Effect of Dietary Supplementation of Trace Elements on the Growth Performance and Their Distribution in the Breast and Thigh Muscles Depending on the Age of Broiler Chickens

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Abstract

This study was designed to determine the effects of dietary supplementation of different doses and forms of Cu, Zn, Fe, Mn and Se on the growth performance and concentration of these elements in the breast and thigh muscle of Ross 308 broiler chickens at the age of 21, 35 and 42 days. The diets for groups 1 and 2 of birds were supplemented with equivalent amounts of trace elements in an inorganic form (Cu sulphate 5 mg·kg⁻¹, Fe sulphate, Zn oxide, Mn oxide 50 mg·kg⁻¹), but sodium selenite or selenized yeast (Sel-Plex) were given at the dose of Se 0.3 mg·kg⁻¹, respectively. Groups 3 and 4 received the same feed as chickens in groups 1 and 2 but with a highly reduced amount of supplemented nutrients in the organic "proteinated" form (Bioplex Cu 2.5 mg·kg⁻¹, Bioplex Fe, Bioplex Zn, Bioplex Mn 10 mg·kg⁻¹), except for selenium which was given at the dose of Se 0.3 mg·kg⁻¹ as sodium selenite or selenized yeast (Sel-Plex). respectively. The diet supplemented with the restricted doses of trace elements in proteinated forms (50% Cu, 20% Fe, Zn, Mn and a regular level of Se) had the same effect on the indexes of growth performance (such as the body weight on days 1, 7, 14, 21, 28, 35 and 42 of life, total feed intake, feed conversion ratio, carcass yield and abdominal fat of chickens) as well as on the concentration of Cu, Fe, Zn and Mn (except for Se) in the breast and thigh muscle of broilers at the age of 21, 35 and 42 days as did the diet with the recommended doses of minerals in the form of inorganic salts. Unlike sodium selenite, the proteinated form of Se (selenized yeast) is able to build a significant deposit of this element in the muscle of broilers at the age of 21, 35 and 42 davs.

Poultry, mineral nutrients, proteinated form, productive indexes, deposition in muscle

There is growing concern how to increase the effectiveness of dietary supplementation of broiler chickens with trace elements on the growth, productive and reproductive performance. The current recommended doses of minerals used in poultry nutrition are taken from the National Research Council documents (Nutrient Requirements for Poultry, 1994) which are based on the results of research conducted in early 1960s and 1970s. Such approach in the nutrition of broilers has the following disadvantages: a) the genetic advancement continually changes the commercial broiler strains, b) inorganic sources of nutrients were routinely used for the dietary supplementation of chickens in those years.

Current experiments on poultry fed with diets enriched with different amounts and forms of Cu, Fe, Zn, Mn and Se show that the doses of organic "proteinated" forms of elements could be significantly reduced compared to their inorganic forms, without compromising the growth performance of broilers (Peric et al. 2006; Nollet et al. 2007; 2008). It is well known that the use of proteinated forms of trace elements increases their bioavailability for the bird organism (Baker et al. 1987; Fly et al. 1989; Wendekind et al. 1990; Van der Klis and Kemme 2002; Cao et al. 2003; Nollet et al. 2005; Lim et al. 2006; Nollet et al. 2008). The feeding study performed by Leeson (2003) showed that the bioavailability of proteinated forms of elements supplemented to the diet for broilers was at least by 30% higher than that of inorganic forms of elements.

204

On the other hand, negative interactions were found between the inorganic forms of trace elements added to the diet (Du et al. 1996; Modal et al. 2007), probably as a result of the competitive inhibition between them during their absorption. In addition, it was concluded that the body retention of inorganic forms of Cu, Fe, Zn, Mn and Se is relatively low (Mohanna and Nys 1998; Van der Klis 1999) and that the excretion of Cu, Zn, Fe and Mn is significantly higher for the inorganic minerals than for the proteinated forms of elements (Nollet et al. 2007; Leeson and Caston 2008).

This study was designed to determine the effects of dietary supplementation of different doses and forms of Cu, Zn, Fe, Mn and Se on the growth performance as well as on the concentration of these elements in the breast and thigh muscle of Ross 308 broiler chickens at the age of 14, 28 and 42 days.

Materials and Methods

Two hundred unsexed Ross 308 broilers, were randomly divided on the day of hatching into 4 groups (n = 50 per group) and fed for 42 days with diets containing different amounts and forms of Cu, Fe, Zn, Mn and Se. The diets were fed *ad libitum* during the whole experimental period for a healthy development of the broiler chickens. The broiler feed "Starter" was fed for days 1 to 21, the broiler feed "Grower" for days 21 to 35 and the broiler feed "Finisher" for days 35 to 42, respectively. The diets and Bioplex Cu, Bioplex Fe, Bioplex Zn, Bioplex Mn and Sel-Plex were prepared and purchased from Alltech Inc., Belgium. All premixes were fortified with phytase and xylanase and a coccidiostatic preparation (Salinomycin) was mixed into the diets for days 1 to 35 of the broiler's life. The composition of diets fed to the broiler chickens during the entire experimental period is presented in Table 1.

During the whole experiment, the broiler chickens in groups 1 and 2 were fed with the diet supplemented with Cu 5 mg·kg⁻¹ DM (Cu sulphate), Fe, Zn, Mn 50 mg·kg⁻¹ DM (Fe sulphate, Zn oxide, Mn oxide) and Se 0.3 mg·kg⁻¹ DM in the form of sodium selenite or selenized yeast, respectively. Groups 3 and 4 received the same feed supplemented with a highly reduced dose of trace elements (except for Se) in the proteinated forms: Cu 2.5 mg·kg⁻¹ DM (Bioplex Cu), Fe, Zn, Mn 10 mg·kg⁻¹ DM (Bioplex Fe, Bioplex Zn, Bioplex Mn) and Se 0.3 mg·kg⁻¹ DM in the form of sodium selenite or selenized yeast (Sel-Plex), respectively.

On the 1st day of life, the broiler chickens were placed in one-level cages. The lighting regime was 23L:1D

Component, g/kg of DM	Starter (day 1-21)	Grower (day 21-35)	Finisher (day 35-42)
Wheat	534.5	566.6	566.6
Peas	100	100	100
Rape seed meal (32% CP)	30	25	25
Soybean meal (46,5% CP, 1.5% fat)	250	220	220
Palm oil	25	35	35
Soya oil	25	25	25
Premix ^a	5	5	5
Limestone	14.5	11.5	11.5
Monocalcium phosphate	8	3.5	3.5
Natuphos 5000G (phytase)	0.1	0.1	0.1
NaCl	2.2	2.2	2.2
NaHCO ₃	2	2	2
L-lysine	1.5	1.5	1.5
DL-methionine	2	2	2
L-threonine	0.5	0.6	0.6

Table 1. Composition of diets fed to the broiler chickens Ross 308 during the entire experimental period

1 kg of basal diet contained: dry matter, 879 g; crude protein, 201 g; crude fat, 73 g; ash, 45 g; crude fibre, 31; ME broiler, 12.31 MJ; lysine, 11.3 g; methionine, 4.8 g; methionine+cystine, 8.2; threonine, 7.6; tryptophan, 2.4 g; valine, 9 g; arginine, 12.9; histidine, 5 g; Ca, 6.8 g; P, 4.5 g; Na, 1.5 g; K, 8.6 g; Cl⁻, 2 g; Cu, 10 mg; Fe, 150 mg; Zn, 35 mg; Mn, 15 mg; Se, 0.1 mg

^aPremix supplied per kg of basal diet: vitamin A, 10000 IU; vitamin D₃, 3000 IU; vitamin E, 20 mg; vitamin K, 3 mg; tiamine, 2 mg; riboflavin, 8 mg; niacin, 15 mg; cholinchlorid, 50 mg; pantothenic acid, 50 mg; pyridoxine, 5 mg; folic acid, 2 mg; cyanocobalamin, 30 µg; biotin, 0.2 mg; I, 2 mg; Co, 1 mg

None of the birds in the treatment groups died during the experiment. The average body weight (BW) of broilers in each group was recorded on days 1, 7, 14, 21, 28, 35 and 42 of life. The total feed intake as well as the feed conversion ratio was calculated at the end of the 42-day experiment. The carcass yield of broilers (% of final BW) and abdominal fat content (in % and g of final BW) were determined.

On days 21, 35 and 42 of experiment, fifteen broiler chickens were slaughtered by the decapitation. Subsequently, samples of breast and thigh muscle within each group were taken and stored at -18 °C till analyzed. After wet mineralization in the microwave oven (Perkin Elmer, MLS 1200), the concentration of copper, zinc and iron in all samples was measured by the flame AAS method and the concentration of manganese was determined by the GFAAS method (Lippo and Sarkela 1995). The concentration of selenium was measured by the same instrument equipped with a hydride generation system according to Bax et al. (1986).

The one-way analysis of variance (ANOVA) with *post hoc* Tukey's multiple comparison test was applied for the statistical analysis between the treatments on days 21, 35 and 42 of life of broiler chickens. Significant changes were found by paired Student's *t*-test comparing the 1st vs. 2nd (day 21 compared to day 35) as well as the 2nd vs. the 3rd sampling (day 35 compared to day 42). The statistical software GrafPad Prism, Version 4.00 (2003) was used in both cases.

Results and Discussion

The diet supplemented with trace elements in proteinated forms that were restricted to 50% Cu, 20% Fe, Zn, Mn and on the regular level of Se had the same effect on the observed indexes of growth performance as follows: body weight at days 1, 7, 14, 21, 28, 35 and 42 of life, final feed intake, feed conversion ratio, carcass yield and abdominal fat as the recommended doses of inorganic elements (Fig. 1 and Table 2). The addition of trace elements (Cu, Fe, Zn, Mn and Se) in the proteinated form to the diet could be reduced to 33% of regular levels as compared to the use of their inorganic salts as the feed supplement, without compromising the growth performance of broilers as afore said.



Fig. 1. The effects of dietary supplementation of the different amounts and forms of Cu, Fe, Zn, Mn and Se on the body weight of broiler chickens. Values are means \pm S.D.; n = 50 in all groups of birds at the age of 1, 7, 14 and 21 days; n = 35 in all groups of birds at the age of 28 and 35 days; n = 20 in all groups of birds at the age of 42 days

The restricted doses of proteinated forms of Cu, Zn, Fe and Mn (except of Se) supplemented to the diet for broilers had the same effect on the amount of these element in both muscles on the 21st, 35th and 42nd day of life of broilers as the recommended doses of their inorganic salts (Tables 3 and 4). Moreover, Bao et al. (2007) found no significant

Index	Group 1	Group 2	Group 3	Group 4
Final body weight (g per bird)	2435 ± 282.7	2297 ± 204.8	2321 ± 204.3	2480 ± 160.8
Total feed intake (g per bird)	3783 ± 145.5	3907 ± 122.9	3780 ± 186.1	3798 ± 157.3
Feed conversion ratio	1.55	1.7	1.7	1.53
Carcass yield (%)	74.05	74.92	73.26	74.15
Abdominal fat (g)	28.7 ± 9.13	33.9 ± 12.26	29.7 ± 15.45	33.6 ± 8.68
Abdominal fat (%)	1.18	1.47	1.28	1.35

Table 2. The effects of supplementation of the diet with different amounts and forms of Cu, Fe, Zn, Mn and Se on the growth performance of broiler chickens after slaughter (day 42 of life)

Results are presented as mean \pm SD, n = 20

differences in the concentration of Cu, Fe, Zn and Mn in the blood plasma and liver of broilers fed with a low-organic diet (2 mg of Cu, 20 mg of Fe, 20 mg of Mn, 20 mg of Zn per kg), a mid-organic diet (4 mg of Cu, 40 mg of Fe, 40 mg of Mn, 40 mg of Zn per kg), a high-organic diet (8 mg of Cu, 80 mg of Fe, 80 mg of Mn, 80 mg of Zn per kg) and an inorganic diet (4 mg of Cu, 40 mg of Fe, 40 mg of Mn, 40 mg of Zn per kg).

On the other hand, the amount of copper, manganese and selenium in the breast and thigh muscle of broilers was found to depend on age. In all groups of birds, the amount of zinc and manganese in both muscles was significantly lower (P > 0.05) and higher (P > 0.001) at the age of 21 days, respectively, than at 35 days. The amount of copper was significantly higher (P > 0.01) at the age of 35 days than at 42 days of life. Mondal et al. (2007) found a

	Index				
	Cu	Fe	Zn	Mn	Se
Day 21					
Group 1	1.04 ± 0.15	4.15 ± 1.83	4.9 ± 0.37	0.17 ± 0.05	$0.07\pm0.01^{\rm A}$
2	1.11 ± 0.28	3.39 ± 1.05	4.72 ± 0.36	0.15 ± 0.01	$0.17\pm0.04^{\scriptscriptstyle B}$
3	0.96 ± 0.16	3.01 ± 1.31	4.68 ± 0.37	0.15 ± 0.05	$0.07\pm0.01^{\rm A}$
4	0.99 ± 0.17	3.42 ± 0.68	4.2 ± 0.39	0.15 ± 0.01	$0.15\pm0.01^{\rm B}$
Day 35					
Group 1	0.95 ± 0.23	3.66 ± 0.72	$6.38 \pm 1.34^{*}$	$0.08 \pm 0.01^{***}$	$0.05\pm0.01^{\rm A}$
2	1.16 ± 0.31	3.19 ± 0.68	$5.22 \pm 0.73^{*}$	$0.07 \pm 0.01^{***}$	$0.16\pm0.01^{\rm B}$
3	1.14 ± 0.22	3.7 ± 1.21	$6.17 \pm 1.91^{*}$	$0.07 \pm 0.01^{***}$	$0.05\pm0.01^{\rm A}$
4	1.09 ± 0.15	3.11 ± 0.63	$5.96 \pm 0.66^{*}$	$0.09 \pm 0.02^{***}$	$0.16\pm0.01^{\rm B}$
Day 42					
Group 1	$0.77 \pm 0.17^{*}$	3.22 ± 0.47	5.43 ± 0.32	0.1 ± 0.01	$0.12\pm 0.02^{\rm A^{***}}$
2	$0.75\pm 0.08^{**}$	3.46 ± 0.82	5.33 ± 0.31	0.09 ± 0.02	$0.30\pm 0.02^{\rm B^{***}}$
3	$0.76 \pm 0.19^{**}$	3.24 ± 0.56	5.36 ± 0.41	0.08 ± 0.01	$0.12 \pm 0.02^{A^{***}}$
4	$0.65 \pm 0.12^{**}$	2.91 ± 0.51	5.4 ± 0.28	0.09 ± 0.01	$0.26 \pm 0.02^{B^{***}}$

Table 3. The effects of supplementation of the diet with different forms and amounts of Cu, Fe, Zn, Mn and Se on the concentration of these trace elements (mg·kg⁻¹ DM) in the breast muscle of broiler chickens at the age of 21, 35 and 42 days

Results are presented as mean \pm SD, n = 15

Significant differences between the experimental groups of broilers at the age of 21, 35 and 42 days: xyP < 0.05; $x^{Y}P < 0.01$; $x^{Y}P < 0.001$

Significant differences between the related experimental groups in the 1st vs. 2nd sampling (day 21 compared with day 35) and the 2nd vs. 3rd sampling (day 35 compared with day 42): *P < 0.05; *P < 0.01; **P < 0.001

	Index				
	Cu	Fe	Zn	Mn	Se
Day 21					
Group 1	1.12 ± 0.40	5.56 ± 0.77	7.73 ± 0.91	0.18 ± 0.04	$0.09 \pm 0.02^{\text{A}}$
2	1.11 ± 0.46	5.49 ± 0.94	8.36 ± 1.60	0.15 ± 0.02	$0.16\pm0.02^{\scriptscriptstyle B}$
3	0.99 ± 0.17	6.53 ± 1.57	8.34 ± 1.07	0.17 ± 0.03	$0.08\pm0.02^{\rm A}$
4	0.96 ± 0.10	6.33 ± 1.07	8.45 ± 1.67	0.15 ± 0.02	$0.16\pm0.01^{\scriptscriptstyle \rm B}$
Day 35					
Group 1	0.92 ± 0.10	5.35 ± 1.92	$10.55 \pm 2.49^{**}$	$0.11 \pm 0.02^{***}$	$0.05\pm0.01^{\rm A}$
2	1.08 ± 0.35	4.34 ± 1.26	$11.62 \pm 2.55^*$	$0.11 \pm 0.01^{***}$	$0.14\pm0.02^{\scriptscriptstyle B}$
3	1.10 ± 0.45	4.96 ± 1.83	$10.90 \pm 2.80^{*}$	$0.13 \pm 0.02^{***}$	$0.07\pm0.01^{\rm A}$
4	1.11 ± 0.29	4.89 ± 1.25	$10.74 \pm 2.10^{*}$	$0.11 \pm 0.02^{***}$	$0.14\pm0.02^{\scriptscriptstyle B}$
Day 42					
Group 1	$0.78 \pm 0.11^{*}$	6.40 ± 1.43	10.54 ± 2.98	0.12 ± 0.04	$0.15\pm 0.03^{\rm A^{***}}$
2	$0.75 \pm 0.05^{*}$	5.42 ± 1.37	12.05 ± 2.63	0.11 ± 0.02	$0.21\pm 0.04^{\rm B^{***}}$
3	$0.74 \pm 0.12^{*}$	5.29 ± 1.37	10.67 ± 2.21	0.10 ± 0.01	$0.12\pm 0.02^{\rm A^{***}}$
4	$0.73 \pm 0.07^{**}$	5.85 ± 1.62	12.19 ± 2.94	0.12 ± 0.02	$0.28\pm 0.02^{\rm B^{***}}$

Table 4. The effects of supplementation of the diet with different form and amount of Cu, Fe, Zn, Mn and Se on the concentration of these trace elements (mg·kg⁻¹ DM) in the thigh muscle of broiler chickens at the age of 21, 35 and 42 days

Results are presented as mean \pm SD, n = 15

Significant differences between the experimental groups of broilers at the age of 21, 35 and 42 days: xyP < 0.05; $x^{Y}P < 0.01$; $x^{Y}P < 0.001$

Significant differences between the related experimental groups in the 1st vs. 2nd sampling (day 21 compared with day 35), and the 2nd vs. 3rd sampling day 35 compared with day 42): *P < 0.05; *P < 0.01; ***P < 0.001

significantly higher concentration of high density lipoprotein cholesterol (P > 0.01) on the day 21 of life compared to 42-day-old broilers which could reflect an increased efficiency of the lipid metabolism. Copper and manganese play an important role in the metabolism of lipids in poultry as demonstrated by Bakilli et al. (1995) and Lu et al. (2006; 2007), respectively. The significantly increased amount of Zn in both muscles could reflect its importance in the nuclei acid metabolism and protein synthesis. Our findings of two times faster increase of the body weight in the 3rd phase of life of broilers (from day 35 to 42 of life) compared to the 1st and 2nd phase (from the day of hatching to day 35 of life) indicate that both last mentioned processes are very effective in the last week of the life of broilers.

On the 21st, 35th and 42nd day, the diet supplemented with selenized yeast significantly increases the concentration of Se in the breast and thigh muscle, contrary to sodium selenite. The apparent advantage of supplementation of poultry feed with the selenized yeast was previously demonstrated by Kuricová et al. (2003), Petrovič et al. (2006), A camovic and Bertin (2006), Pan et al. (2007). Surprisingly, Wang and Xu (2008) found no significant differences in the muscle Se content in chickens whose diets were enriched with the organic or inorganic form of selenium for 21 days. It is generally supposed that the proportion of selenomethionine (SeMet) escaping metabolism to H₂Se is incorporated non-specifically into the proteins of striated muscles which represent over 52-56% of the total body weight in poultry. For this reason the muscle tissue is the most significant body deposit of Se when its proteinated form (selenized yeast naturally containing up to 90% of total Se in the form of SeMet (Schrauzer 2000)) was given to the diet for poultry rather than sodium selenite. As mentioned above, a characteristic feature of the period between days 35 and 42 of life of broilers is the

increased anabolism of muscle tissue which could explain the finding of significantly increased amount of Se (P < 0.001) in both muscles in this phase of life.

In conclusion, the diet supplemented with trace elements in the proteinated form restricted to 50% Cu, 20% Fe, Zn and Mn and on the regular level of Se had the same effect on the body weight on days 1, 7, 14, 21, 28, 35 and 42 of life, total feed intake, feed conversion ratio, carcass yield and abdominal fat of chickens as well as on the concentration of Cu, Fe, Zn and Mn (except for Se) in the breast and thigh muscle of broilers at the age of 21, 35 and 42 days as the recommended doses of inorganic minerals. The results clearly demonstrate that only the proteinated form of Se (selenized yeast) is able to build a significant selenium deposit in the muscle of broilers at the age of 21, 35 and 42 days compared to its inorganic salt (sodium selenite).

Vplyv suplementácie stopových prvkov do diéty na sledované rastové ukazovatele a na ich distribúciu do svaloviny v závislosti na veku broilerových kurčiat

Štúdia sa zaoberá vplyvom dopĺňania Cu, Zn, Fe, Mn a Se do diéty na sledované rastové ukazovatele a ich distribúciu v prsnej a stehnovej svalovine brojlerových kurčiat Ross 308 vo veku 21, 35 a 42 dní. Diéta pre prvú a druhú skupinu bola suplementovaná rovnakým množstvom anorganických foriem prvkov (síran meďnatý 5 mg/kg, síran železitý, oxid zinočnatý, oxid manganistý 50 mg/kg), kým Se bol dotovaný ako seleničitan sodný (1. skupina) alebo selenizované kvasnice (2. skupina) v dávke 0.3 mg/kg. Kŕmna dávka pre tretiu a štvrtú skupinu bola tvorená rovnakou bazálnou diétou, ale organické formy stopových prvkov boli podávané v značne redukovaných množstvách (Bioplex Cu 2.5 mg/kg, Bioplex Fe, Bioplex Zn, Bioplex Mn 10 mg/kg). Selén bol podávaný v rovnakej dávka a formách ako prvým dvom skupinám broilerových kurčiat. Suplementácia redukovaných dávok organických foriem stopových prvkov do diéty pre 3. a 4. skupinu kurčiat mala rovnaký efekt na sledované rastové ukazovatele (živá hmotnosť na 1., 7., 14., 21., 28., 35. a 42. deň, celkový príjem krmiva, konverzia krmiva, výťažnosť a abdominálny tuk), ale aj na koncentráciu Cu, Fe, Zn a Mn (okrem Se) v prsnej a stehnovej svalovine broilerových kurčiat vo veku 21, 35 a 42 dní, ako skrmovanie diéty dopĺňanej doporučovanými dávkami stopových elementov vo forme anorganických solí (1. a 2. skupina). Na rozdiel od seleničitanu sodného, iba selenizované kvasnice vytvorili významné telové zásoby Se vo svalovine broilerov vo veku 21, 35 a 42 dní.

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