup>a</sup> |

<sup>a</sup> Means within row are not significantly different between the groups (P > 0.05).

### Table 6. Egg production and feed consumption/10 eggs of LV chickens up to 38 weeks of age.

| Week of age | Group1 | Group2 | Group3 | Group4 | Group5 | Group6 |
|-------------|--------|--------|--------|--------|--------|--------|
| 25          | 1.53   | 1.78   | 1.92   | 1.83   | 1.77   | 1.54   |
| 26          | 2.92   | 3.13   | 3.16   | 3.32   | 3.18   | 3.19   |
| 27          | 4.06   | 4.28   | 4.39   | 4.15   | 4.32   | 4.35   |
| 28          | 4.52   | 4.79   | 4.86   | 4.63   | 4.78   | 4.64   |
| 29          | 4.72   | 4.96   | 4.91   | 4.94   | 5.06   | 4.90   |
| 30          | 4.75   | 4.86   | 4.69   | 4.62   | 4.72   | 4.77   |
| 31          | 4.09   | 4.32   | 3.64   | 3.92   | 4.00   | 3.97   |
| 32          | 4.21   | 3.97   | 3.88   | 3.86   | 4.20   | 4.07   |
| 33          | 4.63   | 4.34   | 4.39   | 4.48   | 4.50   | 4.59   |
| 34          | 4.80   | 4.67   | 4.66   | 4.79   | 4.86   | 4.63   |
| 35          | 4.75   | 4.72   | 4.64   | 4.84   | 4.67   | 4.68   |
| 36          | 4.52   | 4.42   | 4.23   | 4.65   | 4.63   | 4.64   |
| 37          | 4.10   | 3.93   | 3.79   | 4.09   | 3.89   | 4.02   |
| 38          | 4.41   | 4.29   | 4.02   | 4.27   | 4.27   | 4.41   |
| **Total**   | **58.01**<sup>a</sup> | **58.47**<sup>a</sup> | **57.18**<sup>a</sup> | **58.39**<sup>a</sup> | **58.85**<sup>a</sup> | **58.40**<sup>a</sup> |

<sup>a</sup> Means within row are not significantly different between the groups (P > 0.05).

### Table 7. Embryonic rate and hatchability of LV chickens.

| Farming indices                  | Units     | Group1 | Group2 | Group3 | Group4 | Group5 | Group6 |
|----------------------------------|-----------|--------|--------|--------|--------|--------|--------|
| Total incubating eggs (egg)      |           | 4050   | 3900   | 3900   | 3900   | 4050   | 3900   |
| Total embryonic eggs (egg)       |           | 3778   | 3666   | 3597   | 3592   | 3781   | 3649   |
| Embryonic rate (%)               |           | 93.28  | 94.00  | 92.23  | 92.10  | 93.36  | 93.56  |
| Total chicks (head)              |           | 3458   | 3337   | 3246   | 3299   | 3482   | 3334   |
| Total 1st type chicks (head)     |           | 3394   | 3266   | 3171   | 3221   | 3418   | 3264   |
| Hatchability/embr. eggs (%)      |           | 91.49  | 90.84  | 90.60  | 91.95  | 92.21  | 91.75  |
| Hatchability/incub. eggs (%)     |           | 84.96  | 85.35  | 83.44  | 84.84  | 85.84  | 85.91  |
| 1st type hatchability/embr. eggs (%) |       | 89.54  | 88.89  | 88.47  | 89.80  | 90.58  | 89.83  |
| 1st type hatchability/ incub. eggs (%) |   | 83.18<sup>a</sup> | 83.52<sup>a</sup> | 81.49<sup>a</sup> | 82.87<sup>a</sup> | 84.33<sup>a</sup> | 84.11<sup>a</sup> |

<sup>a</sup> Means within row are not significantly different between groups (P > 0.05).
chicken lines: the hatchability per total incubating eggs of LV chickens ranged from 84.6–85.86%.

3.5. Biochemical and physiological indices of parent LV chickens

Physiological and biochemical indices of poultry blood are quite stable. However, research on these criteria is of importance in the detection, prediction or giving exact conclusions about the production traits of livestock and poultry in particular. The results presented in Table 8 show that the number of red and white blood cells peaked at group 3: 3.17 million/mm$^3$ and 8.06 thousand/mm$^3$, respectively, while these indices in the group 1 were lowest: 2.81 million/mm$^3$ and 4.74 thousand/mm$^3$, respectively.

The content of protein peaked in group 4 with 20% metal nanoparticles used and equal 49.78 g L$^{-1}$ and the lowest content (42.56 g L$^{-1}$) was found in group 1 which was supplemented with inorganic minerals. The content of albumin and globulin peaked in group 4 (18.11 g L$^{-1}$ and 31.67 g L$^{-1}$, respectively), while their lowest content was found in group 1 (16.11 g L$^{-1}$ and 27.44 g L$^{-1}$, respectively).

4. Conclusion

Reproductive LV chickens’ diet using 25% of nanoscale metals with respect to the standard inorganic mineral supplement gave distinct poultry farming effects. Chickens at an age of 38 weeks showed highest livability and laying rate (94% and 60.05%, respectively), egg and 1st type chicks/incubating eggs production were 58.85 eggs/head and 84.33%, respectively, while feed consumption was the lowest. Besides, the obtained results claimed that using nanoscale metal powders instead of inorganic mineral component allows animals to more effectively absorb feed minerals, hence considerably decreasing environmental pollution risks.

Acknowledgments

The authors appreciate the financial support of the Ministry of Science and Technology, Vietnam.

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Table 8. Effect of nanocrystalline metals (Fe, Zn, Cu, Se) on biochemical and physiological indices of LV chickens’ blood.

| Biochem. indices          | Units     | Group1 | Group2 | Group3 | Group4 | Group5 | Group6 |
|---------------------------|-----------|--------|--------|--------|--------|--------|--------|
| Red blood cells           | million/mm$^3$ | 2.81$a$ | 3.00$b$ | 3.17$a$ | 2.84$a$ | 2.94$a$ | 2.88$a$ |
| White blood cells         | thousand/mm$^3$ | 4.74$b$ | 5.21$b$ | 8.06$a$ | 5.52$b$ | 6.23$b$ | 7.81$a$ |
| Protein                   | g/L       | 42.56$a$ | 48.56$a$ | 47.89$a$ | 49.78$a$ | 44.11$a$ | 48.33$a$ |
| Albumin                   | g/L       | 16.11$a$ | 16.56$a$ | 16.67$a$ | 18.11$a$ | 16.56$a$ | 17.00$a$ |
| Globulin                  | g/L       | 27.44$a$ | 30.89$a$ | 31.22$a$ | 31.67$a$ | 31.67$a$ | 31.11$a$ |

$a$ Means within row are not significantly different between the groups (P > 0.05).

$b$ Means within row differ remarkably between the groups (P < 0.05).