Impact of three different trace mineral sources on performance, digestibility and retention of zinc, manganese, copper, and iron in broiler chickens

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Para investigar el efecto de tres fuentes de Cu, Fe, Mn y Zn sobre el rendimiento zootécnico, así como, sobre la digestibilidad aparente total (ATTD) y la acumulación de los microminerales (TM) en hígado, tibia, pechuga y piel en pollos broiler, se realizó un estudio de depleción-repleción, de 28 días de duración. Durante los primeros 14 días, 88 pollitos macho Cobb 500 de un día se alimentaron con un pienso de depleción, es decir solo con el contenido mineral de las materias primas. A día 14 se tomaron muestras de dieciséis aves para analizar los tejidos. En los siguientes 14 días del periodo de repleción los 72 animales restantes se dividieron en tres tratamientos distintos, 8 corrales por tratamiento, y se suplementaron con las distintas fuentes de TM (34 ppm Fe, 44 ppm Mn, 24 ppm Zn, 3 ppm Cu), en forma de sulfatos (SUL), quelatos de glicina (GC) o quelatos de aminoácidos de soja (AAC). La ATTD y el contenido mineral en los tejidos corporales se midió en muestras mezcladas (n=8) los días 27 y 28. El peso vivo (BW) promedio de las aves fue 382 ± 25 g al día 14. Se observó una tendencia en el BW a día 28 (SUL: 1501 ± 33 g; GC:1523 ± 28 g; AAC: 1528 ± 21 g; P=0,07). Todos los grupos mostraron un índice de conversión similar durante la fase de repleción (días 15 – 28; SUL: 1,28 ± 0,04 g; GC:1,25 ± 0,04 g; AAC: 1,26 ± 0,03 g; P=0,22). La ATTD del Fe, Mn, Cu y Zn mejoró numéricamente, de media, un 12%, 20%, 19% y 19%, respectivamente, con el uso de quelatos en vez de sulfatos, observándose diferencias significativas entre el SUL y el AAC para el elemento Mn (SUL: 14,3 ± 1,9%, AAC: 17,7 ± 2,3%, P<0,01) y entre el SUL y el GC para el elemento Zn (SUL: 17,9 ± 3,0, GC: 21,9 ± 2,9, P<0,05). No hubo diferencias en la ATTD entre el GC y el AAC. De media, la concentración de TM en los tejidos corporales a día 28 fue un 4% mayor en las aves de los grupos GC y AAC que en el grupo SUL. Se detectó un efecto significativo de la fuente mineral sobre la concentración de Fe y Mn en hígado, Cu en pechuga y Fe en tibia, con mayores valores en el grupo AAC que en el grupo SUL. Este estudió confirmó que el Fe, Mn, Zn y Cu son más disponibles para los broilers cuando se suplementan en forma quelada que en forma inorgánica, en situaciones de deficiencia. Las diferencias en ATTD de los TM se reflejaron consistentemente en los datos de retención corporal. El uso de fuentes minerales queladas permite reducir la excreción de TM y en consecuencia el impacto ambiental.

Keywords: trace mineral source, broiler chickens, performance, digestibility, retention

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Introduction

Trace minerals are well known to play an important role in a variety of body functions, although their requirement is compared to other nutrients less than 100 mg per kg dry matter (Yatoo et al., 2013). Deficiency of trace minerals is difficult to detect under practical conditions. That makes it even more important to ensure a sufficient supply of the animal. The steady improvements in animal performance on the one hand, and environmental concerns on the other hand, have led to a risen interest for efficient trace mineral sources in animal feeding. Due to the chemical bonding and stability in the gastrointestinal tract (GIT), the bioavailability of various trace minerals is determined. Commonly inorganic sources like oxides and sulfates are used in animal production. But it is widely accepted, that trace minerals iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) can be absorbed to a higher extent when supplemented in organically bound forms compared to inorganic forms (Ammerman et al., 1995; Jongbloed et al., 2002). This can be confirmed by the results out of the experiment conducted by Abdallah et al. (2009). Chickens fed with organic trace minerals showed a significant higher body weight, better feed conversion and higher tibia ash compared to those who were fed with inorganic trace minerals.

In order to evaluate bioavailability of trace minerals from different sources in vivo, studies using a depletion-repletion trial design have been performed, mostly in piglets (Schlegel, 2006). Respective data with broiler chickens as model animal are scarcely available (Männer et al., 2016). Moreover, the effect of trace mineral source on differences in accumulation of trace minerals in specific tissues and organs of broiler chickens after a depletion period is not fully clarified.

The aim of this research was to investigate the effect of three sources of Cu, Fe, Mn, and Zn (sulfates, glycine chelates, soy amino acid chelates) on zootechnical performance, as well as apparent total-tract digestibility (ATTD) and accumulation of these trace minerals in several body tissues of broiler chickens by a depletion-repletion study design.

Materials and methods

The study was carried out in eighty-eight 1-day-old male broiler chickens (Cobb 500) at the Institute of Animal Nutrition at Freie Universität Berlin. All birds were fed a basal diet containing only native contents of trace minerals for the first 14 days (depletion period). The birds were housed in floor pens with bedding of softwood shavings and were given *ad libitum* access to water and feed in mash form. After the first 14 days of life, the birds were divided in 3 feeding groups to stainless steel metabolic cages with 3 birds per cage. In the repletion period from day 15 to 28 of age, animals were supplemented with Cu, Fe, Mn, and Zn either from sulfates (SUL), glycine chelates (GC, E.C.O.Trace®, Biochem Zusatzstoffe GmbH) or soy amino acid chelates (AAC) according to official feeding recommendations (NRC, 1994). De- and repletion diets were manufactured in the institute (registration number: DE-BE-100001) and prepared without inclusion of enzymes, growth promotors, acidifiers, probiotics or coccidiostats. Diet composition is shown in Table 1.

Table 1: Diet composition

Ingredients	Depletion period[3)]	Repletion period[4)]
Corn (%)	47.80	60.80
Soybean meal (49% CP)	35.80	24.00
Soybean-oil (%)	8.20	6.50
Wheat bran (%)	4.00	4.00
Limestone (%)	1.68	1.60
Monocalcium-phosphate (%)	0.80	0.65
Premix[1)] (%)	1.20	1.20
Titanium(IV)-dioxide (%)	-	0.30
DL-Methionine (%)	0.27	0.25
L-Lysine–HCL (%)	0.25	0.35
L-Threonin (%)	-	0.07
Trace mineral mix[2)] (%)	-	0.117

[1)] Contents per kg Premix: 600000 I.U. Vit. A (acetate); 120000 I.U. Vit. D_3; 6000 mg Vit. E (α-tocopherol acetate); 200 mg Vit. K_3; (MSB); 250 mg Vit. B_1 (mononitrate); 420 mg Vit. B_2 (cryst. riboflavin); 300 mg Vit. B_6 (pyridoxin-HCl); 1500 µg Vit. B_{12}; 3000 mg niacin (niacinamide); 12500 µg biotin (commercial, feed grade); 100 mg folic acid (cryst., commercial, feed grade); 1000 mg pantothenic acid (Ca d-pantothenate); 60000 mg choline (chloride); 45 mg iodine (calcium-iodate); 20 mg selenium (sodium-selenite); 140 g sodium (NaCl); 55 g magnesium (magnesium sulfate); carrier: calcium carbonate (calcium min 38%);
[2)] per kg trace mineral premix: 29231 mg Fe, 20342 mg Zn, 37692 mg Mn, 2479 mg Cu from SUL, GC, or AAC
[3)] day 1 to 14 of age
[4)] day 15 to 28 of age

TiO_2 was used as a marker for ATTD measurements. Analyzed nutrient contents in the de- and repletion diets are shown in Table 2.

Table 2: Analyzed nutrient contents in the diets

	Depletion period[1)]	Repletion period[2)]		
		SUL	GC	AAC
Dry Matter (g/kg)	940	908	912	908
Crude protein (g/kg)	228	193	195	196
Crude fiber (g/kg)	26.2	28.5	29.0	28.4
Crude fat (g/kg)	105	99.2	98.3	98.7
Ash (g/kg)	55.1	49.2	49.1	48.9
Starch (g/kg)	319	384	387	385
Sugars (g/kg)	43.8	37.9	37.5	37.3
Calcium (g/kg)	9.0	7.9	8.0	7.9
Total phosphorus (g/kg)	6.0	5.4	5.5	5.3
Sodium (g/kg)	1.6	1.7	1.8	1.7
Iron (g/kg)	43.5	103.8	99.6	104.7
Manganese (g/kg)	14.2	61.6	62.0	61.3
Zinc (g/kg)	16.0	50.8	52.3	53.1
Copper (g/kg)	4.8	8.9	8.6	8.3

[1)] day 1 to 14 of age
[2)] day 15 to 28 of age

Body weights per cage were recorded weekly. Feed consumption per broiler chicken was estimated as the total feed supplied per cage and period corrected for dispersed/leftover feed and number of birds per cage. Feed conversion ratio (feed:gain) was calculated from average feed intake and body weight gain per cage. Excreta of all birds were collected at day 27 and day 28 of age and were pooled per pen, freeze-dried and analyzed for the trace mineral contents. At day 14, a number of 16 birds and at day 28 all birds were stunned and culled by bleeding (approval number A 0100/13). Liver, left breast muscle, left tibia bone (TB) and overall breast skin were excised, whereby the tissues were pooled per cage. Samples of feeding stuffs as well as excreta and body tissue samples were analyzed for dry matter, crude ash, Fe, Mn, Zn and Cu in accordance to the methods given by VDLUFA (dry matter: VDLUFA III 3.1; crude ash: VDLUFA III 8.1; Fe: VDLUFA III 11.1.2; Mn: VDLUFA III 11.4.2, Zn: VDLUFA III 11.5.2, Cu: VDLUFA III 11.3.2). Feed samples were additionally analyzed by the following methods: crude protein: VDLUFA III 4.1.1 modified according to macro-N determination (vario Max CN); crude fiber: VDLUFA III 6.1.4; ash: VDLUFA III 8.1; crude fat: VDLUFA III 5.1.1; starch: VDLUFA III 7.2.1; total sugars: VDLUFA III 7.1.1; calcium: VDLUFA VII 2.2.2.6; phosphorus: VDLUFA VII 2.2.2.6; sodium: VDLUFA VII 2.2.2.6. Trace minerals in bones were measured after defatting. TiO_2 content in diets and excreta were measured using a UV spectrophotometer following the method of Myers et al. (2004). Statistical analysis was carried out by one-way ANOVA. Multiple comparisons between groups were made by LSD Post-hoc test and significant differences were declared at P<0.05.

Results and discussion

Body weight (BW) at start of repletion period was not different between feeding groups and averaged 383.2 ± 20.5 g. At trial end, trace mineral supplementation in form of glycine or soy amino acid chelates improved the feed conversion ratio and body weight gain (BWG) numerically as shown in Figure 1. The final BW at day 28 of the feeding groups showed only a tendency for the AAC group compared to the SUL group (SUL: 1501 ± 33 g; GC:1523 ± 28 g; AAC: 1528 ± 21 g; P=0.07).

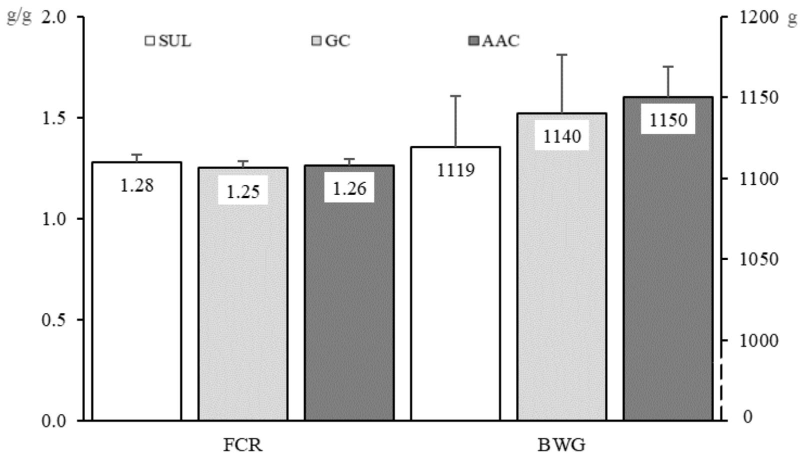

Figure 1: Performance in broiler chickens during repletion period, feed conversion ratio (FCR) and body weight gain (BWG, g), (Means, ±SD, n=8)

Similarly, in another depletion-repletion study the use of a mixture of Zn, Mn, Cu, and Fe from GC tended to be more efficient than respective sulfate forms (P<0.05) in improving BWG within 21-days repletion period (Männer et al, 2016). In line with these observations, De Marco (2017) reported a significantly improved BW of broilers after 35-days supplementation of GC trace minerals compared to the control group, which was supplemented with a mixture of sulfates and oxides, however without a preceding depletion phase. Numerical improvements of BWG of AAC compared to SUL fed broilers after 14 days repletion period was shown in Bao et al. (2007) with slightly lower and higher supplementation of organic trace minerals than inorganic. Nollet et al. (2008) determined numerical higher BW of broilers after 21 days feeding with a total replacement of inorganic trace elements with AAC. Moreover, numerical improvements in zootechnical performance are known from studies investigating single elements in broilers by exchanging inorganic forms by GC forms (Ma et al., 2013; Kwiecien et al., 2015; Kwiecien et al. 2016) or by AAC forms (Hudson et al., 2003). Controversial, no effect on performance was reported in studies using GC (Shi et al. 2015, Untea and Panaite et al. 2016) or AAC forms (Nollet, 2007; Star et al., 2012) compared to inorganic supplements.

The ATTD of Fe, Mn, Cu and Zn in the total diet on average was numerically improved by 12%, 20%, 19% and 19% respectively, when using chelated instead of sulfate forms. Significant differences were measured between SUL and AAC for the element Mn (SUL: 14.3 ± 1.9%, AAC: 17.7 ± 2.3%, P<0.01) for the element Mn and between SUL and GC for the element Zn (SUL: 17.9 ± 3.0, GC: 21.9 ± 2.9, P<0.05). Numerical variations were detected for Fe and Cu (Figure 2), whereas between SUL and GC, Cu tended to be significant higher digestible for the GC group (P=0.08). Studies in other animal species have shown that AAC bound trace minerals are more digestible than inorganically bound trace minerals (Spears et al., 2004; Männer et al., 2006; Hildebrand and Männer, 2013). In line with these findings, significantly higher apparent ileal digestibility of Fe, Zn, Mn and Cu after 21 days of repletion has been reported when using glycinates or AAC instead of sulfates (Männer et al. 2016). From studies investigating single elements exchanging inorganic forms by AAC forms, Miles et al. (2003) determined a less bioavailability of Cu and Zn as AAC in broiler, what was refuted in the present study. The absorption of Zn was measured by *in situ* ligated loops of broiler in the study of Yu et al. (2010), where Zn absorption in duodenum was higher in AAC group compared to GC group, but in jejunum and ileum the GC group showed higher absorption rates. In the present study, the GC group showed even a numerical higher ATTD of Zn and Cu compared to the AAC group. A less ATTD of AAC in broiler was assumed from De Marco (2017), because of higher excretion levels compared to glycinates.

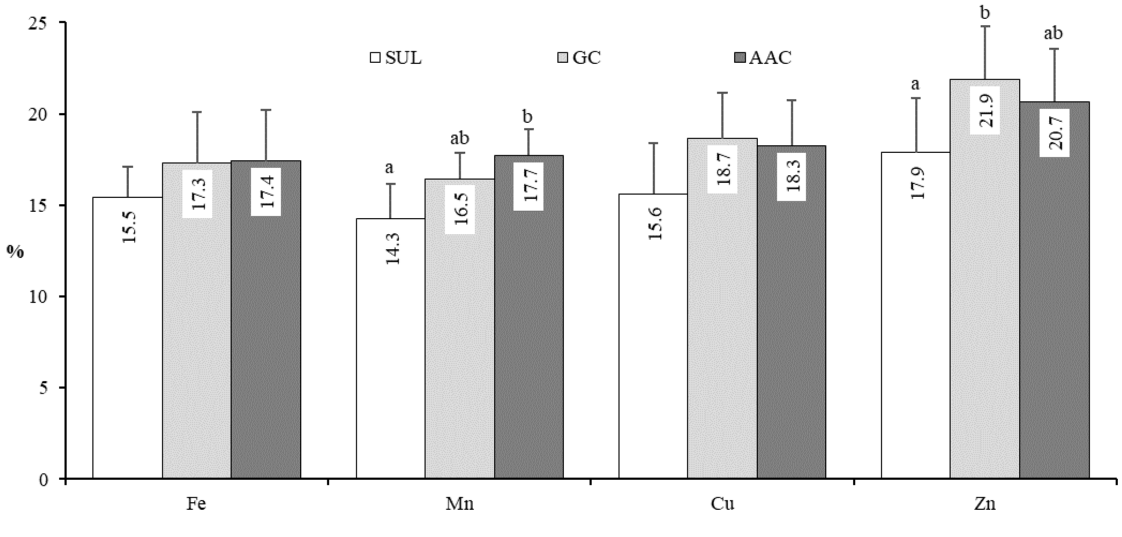

Figure 2: Apparent total-tract digestibility (ATTD) of complete diet (%) at day 28, (Means, ±SD, n=8)

Body tissue retention after depletion (d14) and repletion period (d28) are shown in Table 3. The differences in ATTD of trace minerals were partly reflected in metal concentrations in body tissues at the end of the trial. In body tissues of GC and AAC group, the retention of Fe, Mn, Zn and Cu in different tissue samples was numerically higher than in SUL group. Significant differences between AAC group vs. SUL group were detected in retention of Fe and Mn in liver, Cu in breast muscle and Fe in TB. This is in line with the ATTD data of the current study. Most studies examining the accumulation of trace minerals in broilers focused on the element Zn. In the present study Zn concentrations in liver, skin and TB were numerically higher with chelated forms compared to group SUL. According to the Zn content of 42-days old broiler chickens, a significantly higher retention was shown when 30 ppm Zn as GC where used instead of 40 ppm Zn as SUL (Sridhar et al., 2015). In another depletion-repletion study in piglets, a significant increase in the Zn content of skin by using glycinate form instead of sulfate form has been demonstrated (Hildebrand and Männer, 2013). Effects of Zn supply level on TB are well documented. The use of glycine-bound Zn allows a higher Zn concentration in the TB of 42-days old broiler chickens compared to an inorganic Zn form and allows a reduction in Zn dosage (Kwiecien et al., 2016). Supplemented Zn complexed with amino acids (10 and 15 ppm) showed significantly higher accumulation rates in TB of broiler compared to a group without any supplementation. In the same study, the supplementation of Zn as AAC (15 ppm) compared to Zn as sulfate (10 ppm) showed significantly higher accumulation rates in TB only with a higher amount of supplemented trace mineral (Star et al., 2012). Higher levels of Zn in liver have been reported when using a glycine-bound form instead of an inorganic one (Untea and Panaite, 2016), however it must be considered that liver Zn concentration is not assumed as first choice criteria for evaluation of bioavailability of Zn sources (Jongbloed et al., 2002). The current study showed clear effects of Fe supply on Fe retention. As shown by Shi et al. (2015) replacing Fe sulfate with Fe glycinate can effectively improve hemoglobin status. In the studies of another working group, the use of Fe glycinate instead of sulfate form had a positive effect on the hematological and biochemical parameters of broilers (Kwiecien et al., 2015a), whereas no clear effects of Cu source (glycinate vs. sulfate) on blood parameters and Cu level in liver were detectable (Kwiecien et al., 2015b). Higher Cu retention in blood plasma and liver after 21 days was detected by supplementation of 120 ppm Cu from AAC compared to sulfate (Liu et al., 2012). Studies on body retention of manganese from glycinate forms in broiler chickens are scarcely available. No effect of Mn source (chelated forms vs. sulfate form) on bone Mn concentration was detected after 42 days, whereas concentration in the heart muscle tissue indicated a higher bioavailability of Mn from the organic sources (Li et al., 2004). Contrary to results in TB of this study, Bao et al. (2007) detected lower Cu, Mn and Zn retention rates in the TB of AAC-fed compared to sulfate-fed broilers even in high or low supplementation of trace minerals, whereby the retention of Fe showed slightly higher values with 20 ppm organic Fe compared to 70 ppm inorganic Fe. Numerically increased levels of Mn in the muscle tissue were also reported by Hildebrand and Männer (2013) in piglets when glycine-bound Mn was supplemented instead of sulfate form. These observations contrast with the current study, where Mn content of TB was higher in the GC group than in the SUL group, but no difference in breast muscle tissue were detectable.

Table 3: Trace mineral concentration in liver, breast-muscle, skin and TB of broiler chickens at the end of depletion (day 14) (n=16) and end of repletion (day 28), (Means, ±SD, n=72)

	Day 14	Day 28			
		SUL	GC	AAC	P-value Effect of source
	Liver				
Fe (mg/kg)	263 ±47.9	440 ±25.8[a]	464 ±19.4[ab]	485[b] ±44.7	0.031
Mn (mg/kg)	10.5 ±0.21	10.2 ±0.66[a]	10.6 ±0.55[ab]	11.3[b] ±0.57	0.005
Cu (mg/kg)	16.8 ±4.12	12.0 ±1.66	12.4 ±1.52	12.0 ±2.17	0.879
Zn (mg/kg)	80.4 ±11.6	67.4 ±3.36	69.8 ±3.87	69.4 ±3.44	0.366
	Breast muscle				
Fe (mg/kg)	29.3 ±7.84	17.7 ±1.70	18.2 ±1.74	18.1 ±1.14	0.810
Mn (mg/kg)	1.09 ±0.32	0.52 ±0.03	0.53 ±0.10	0.51 ±0.18	0.974
Cu (mg/kg)	2.18 ±0.43	1.77[a] ±0.48	1.87[ab] ±0.56	2.36[b] ±0.17	0.031
Zn (mg/kg)	25.8 ±1.81	20.4 ±1.80	19.4 ±1.41	19.5 ±1.03	0.311
	Skin				
Fe (mg/kg)	25.8 ±3.82	33.2 ±3.6	34.0 ±2.44	33.9 ±3.01	0.851
Mn (mg/kg)	1.69 ±0.14	2.99 ±0.20	3.17 ±0.26	3.19 ±0.26	0.288
Cu (mg/kg)	5.89 ±1.03	5.07 ±0.45	5.13 ±0.75	5.40 ±0.26	0.734
Zn (mg/kg)	23.7 ±1.96	45.3 ±3.43	46.6 ±3.87	46.1 ±2.03	0.748
	Tibia bone				
Fe (mg/kg)	82 ±1.41	133 ±6.0[a]	136 ±5.0[ab]	143[b] ±10.3	0.033
Mn (mg/kg)	2.64 ±0.24	4.01 ±0.48	4.19 ±0.6	4.11 ±0.37	0.752
Cu (mg/kg)	7.73 ±1.36	4.76 ±0.18	4.87 ±0.29	4.71 ±0.32	0.502
Zn (mg/kg)	108 ±7.92	110 ±8.4	113 ±11.2	116 ±8.0	0.499

This study confirmed that Fe, Mn, Zn and Cu are available in broilers to a higher amount when supplemented in chelated form instead of sulfates in a deficient situation. Differences in ATTD of TM were not consistently reflected in body retention data. The use of chelated TM sources allows to reduce TM excretion and respective environmental impact.

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